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# **2021 YEARBOOK**



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### Aim and Scope

Archives of Breast Cancer (ABC) is an open access, peer-reviewed journal that publishes articles on all aspects of breast cancer research, including the pathophysiology, prevention, early detection, diagnosis, treatment, molecular and cellular biology, genetics, epidemiology, psychological issues, rehabilitation and quality of life. Although the main focus of the journal is breast cancer, some important topics among benign breast diseases and breast health such as breastfeeding will be considered for publication.

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### Editorial Office:

No 1437, Everest St, Alexis Nihon Blvd., Montreal, Canada Tel: +1 514-643-0969

Email: office@archbreastcancer.com

Website:

www.archbreastcancer.com



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## **ARCHIVES OF BREAST CANCER**

### Dear Scientists, Researchers, and Colleagues

### Happy 2022,

We are starting the new year while the COVID-19 outbreak is still a significant health problem in many countries worldwide. In 2021 we published several articles on breast cancer management during the pandemic. I am so proud to state that despite the limitations caused by COVID-19, the journal's promotion continues in 2021. ABC now publishes at least 14 articles per issue from various medical centers all around the world. We have published high-quality articles from Asia, Africa, Australia, Europe, and America this year. We have joined a Medical publisher, Farnam, and now applied to be indexed in Thompson-Reuters and Scopus as predicted.



Processing the submitted manuscripts is now more professional. Despite a 135 percent increase in the submissions, we succeeded in reducing the mean duration from submission to first decision to less than three weeks.

In 2022, we plan to improve the quality and quantity of the published articles and develop the infrastructure to accelerate the article processing time and facilitate the peer-review process. Also, we will make all necessary efforts to subclassify the editorial board to the subspecialty disciplines and increase the smart participation of the editorial board members in the whole process of the publication.

In general, I wish 2022 would witness the controlling of the covid outbreak and as for ABC, I hope our application to Scopus and Thompson Reuters will be accepted based on the quality of the published articles and our professional publication process.

ABC asks the esteemed scientists to contribute to this international multidisciplinary forum as a reader, reviewer, and editorial board.

Ahmad Kaviani, MD, Editor-in-Chief, Archives of Breast Cancer

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DOI: 10.32768/abc.202183162i-162ii Incredible Oncologist, Productive Researcher; Peiman Haddad

> Professor Peiman Haddad (1960-2021)



Professor Peiman Haddad was born on December 6, 1960 in Kashan, a small town in Iran, where his father was serving as the attorney general and his mother was a high-school teacher. Peiman went to elementary school in Firoozkooh and attended the prestigious Kharazmi high-school in Tehran. After receiving his high-school diploma, he started medicine at Tehran University of Medical Sciences in 1984. He finished Radiation Oncology specialty at the same university by 1994. Soon, he joined Iranian Cancer Institute and start working there from 1996. Based on his incredible scientific activities he was promoted to full professor in radiation oncology in 2011, and was appointed to the position of the head of the Radiation Oncology department at Tehran University of Medical Sciences in 2015. Throughout his life, Peiman followed his great ambitions in scientific and research activities and his passion toward the community, his family and nature, climbing numerous and volcanos in Iran and in Asia.

### John Yarnold, Emeritus Professor of Clinical Oncology, London, UK

I first met Professor Peiman Haddad almost 30 years ago when I arrived in Tehran to visit centres collaborating in an international trial testing adjuvant chemo-endocrine therapies in women with early breast cancer. He later visited the Royal Marsden Hospital, London, UK, where he spent several months in clinic with me and other colleagues. I have just looked at a photograph of him from the late 1990s taken in the room at home where I am writing this. I remember he described his passion for climbing mountains, an activity that struck me at the time as consistent with his strength of character and multiple talents. He was never afraid to challenge received wisdom, not least mine, and his questioning was always probing in original ways. As his many publications show, his academic interests were not restricted to breast cancer and included important challenges in radiotherapy. On visits to Iran in the 2000s, I kept in touch with Professor Haddad and visited the Radiation Oncology Research Centre, of which he was Director, at the Tehran University of Medical Sciences. As recently as February 2021, I had the privilege and pleasure of contributing to a webinar he organised discussing recent advances in treatment of breast cancer, a meeting which he chaired with typical flare and confidence. He was a wonderful representative of our global oncology community and a colleague whom we shall keep in our hearts and minds.

### Dr Andrea Bezjak, Professor, Department of Radiation Oncology, University of Toronto, and Princess Margaret Cancer Center

Dr Peiman Haddad had strong professional relationships with many at the Department of Radiation Oncology, University of Toronto, and the Princess Margaret Cancer Center. He spent a very productive year of fellowship with us in Toronto in 2002-2003, where he impressed everyone with his diligence, academic productivity and enthusiasm for improving outcomes of patients and knowledge within our field. During that year, he forged relationships and friendships that continued to grow despite the geographical distance. It was obvious then, and even more obvious over the ensuing years and decades, that we shared the same passion for radiation oncology, and for academic pursuits.

Peiman was a vocal and effective advocate for enhancements in radiation oncology in his center and in Iran in general, through international research collaborations, conferences, workshops and other ways to grow the quality and safety radiotherapy



agenda within his country. He was well respected and well liked by all of us who interacted with him, and we were very saddened to hear of his sudden passing. His legacy will remain, and collaborations between our centers continue, as created by him. Our condolences go to his dear wife and daughters, his colleagues and friends. We have lost a wonderful person, oncologist, colleague and friend.

### Dr. Mohammad Ali Mohagheghi, Professor of Surgical Oncology, the Head of Cancer Research Center

Prophet Mohammad said: "When a scientist passes away, the damage done is so great that nothing can compensate for it." The loss of Dr. Peiman Haddad, who was a great scientist and a philanthropist physician is very sad and heartbreaking and the academic community, patients and his family have suffered a lot, with whom I sympathize whole-heartedly. Dr. Peiman Haddad who was a man of great character in terms of medical ethics, compassion, and service left us and I ask Almighty God' mercy for him.

The founding fathers of Radiotherapy and Radio-Oncology Department at Iran Cancer Institute, Dr. Kamaledin Dehshiri, Dr. Akbar Ghaffarian, Dr. Abbas Etemad, Dr. Sajjadi, and Dr. Peiman Haddad always kept up-to-date with the latest scientific advancements in the world to solve numerous problems faced by cancer patients. As a veteran member of this unique community in the Middle East, I am confident that the Radio-Oncology Department will continue its progress in the right way envisioned by honest and philanthropic efforts of the late professors at this center including Dr. Peiman Haddad.

### Shahin Akhondzadeh PhD., D.Sc., FBPhS, Professor of Clinical Psychopharmacology, Department of Psychiatry, Tehran University of Medical Sciences

In the late 1990's when Iran's scientific growth started, I was responsible for Tehran University of Medical Sciences' publications and I also conducted the university's scientometric activities. I learned that one of the faculty members at the Radio-Oncology Department of the University, Dr. Peiman Haddad, was publishing very influential articles in highly credible international journals and thus I was acquainted with Dr. Peiman Haddad. Later on, he helped me with the School of Medicine's International Relations tasks, and in those years. I realized that in addition to his scientific abilities, he is also very nice, good-hearted, and a family man. In spite of his numerous scientific abilities, he was very modest and I believe those who live with their hearts, like Dr. Haddad, have a short life expectancy just as it happened to him. I have lost a very dear friend. I hope all the kindness that Dr. Haddad dedicated to

his patients and his colleagues would make full circle and come back to his family by God's will.

### Farnaz Amouzegar Hashemi, Professor of Radiation Oncology, Tehran University of Medical Sciences

As I am writing this, I still find it extremely difficult to believe that Dr. Peiman Haddad is no more among us. My residency coincided with the beginning of his career as an Assistant Professor in 1994. Our Late Professor, Dr. Dehshiri, had mentioned how smart Dr. Haddad was and what a bright future he would have in radiation oncology, and he was indeed a truly brilliant individual.

He invented a practical wooden device for craniospinal irradiation in children which helped to significantly improve fixation and reproducibility of treatment, in an era that cobalt units and twodimensional treatments were the only facilities. It was through his efforts that our department participated in two big international trials: ABC and ATLAS. In addition to completing an official fellowship at Princess Margaret Cancer Center, Professor Haddad visited many radiation oncology departments all over the world and made a positive contribution to our community by bringing back new research and teaching ideas.

He was very honest, kind-hearted, and respectful to his patients, residents and all the staff. Moreover, he was truly a well-rounded and athletic individual. I was astonished when he first told us about his hike from Tehran all the way to the North of Iran for 3 consecutive days. He was a motivation for residents and colleagues, and a true role model for all.

"Instead of running away from difficulties, I prefer to encounter and deal with them." This is one of his quotes that I will never forget. Though the Iranian Radiation Oncology Society has lost one of his most effective members, Dr. Haddad's story is not over yet. He will always be remembered.





### DOI: 10.32768/abc.2021811-3 Classification of the Clinical Presentation, Severity, and Response to Treatment in Idiopathic Granulomatousis Mastitis

Ahmad kaviani\*<sup>a,b,c</sup>, Mahtab Vasigh<sup>a,c</sup>

<sup>a</sup> Department of Surgery, Tehran University of Medical Sciences, Tehran, Iran

<sup>b</sup> Department of Surgical Oncology, University of Montreal, Montreal, Canada

<sup>e</sup> Breast Disease Research Center, Tehran University of Medical Sciences, Tehran, Iran

Idiopathic granulomatous mastitis (IGM), also called idiopathic lobular mastitis, is a rare, benign inflammatory condition of the breast with currently unknown etiology. The cornerstone of the design of a clinical trial relies on the type and the severity of the clinical findings and the response to the treatment strategy applied. Without a standard classification, we may not expect to accomplish uniform investigations in different centres. According to the considerable experience of the authors and considering our previous publications, we suggest a classification in four grades according to the clinical manifestation (inflammatory, cutaneous, soft tissue, and systemic findings). Severity can predict the treatment response, so we suggest the therapeutic modalities be chosen based on this factor.

Idiopathic granulomatous mastitis (IGM), also called idiopathic lobular mastitis, is a rare, benign inflammatory condition of the breast with currently unknown etiology.<sup>1</sup> The course of the disease is sometimes chronic and devastating for the patients.<sup>2</sup> It mostly affects women of childbearing age with a history of pregnancy or lactation within the last five years of the onset of the disease.<sup>3-5</sup> This is a rare condition in developed countries; however, it is more prevalent in developing counties. Recently there has been an increase in the number of studies in this regard. The reports mostly come from middle-east countries, including Iran and Turkey.<sup>4</sup>

Although an increase in the number of studies is evident, and it is a well-known clinical condition since 1972<sup>6</sup>, there is not yet a general consensus regarding the management of this disease.<sup>7,8</sup> In the

\* Address for correspondence: Ahmad Kaviani, MD Professor of Surgery, Address: Imam Khomeini Hospital Complex, Tehran, 1419733141, Iran Tel: +98 21 88871785 Fax: +98 21 88871698 Email: akaviani@archbreastcancer.com literature, there is a disparity in clinical findings, diagnosis, management, and follow-up of the disease in different studies.

The cornerstone of the design of a clinical trial relies on the type and the severity of the clinical findings and the response to the treatment strategy applied. Without a standard classification, we may not expect to accomplish uniform investigations in different centres.

The clinical presentation of the disease contains a wide range of signs including inflammatory symptoms resembling infectious mastitis or inflammatory carcinoma depending on the severity and extent of the inflammation. On the other hand, some patients would refer to medical centres with breast mass and tissue thickening. This group of symptoms are mostly related to the collection with or without inflammation that may mimic breast cancer. Finally, in some situations, the breast complaints are a part of a systemic disease. The clinical manifestation of the disease is summarized in Table 1.

Three hundred seventy-four patients were analysed in our previous work.<sup>9</sup> The severity of the disease in that study was categorized into three levels. Considering our previous publications<sup>9-14</sup>, we made some modifications to the classification system we used. We classify the severity of the disease in Table 2, based on the clinical manifestations and radiologic findings. We have conducted another investigation on the radio-pathologic findings of the disease in 224 patients and the severity of the disease is classified based on the description presented in this paper (Table 2). The clinical findings of the disease, along with radiologic and pathologic assessment of this large cohort of patients, will be presented soon.

Also, as an outcome measure, the researchers need a consistent classification of the response rate. This classification is crucial to evaluating the efficiency of each treatment modality, so we categorize the response to treatment based on the rate of improvement in the signs and symptoms as well as

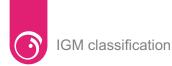


Table 1	. Classification	of the signs	and symptoms	

Inflammatory	Pain, Redness, Erythema, peu d'orange, skin thickening, axillary lymphadenopathy
Soft tissue*	Deep collections, tissue thickening, mass, skin dimpling, nipple retraction
Cutaneous Destructive	Thin red skin, superficial collection, Ulcer, fistula
Extramammary	Systemic lymphadenopathy, Arthralgia, Arthritis, Erythema nodosum, etc
* This group needs to be studi	ied by clinic-radiologic investigations. They can be considered mild when the collection is single with

minimal inflammatory reaction around it, moderate when the collections are multiple in a single quadrant and severe when the collections can be seen in more than one quadrant.

Grade	Inflammatory	Soft tissue	Cutaneous destructive	Extramammary	Suggested Treatment
Ι	Mild	Mild	No	No	NSAIDs*
Π	Mild to Moderate	Moderate to Severe	No	No	Percutaneous drainage <sup>6</sup> ANB <sup>9</sup> NSAIDs*
III	Sever	Moderate to Severe	Yes	No	Open drainage $ANB^{9}$ NSAIDs * $+ CS^{\epsilon}$ $+ _{TSM^{\eta}}$
IV	Any	Any	Any	Yes	Based on the protocols for systemic disease

\* Non-Steroidal Anti Inflammatory Drugs for three months

 $^{\circ}$  Should be done with large born needles, preferably with the guide of ultrasonography

<sup>8</sup> Widespectrum oral antibiotics with coverage on gram-positive microorganisms for 10-14 days

<sup> $\epsilon$ </sup> Corticosteroids can be used based on clinical impression with 50 mg prednisolone in a tapering dose, discontinue in 10 weeks. Can be continued in low dose (5-10mg/day) for a maximum of 3 months

<sup>1</sup> Immunosuppressive medications can be used if only the disease in persistent in Grade III for 6 months

Table 3. Classification of "Resp	onse to Treatment" in breast-limited IGM	Λ
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	Initial Control of symptoms	No Recurrences*	Severity of the recurrences*
Excellent Control	Complete <sup>e</sup>	0	-
Good Control	Complete <sup>e</sup>	1-2	Grade I-II
Fair Control	Partial <sup>9</sup>	3-4	Grade I-II
Poor Control	Fair <sup>ε</sup>	Persistent disease	Grade III

\* During a 12-months follow up

 $^{\theta}$  Resolution of >90 percent of the signs and symptoms

<sup>9</sup> Marked reduction in the signs and symptoms

 $^{\epsilon}$  Not a big resolution in signs and symptoms

the frequency of the recurrences (Table 3). It is clear that the clinical classifications will be authenticated if they are concordant with the imaging (ultrasonographic) findings.

### **Conflict of Interest**

None.

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## Archives Of Breast Cancer

### Personalization of Ductal Carcinoma In-Situ Management: Large Databases and an Emerging Role for Global Data Sharing

Thomas O'Keefe\*<sup>a</sup>, Olivier Harismendy<sup>b</sup>, Laura Esserman<sup>c</sup>, Anne M Wallace<sup>a</sup>

<sup>a</sup> Division of Breast Surgery and the Comprehensive Breast Health Center, University of California, San Diego, CA, USA <sup>b</sup> Department of Medicine, Moores Cancer Center and Division of Biomedical Informatics, University of California, San Diego, CA, USA

<sup>°</sup>Carol Franc Buck Breast Care Center, University of California, San Francisco, CA, USA

We have read with great interest recent DCIS observational studies by Mannu et al.<sup>1</sup> and Giannakeas et al.<sup>2</sup> regarding invasive breast cancer risk and breast cancer mortality after ductal carcinoma in situ and the association of a diagnosis of ductal carcinoma in situ with death from breast cancer. In this paper, we explore some of the shortcomings of the current landscape of DCIS literature and make a call for global data sharing for sources of DCIS outcomes data.

### I. Ductal Carcinoma in Situ Outcomes

A major challenge posed by ductal carcinoma in situ (DCIS) research is the significant heterogeneity of the disease coupled with the rarity of relevant outcomes of interest such as progression to invasive disease and breast cancer mortality (BCM). Several randomized controlled trials (RCTs) have been conducted on DCIS patients with lumpectomyamenable disease to assess the effect of adjuvant radiation therapy (RT) and endocrine therapy (ET) on outcomes. These studies consistently demonstrated that while such adjuvant therapies reduced second breast cancer events (SBCE), they neither influenced BCM nor overall survival (OS).<sup>3-5</sup>

The full picture is more complicated. There is evidence that patients who suffer invasive SBCEs have higher rates of BCM<sup>6</sup>, a very rare outcome for DCIS patients. This suggests that either the previously mentioned RCTs were insufficiently powered to detect the impact of adjuvant therapies on BCM, or that the adjuvant therapies administered

\* Address for correspondence: Thomas Joseph O'Keefe V, Address: Mailbox 0819, 3855 Health Sciences, La Jolla, San Diego, 92037 CA, USA Tel: +1 858 622 1771 Email: <u>tokeefe@ucsd.edu</u> are preferentially reducing rates of non-lethal invasive SBCEs, or that there is a small proportion of DCIS lesions that are associated with substantially high risk for a metastatic event, even when an invasive component has not been found.<sup>6,7</sup> What we traditionally refer to as high risk factors for DCIS lesions are predominantly related to the risk of progression to invasive disease or recurrence. It is unclear, however, whether these factors are also associated with high risk of BCM. We anticipate that investigation of trends and patterns within subgroup analyses of large cohorts may shed light on the influences of such factors on these respective outcomes of interest.

II. Frequently Overlooked Covariates in DCIS Outcomes

### Race

Black race has been repeatedly shown to be associated with increased risk of invasive progression and worse prognosis in DCIS patients in the US, which has been attributed to various causes, including delay in adjuvant therapy, biological differences such as differing rates of hormone receptor positivity, and, of relevance to the referenced studies, reduced access to high quality screening facilities.<sup>8-11</sup> In the study by Mannu et al., race was not incorporated into the outcomes analysis.<sup>1</sup> The proportion of black Britons relative to the overall UK population may be smaller than that in the US, but any information obtainable from the National Health Service Breast Screening Programme (NHSBSP) regarding the proportion of patients attending their invited screening mammogram would allow for a rough comparison of these numbers to the proportion of black Britons in the general population to help shed light on this question.

*HER2 Overexpression* We recently showed that patients with HER2

3

overexpressing DCIS had increased rates of ipsilateral invasive SBCE on an analysis of data from the Surveillance, Epidemiology and End Results Program (SEER), a large US cancer database, but the significance of HER2 overexpression on invasive recurrence has been debated, with some studies demonstrating increased, some decreased, and some no change in risk based on this marker.<sup>12-16</sup> At most institutions, HER2 is not routinely assessed for DCIS; however, at one of the institutions represented by the authors, it has been routinely tested for DCIS patients in recent years, and at the other, it has been assessed for patients entering an active surveillance protocol due to the higher observed rate of invasive progression in patients declining surgical therapy at that institution. HER2 status was not accounted for in either of the referenced studies.

### Microinvasion

Patients who received chemotherapy are frequently excluded from DCIS outcomes studies, as chemotherapy is not indicated for pure DCIS. One of the possible limitations of the referenced study by Mannu et al. is that it is unclear whether this was done with or without knowledge of microinvasion status. If this was done without the knowledge of microinvasive status, it is possible that a subgroup of patients with microinvasive disease would remain, because not all patients with microinvasive disease, or even those with triple negative microinvasive disease, are necessarily required to undergo chemotherapy. Microinvasion is associated with worse prognosis<sup>17</sup>, so an incomplete inclusion of these patients based on chemotherapy receipt may have unanticipated influences on outcomes analysis.

### Surgical Laterality and Breast Reconstruction

Another concern particular to the study by Mannu et al. is that patients undergoing mastectomy were grouped together for analysis; we are interested in whether surgical laterality for mastectomy with unilateral DCIS was available, as this would be expected to significantly influence the results of the ipsilateral-contralateral rate comparison.

Furthermore, breast reconstruction may be associated with improved breast cancer specific survival in invasive breast cancers <sup>18-20</sup>, though it is unclear whether this is secondary to biology or socioeconomic factors.<sup>19</sup> However, it is possible that patients with DCIS may also have a survival benefit with reconstruction and represent an additional confounder.

### III. Additional Analyses for Consideration

Breast Cancer Mortality without an Intervening Invasive Lesion

In Steven Narod's 2015 analysis on BCM among DCIS patients, roughly half of patients who suffered

BCM after an initial diagnosis of DCIS did so without documented evidence of an intervening SBCE.<sup>6</sup> In the study by Mannu et al., it would be intriguing to see whether this trend was also present among the cohort in this study, as it is possible that Narod's observation could have been related to one of the many sometimes rather opaque abstracting guidelines for tumor registrars, or it could be secondary to biology, in which case, learning more about these types of high risk DCIS would be very important.

### Molecular Phenotype Changes after Adjuvant Endocrine Therapy

If data regarding the molecular phenotype of invasive recurrences were available, it would be interesting to investigate whether RT or ET influence rates of changes in molecular phenotype from the initial lesion to the invasive recurrence. And in particular, if a subset of HR- patients were identified who are more likely to have a HR+ invasive recurrence, it may be possible that these patients could benefit more from adjuvant or prophylactic ET.

### IV. Point of Interest

### Contralateral Invasive SBCE Rates

One of the most interesting findings to us in the study by Mannu et al. was the association of BCS without adjuvant RT as well as involved margins with invasive contralateral SBCE, where BCS without RT reduced contralateral invasive risk, and involved margins increased contralateral invasive risk.<sup>1</sup> Involved margins are classically thought of as conferring risk of local recurrence, and adjuvant RT has only been shown to influence ipsilateral recurrences. There are many possible explanations for this interesting finding. It is possibly as simple as exposure to less intense radiation in the contralateral breast predisposing to a new focus of disease in that breast, though at least for invasive lesions, this does not appear to be a significant influencer.<sup>21</sup> Alternatively, the presence of involved margins may represent some lesion related factor suggestive of a predisposition to further disease at other sites.

### V. Minor Clarification

### Tumor size

One issue we have noted in DCIS registry data in the US, including the SEER based study by Giannakeas et al., pertains to the method by which lesion sizes are coded when there is more than one focus of disease.<sup>2</sup> In our local tumor registries, DCIS size may be abstracted as either the largest contiguous focus of disease or as the overall extent of disease. This could have a significant impact on the outcomes analyses performed. For example, a tumor coded as one centimeter, due to the pathologist stating the largest contiguous focus of disease, when that disease actually extends over a much larger area of breast tissue, could result in an inappropriate proxy for tumor size. If tumor size reporting is inconsistent in the UK as it seems to be here in the US, a better surrogate may be the lesion size on screening images, assuming the entire lesion is mammographically visible or otherwise that the patient's lesion was appropriately characterized by adjunct imaging methods such as ultrasound or magnetic resonance imaging.

### VI. A Call for Global Data Sharing

In the US, there are two large publicly available cancer databases that can be readily queried for the purposes of BCM analysis. Both have advantages and disadvantages.<sup>22-24</sup> The previously mentioned SEER database allows for analysis of invasive SBCE, BCM, and OS, but does not include data on ET and captures a smaller percentage of DCIS patients. The National Cancer Database (NCDB), on the other hand, includes a larger sampling of patients and offers a better characterization of treatment related factors including ET and immunotherapy, but problematically the only outcome made available is OS, not BCM or invasive SBCE.

An equally important limitation from the data science standpoint pertains to the relationship between these two databases. While many patterns may be identified within a given individual dataset, not all of them are necessarily of clinicopathologic consequence. We recently showed, for example, that a coding idiosyncrasy within SEER guidelines results in its underestimation of breast cancer mortality, even though it has previously been used to estimate this outcome.<sup>6,25</sup> Another possible source of error is that some patterns may be identified due to confounding variables that are either not available within a given dataset or not utilized within a particular analysis. Ideally, to combat such unanticipated influences, one of these sources could be used as a discovery database, analogous to the training datasets utilized in machine learning techniques, and the other could be used to verify these findings, analogous to validation datasets. This is unfortunately unrealistic with respect to outcomes analysis using the current publicly available datasets because of the lack of overlap between endpoints such as SBCE and BCM, as well as the fact that there are shared patients between some datasets such as SEER and NCDB.

The two University of California institutions representing our authors have been in the process of making a publicly accessible DCIS resource. This dataset will obviously be smaller in size than such national datasets as SEER and NCDB. However, it will incorporate not only clinical, histopathologic, and outcomes data, but also curated data on exposure to hormone therapy and findings from our molecular biology and imaging studies on these deidentified patients. This will allow data scientists interested in subgroup analyses to investigate trends that may be missed in more comprehensive analyses. We are planning a system whereby any researcher or data scientist with a legitimate question can gain access through us to this database, similar to the systems utilized by SEER and NCDB.

We wish to invite all researchers with access to such unique DCIS datasets to work to make deidentified, publicly available versions. Such publicly accessible versions of these datasets would be highly valuable from the standpoint of outcomes analysis, as it would allow for validation of hypotheses generated regarding BCM from other large, public datasets which may be more limited with respect to characterization and outcomes.

Translational scientists may someday provide the medical community with the tools to provide truly individualized patient care for DCIS patients. In the interim, we believe that more can be done to personalize management for the hundreds of thousands of women diagnosed with this condition each year by more carefully elucidating the relevance of different clinicopathologic features to different possible outcomes.

### **Conflict of Interest**

None.

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### By the Metrics Surgical Oophorectomy Plus Tamoxifen is the First Adjuvant Treatment Option for Premenopausal Women with Hormone Receptor Positive Breast Cancer

Richard R. Love\*<sup>a</sup>

<sup>a</sup> Department of Computer Science, Marquette University, Milwaukee, WI, USA

"The right measure for successful health care isn't about the maximum possible for a few, but the average for everyone... and the minimum opportunities available to even those with the fewest resources and privileges"<sup>1.</sup>

"How you define the problem determines whether you solve it."<sup>2</sup>

Outcomes for the majority of women with breast cancer, most of whom, but hardly all, do not live in high-income countries, are poor.<sup>3</sup> The breast cancer sub-group of premenopausal women with hormone positive tumors is large: at a minimum: 550,000 total new cases/year, 420,000 of whom come from low-and middle-income countries (LMICs), with 82% of the global population. Based on Globocan estimates for all new breast cancer cases for 2018 at 2.088 million, this subgroup number may be as high as 700,000 cases.<sup>3</sup>

For this specific population, the impact of optimal treatment is large. The Early Breast Cancer Trialists' Group found a risk reduction of 25% in death at 10 years with 5 years of tamoxifen treatment.<sup>4,5</sup> The additional benefits of ovarian suppression or ablation added to tamoxifen suggest risk reductions for death of as much as 42%.<sup>6-8</sup> These data suggest that in a premenopausal population of half axillary node-positive patients with a 10-year overall survival of 55-60% without any adjuvant treatment, with optimal 5-year adjuvant endocrine therapy this figure might increase to 75-78%. These estimates, therefore, suggest that 80-100,000 of the 420,000 (minimally) low-and middle-income country women diagnosed annually, at greater absolute risk for death without optimal treatment,

\* Address for correspondence:

Richard R. Love, M.D. Address: 2708 Columbia Road, Madison, WI 53705, USA. Tel: +1 6086987881 Fax: +1 608 238788 Email: richardibcrf@gmail.com who could be saved for 10 years, instead die.

What is the problem? Among many issues, the following stand out:

• Human rights challenges. In many countries, women do not have permission to seek medical care for themselves. If it were widely known that attainable, affordable, and effective treatment was available, this might mitigate some of these restrictions.<sup>9</sup>

• Barriers to seeking care. Women do not seek interventions for breast problems they know they have. Many women in LMICs don't seek care because they know that in their medical systems, financial resources will be demanded for diagnosis and treatment, money their families do not have.<sup>9,10</sup>

• Financial and health system operational issues. Increasingly, health care systems globally operate on business models, with high levels of corruption in many LMICs. For most patients, their systems are byzantine, and timeconsuming.<sup>9</sup>

In these broad contexts, for premenopausal women with hormone receptor-positive breast cancer:

• Following from leading cancer organization guidelines, SO (surgical oophorectomy) plus tamoxifen as a treatment choice is not offered.<sup>11</sup>

• Because of financial barriers and treatment nonadherence, many patients, particularly those in LMICs (the majority), if they have operable disease and do undergo primary surgery, appear not to get any or enough adjuvant treatment to provide maximally achievable outcome benefits: prevention of disease recurrence and death.<sup>12</sup>

• Together with assumptions that SO or ovarian ablation and ovarian function suppression are equivalent clinical treatments, SO and GnRH treatments are inappropriately considered as biologically and therapeutically equivalent.<sup>11</sup>

• When offered, there is incomplete consideration of the SO + T option by patients because of no mention by physicians of data regarding multiple key metrics of value and quality of this intervention.<sup>11</sup>

• Incomplete/immature data on SOFT/TEXT investigated/ recommended treatments regarding key metrics, particularly long-term secondary effects.<sup>8</sup>

• Unrealistic considerations and discussions of host differences about symptoms.

• Much lower clinical practice compliance with treatment programs than in research studies, and limited approaches to treatment in-adherence problems.<sup>12</sup>

• A dominant tumor-biology-focused treatment paradigm exists when there are strong suggestions that a host biology-focused treatment paradigm is also likely.<sup>13</sup>

• Individual patient (in researchers' experiences) versus global public health treatment paradigms, with limited consideration of the equity issues associated with these paradigms.<sup>14</sup>

The breadth of these problems as causal explanations emphasize the importance of patientcentric issues in clinical care. In many ways, they are all of a whole. This lengthy introduction has been offered to frame appropriately, constructively, broadly, comprehensively, and for women globally, the rationale and substance of this communication.

Consider then with the six Institute of Medicine (IOM) quality of health care metrics, relevant SO+T data from the author's two phase III randomized clinical trials (and peripherally one additional trial in metastatic disease in consideration of one issue -- host paradigms), an ECOG trial, and the updated SOFT/TEXT trial report.<sup>4, 5, 8, 14-18</sup> Additionally, note data from other earlier adjuvant trials where SO has been a treatment: the very first adjuvant trials considered in meta-analysis, and Scottish, Danish, and French trials.<sup>4,5,19-21</sup>

The six IOM quality of care metrics are efficacy, safety, efficiency, patient-centeredness, timeliness, and equity.<sup>22</sup> Addressing the data about these measures with respect to SO+T in order:

### A. Efficacy/Effectiveness

A data-supported place for SO in the adjuvant therapy of breast cancer was created by the EBCCTG meta-analysis which included 4 trials, first individually reported on beginning in 1970.<sup>4, 5</sup> The individual patient data from these trials and those involving radiation to the ovaries, with what would be considered significantly less-thanrigorous methodologies today, and in the absence of patient tumoral hormone receptor data, looked at together, suggested that ovarian function ablation or suppression with radiation, conferred long-term recurrence free (DFS) and overall survival (OS) A Scottish trial in women with axillary node positive breast cancer, found ovarian ablation provided equivalent outcome benefits to those from CMF chemotherapy.<sup>21</sup> In the sub-set of patients whose tumors were later assessed for hormonal receptors, those patients with hormone receptor positive tumors benefitted more from ovarian ablation. A Danish trial in hormone receptor positive patients found ovarian ablation and CMF to be equivalent therapies in efficacy.<sup>19</sup>

A trial in premenopausal women in Vietnam and China with operable breast cancer, 52% of whom were axillary node positive, unselected for hormone receptor status at the time of primary treatment, found that in the patients subsequently determined to be estrogen receptor positive, there were 7 year disease-free and overall survival risk reductions of 0.46 and 0.54, respectively.<sup>6,17</sup>

A French trial in axillary node-positive, tumor hormone receptor- positive patients found ovarian ablation plus tamoxifen to be superior to FAC chemotherapy.<sup>20</sup>

An underpowered American Intergroup study in axillary node negative, hormone receptor positive patients found SO plus tamoxifen to give better overall survival at 5 years than tamoxifen alone with a risk reduction of 0.5 (OS 97.6% versus 95.2%) (N.S.).<sup>16</sup>

A trial in hormone receptor positive Vietnamese and Filippine women found, in explanatory analyses, that patients with true follicular or luteal menstrual phase status confirmed by blood progesterone testing, had significantly better DFS and OS compared to patients with unconfirmed luteal (or prolonged follicular or anovulatory) status (history of luteal phase, but low progesterone levels).<sup>15</sup> This same observation was made in a SO plus tamoxifen study in women with hormone receptor positive metastatic breast cancer.<sup>18</sup>

In the recently updated SOFT/TEXT trials analyses of ovarian suppression by GnRH agonist treatment plus tamoxifen or GnRH treatment followed by SO plus tamoxifen, versus tamoxifen alone, 8-year OS risk reductions of 0.33 without, and 0.41 with chemotherapy (higher risk patients), were observed with the combined ovarian plus tamoxifen treatments, for both p=0.01.<sup>8</sup>

In two trial data sets, the combination of SO or GnRH plus tamoxifen has been suggested to be more beneficial in Her-2neu positive patients.<sup>8,23</sup>

It is self-evident, but important to note that assigned SO in all of the above trials was received by practically all of the studied patients, while in the SOFT/TEXT trials early discontinuation of the GNRH plus tamoxifen treatment occurred in 19.3% of participating subjects in contexts where treatment for 5 years was being studied.<sup>8</sup> Based on data about nonadherence to tamoxifen alone treatment (see



below), in non-research settings early discontinuation rates might be expected to be even higher.<sup>12</sup> This is but one of several differences between patients treated with GnRH therapies versus SO. In the SOFT/TEXT trials' interventions, GnRH was given as noted for variable periods less than the planned 5 years. There is no ability to monitor for GnRH efficacy in individual patients, so there is no certainty from month to month of biological effect. GnRH followed by SO beyond 6 months was a treatment option in SOFT/TEXT trials, but SO was done variable times over the 5 years with unspecified treatment gaps between treatments and with uncertain hormonal signaling effects. The duration of follow up in the SOFT/TEXT trials provided limited long-term allcause mortality information.8

While the SOFT/TEXT trials investigated the issue of ovarian suppression plus aromatase inhibitor treatment, at 8 years there was no significant difference in overall survival compared with ovarian suppression plus tamoxifen treatment, this at a timepoint 3 years after completion of the adjuvant therapies.<sup>8</sup>

Finally, regarding efficacy of SO plus tamoxifen in the contexts described above, population data on tamoxifen nonadherence strongly suggest that in clinical practice tamoxifen consumption falls dramatically over the 5 years currently prescribed period, and that perhaps but 1/3<sup>rd</sup> of women, even in high-income countries, take the medication for this period.<sup>12</sup> Further, regarding absolute overall benefits of adjuvant hormonal therapies, these have to be understood in the contexts now of additional benefits to these patients from adjuvant radiation therapy, and long term (between year 5 and 10) hormonal therapies, particularly tamoxifen.<sup>24-26</sup>

### Conclusions regarding efficacy of SO+T

The above reviewed specific SO studies demonstrate a consistent overall, and withinseveral-studies-consistent picture of efficacy from SO plus T greater than that from SO alone or tamoxifen alone, and equivalent or perhaps with some twists (timing of surgery, for example; or better treatment adherence), efficacy to standard widely available chemotherapy regimens. These superior benefits are seen in node positive as well as node negative patients. Over the first 10 years following diagnosis and treatment, SO plus tamoxifen is clearly and significantly an optimally effective treatment, in major part, at the population level, because when chosen, surgical oophorectomy is always received.

### B. Safety and toxicity side effects

In high-income country studies, with often limited follow-up periods of 5-10 years, immediate symptomatic side effects are well-reported, but longterm clinical outcomes are less documented. For the symptoms data, what is important to note is that particularly the vasomotor symptoms vary in frequency, intensity, and duration among populations. In Asian populations, the intensity and duration of vasomotor symptoms following SO plus tamoxifen were indistinguishable from these metrics in untreated women after one year.<sup>27</sup> In contrast, in American and European populations, these symptoms were more significant.<sup>8,16</sup>

For bone mineral density, SO plus tamoxifen causes bone loss at only the lumbar spine site, for one year, and is associated with no significant loss at the hip at all.<sup>28</sup> This is a significant salutary benefit, suggesting that such treatment does not need to be supplemented with bisphosphonate therapies with their own financial costs and toxicities. This is not the case with GnRH plus tamoxifen or aromatase treatments.<sup>28</sup>

Because of the use of tamoxifen in postmenopausal women with breast cancer and as a chemoprevention drug, there are considerable data to allow estimates of the long-term secondary effects of SO plus tamoxifen, summarized in Table 1.

As can be read here, these data are remarkably reassuring that this treatment has long-term overall benefits which far exceed those often overemphasized for endometrial cancer and thromboembolism. What

All-cause mortality	Decreased <sup>29</sup>
CHD mortality**	Decreased <sup>29, 30</sup>
Myocardial infarction	Decreased <sup>29,31</sup> (in 2/5 studies)
Stroke	Decreased <sup>31</sup> (in 3/6 studies)
VTE/PE***	Limited in women under age 501 <sup>5, 17, 32</sup>
Lung cancer	Decreased <sup>29</sup>
Colon cancer	No impact <sup>33</sup>
Endometrial cancer	Excess affecting 0.2%/ year with very rare deaths <sup>15, 17, 34, 35</sup>
Ovarian cancer	Decreased

Table 1. Estimated long-term secondary effects of SO+T\*

\*Based on studies of tamoxifen in postmenopausal women

\*\* C.H.D.=Coronary Heart Disease

\*\*\*Venous thromboembolism, pulmonary embolism

is also clear is that at present such long-term data are not available for GnRH plus tamoxifen or aromatase inhibitor treatments, and also that there are multiple reasons to expect that when available data on the outcomes listed in Table 1, will offer a much less favorable picture for GnRH treatments than those for SO plus tamoxifen.

Other than vasomotor symptoms, other specific side effects of SO should be noted. Among 1101 patients who received SO in two adjuvant studies primarily in Vietnam, China, and the Philippines, there was no 30-day mortality, and 4 patients only developed pneumonia (2) or deep vein thromboses (2).<sup>15,17</sup> Most of these SO procedures were done under the anesthesia done also for the primary breast surgery. All of these patients were fully informed of the irreversible nature of this procedure on their menopausal status and ability to conceive, and provided written informed consent.<sup>15, 17</sup> In one major clinical trial site in Manila, 3 patients of 336 (<1%), refused SO plus tamoxifen treatment, possibly because of irreversible and inability-to-conceive treatment consequences.

In summary, with respect to the IOM safety metric, SO plus tamoxifen is comprehensively described over short and long terms and the overall impact on women's health is very favorable.

### C. Cost efficacy/efficiency/net benefit for cost

For patients, SO plus tamoxifen is much more cost-effective or gives more net health benefit for much lower patient payment, than GnRH plus tamoxifen. SO plus tamoxifen treatment maximizes the impact of available medical resources, when both indirect and direct costs for both providers and patients are considered. Perhaps more so in low- and middle-income than in high-income countries, financial issues come to the fore in treatment decisions. A breast self-examination trial in the Philippines was abandoned because patients who had breast tumors did not seek care because they assessed that they did not have the financial resources to have treatment.<sup>36</sup> In the United States, the American Society of Clinical Oncology considers delivering value to be a major driver of change in health care delivery, and has expressed concerns regarding the financial toxicity of expensive cancer therapies.<sup>37</sup> The cost -efficacy of SO plus tamoxifen treatment

has been estimated at \$351 per year of life saved, a level of return comparable to those suggested for very effective vaccinations.<sup>17</sup>

SO plus tamoxifen requires inpatient surgery accomplishable in most settings globally together with the primary breast surgery, with financial costs covered. Tamoxifen, in most settings, requires out of pocket costs for patients, but of manageable levels. No additional therapies are required in particular because of an absence of bone loss toxicity. In contrast, GnRH therapies require monthly (recommended) or 3 monthly physician visits with associated significant direct and indirect patient costs, and as noted above, 5 years of treatment are the standard of care.

### D. Patient-centeredness

This important metric addresses tailoring treatment to patient needs, values, and preferences. SO treatment absolutely requires open discussion with patients about this option in detail, and as such, if chosen by patients, meets the metric requirements. In contrast, full exposition of GnRH treatment with all its components, clearly is not well patientcentered, and is significantly impractical.

#### E. Timeliness

SO, treatment is a one-time intervention done together with primary surgery. Once completed, patients must incur the benefits. There are no delays in treatment associated with unavailability of medicines and finances, or schedules of patients and caregivers, as occur regularly with GnRH treatments. This "one stop" metric of SO makes it a better treatment than GnRH.

#### F. Equity

Essentially all women globally can get SO treatment, which meets the need offered by Tufeki for successful health care.<sup>1</sup> SO treatment limits corruption factors which play out over time with GnRH treatments. SO plus, tamoxifen treatment provides consistent quality of care to all patients and is a socially just treatment. With GnRH treatments, major financial issues and dysfunctional health systems interfere with delivering this treatment to populations for their maximal benefit. Table 2 summarizes the fore-presented data on IOM metrics.

Table 2. Summary: Adjuvant SO+T by the 6 IOM metrics

**Efficacy:** When taken for prescribed 5 years, efficacy is equivalent to (or possibly significantly better) than guideline-recommended GnRH plus tamoxifen treatment. Globally, SO as a treatment is practical and when chosen by patients is always received.

**Cost efficacy:** With SO performed together with primary breast surgery and generic tamoxifen, cost/year of life saved is remarkably low. Value as a treatment is high to patients and for medical systems.

Patient-centeredness: SO plus tamoxifen treatment is practical.

Equity: SO+T is a consistent, high-quality achievable intervention for women everywhere. Socially just.

**Safety:** The organ and tissues effects of SO+T are well-known and more favorable than those for any other hormonal therapies. The symptomatic effects vary among patients, and dissipate over one year.

Timeliness: No delay in getting some adjuvant treatment administered.



Globally, all 550,000-700,000 women annually (1/3rd of all new cases) for whom adjuvant hormonal therapies are strongly indicated, should be provided information on the 6 metrics of quality of care for adjuvant treatments, and given the option of SO plus tamoxifen treatment. Treatment standards are local not global; thus, particular circumstances, both patient and medical system may determine whether SO+T is a reasonable option.<sup>38</sup>

### **Conflict of Interest**

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### DOI: 10.32768/abc.20218114-15 A Concise Viewpoint on Menopause, Hormone-Replacement Therapy and Breast Cancer Risk

Vaishnavi Natarajan<sup>a</sup>, Anindita Chakrabarty<sup>\*b</sup>

<sup>a</sup> Amity University, Noida, Uttar Pradesh, India
<sup>b</sup> Shiv Nadar University, Greater Noida, Uttar Pradesh, India

Menopause is accompanied by numerous physiological and psychological symptoms degrading the quality of life.<sup>1</sup> Hormone Replacement Therapy (HRT), introduced in the 1960s, effectively manages many menopausal symptoms. During the 1970s, a link between estrogen therapy and increased incidence of endometrial cancer was found and resolved by combination-HRT containing estrogen and progestin.<sup>2</sup> Nevertheless, its usage declined dramatically when the Women's Health Initiative (WHI) published its findings in 2002 reporting excess risk of breast cancer (BC) with HRT usage, particularly with the combination regime.<sup>3</sup> Eventually, studies contradicting this finding were published.<sup>4-6</sup> Recently, significantly higher BC risk in combined HRT users has been re-established.<sup>7,8</sup>

Menopause, generally commencing in the forties, consists of three stages; perimenopause or transition phase, menopause, and post-menopause marking the cessation of menstrual cycle.<sup>9</sup> Menopausal symptoms can be somatic (vasomotor and psychic disorders), organic (skin, urogenital and weight changes) and metabolic (changes in lipid spectrum, atherosclerosis and osteoporosis).<sup>10</sup>

HRT is effective in alleviating menopausal symptoms and preventing diseases associated with long-term E2 deprivation and categorized as E2-only therapy (ERT) or a combination of E2+P4 (EPT) (Table 1). The American Association of Clinical Endocrinologists (AACE)/American College of Endocrinology (ACE) clinical practice guidelines provide recommendations for patients based on medical history and specific needs.<sup>11</sup>

HRT preparations and doses		Doses (mg/d)
Estrogen based preparation and doses Oral		· · · ·
	17 β- ESTRADIOL	0.5
	ETHINYL ESTRADIOL	2.5 mcg/d
Transdermal	CONJUGATED ESTROGEN	0.3-0.45
	17 β- ESTRADIOL PATCH	0.014-0.0375
	17 β- ESTRADIOL GEL	0.25 or 0.75
	17 β- ESTRADIOL SPRAY	1.53
Vaginal	17 β- ESTRADIOL EMULSION	8.7
	17 β- ESTRADIOL VAGINAL CREAM	0.2
	CONJUGATED ESTROGEN VAGINAL CREAM	0.3125
	17 β- ESTRADIOL VAGINAL TABLET	10 mcg/d
Progestogen based preparation and doses Oral	17 β- ESTRADIOL VAGINAL RING	2mg/ring or 12.4mg/ring
	MEDROXYPROGOSESTERONE ACETATE	1.5-2.5
	NORETHINDRONE ACETATE	0.1
	DROSPIRENONE	0.25
Transdermal	MICRONIZED PROGESTERONE	100-200
	NORETHINDRONE ACETATE	0.14
	LEVONORGESTRIL	0.015

Table 1. Different estrogen and progesterone prescriptions for HRT

\* Address for correspondence:

Anindita Chakrabarty, Ph.D. Address :Shiv Nadar University, Greater Noida, Uttar Pradesh 201314, India. Tel: +91 1800 102 1768 Email: <u>anindita.ac@gmail.com</u> In 2002, the WHI reported a 26% increase in BC in women receiving EPT with a relative risk (RR) of 1.26.<sup>3</sup> This caused widespread panic.<sup>6,12</sup> However, in the WHI study, the RR was calculated from 30/10,000 non-users versus 38/10,000 HRT users, representing a very minute absolute risk of 8/10,000



or 0.08% per year. Later, re-analysis of the WHI data demonstrated that the benefits of HRT exceed the risks in a majority of women entering menopause.<sup>13</sup> Studies analyzing the effects of EPT for 5 years or less on BC risk identified only 6% of the tumors diagnosed over a 5-7-year period occurring de novo, leaving 94% pre-existing tumors growing in size reaching the diagnostic limit. It was also found that natural P4 rather than synthetic progestogens is a safer choice in this regard.<sup>4,5</sup> In 2019, a meta-analysis with worldwide epidemiological evidence collected between Jan 1, 1992 and Jan 1, 2018 analyzed the BC risk with different HRT types. According to this study, in women starting HRT at 50 years of age and continuing it for 5 years, the BC incidence at 50-69 years would increase by 1 in 50 in the  $E_2$  plus daily  $P_4$ group, 1 in 70 in the  $E_2$  plus intermittent  $P_4$  group and 1 in 200 in the  $E_2$  only group. With 10 years of HRT use, these risks would double. This study found no significant differences among progestagenic constituents contributing to the risk, including the commonly preferred micronized progesterone preparation. Also, very little risk was associated with local (vaginal) E<sub>2</sub> application.<sup>7</sup>

Finally, in 2020, a UK-based study confirmed a definitive BC risk with different HRT preparations. While the extent of risk varies with the type of HRT used, higher risk is almost always associated with combined HRT and longer duration of use.<sup>8</sup>

A strong association between HRT use and increased BC risk exists. This is more prominent with EPT than the ERT and persists even a decade after discontinuation. Therefore, doctors and health care professionals should take the medical history and existing BC risk of the patients into account prior to making treatment decisions.

### **Conflict of Interest**

The authors declare no conflict of interest.

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### DOI: 10.32768/abc.20218116-20 Surgical Margins Status Assessment After Breast Conserving Surgery: Is There a Difference Between Surgeon and Radiologist Evaluation?

Alessandra Surace\*<sup>a</sup>, Stephanie Gentile<sup>b</sup>, Aurora Raponi<sup>b</sup>, Giorgia Pasquero<sup>a</sup>, Donatella Tota<sup>b</sup>, Maria Grazia Baù<sup>a</sup>

<sup>a</sup> AOU Città della Salute e della Scienza, Department of Gynecology, University of Turin, Italy <sup>b</sup> AOU Città della Salute e della Scienza, Department of Radiology, University of Turin, Italy

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Key words: Breast cancer, mammography, intra-operative imaging, Faxitron, lumpectomy

### Introduction

Breast conserving surgery (BCS) is the first-choice treatment for early breast cancer (BC), but tumor-free

\* Address for correspondence: Alessandra Surace, MD Address: Gynaecology and Obstetrics 2, Città della Salute e della Scienza, University of Turin, Corso Spezia, 60- 10126, Turin, Italy. Tel: +39 011 3131822 Email: alessandra.sur@gmail.com

ABSTRACT

**Background:** The aim of this study is to evaluate the accuracy of intra-operative specimen mammography (ISM) in surgical margins status assessment and highlight the concordance between the interpretations of the surgeon and the radiologist.

Methods: Our cross-sectional study included 130 patients with early breast cancer, surgically treated between October 2013 and September 2017 in the multidisciplinary breast center of the A.O.U. City of Health and Science (which is a complex of several hospitals) in Turin, Italy. All recruited patients underwent breast conservative surgery. Surgical margins were evaluated intraoperatively, using intra-operative specimen mammography. A standard compression intraoperative specimen mammography was obtained by the surgeon using the dedicated radiological equipment (Faxitron®, BioVision). After the surgeon's evaluation of the margins, Faxitron images were sent to PACS. All ISMs images were analyzed by the same specialized radiologist in remote access to confirm the surgeon evaluation. We used kappa formula to report concordance.

Results: The discordance rate of positive readings between the surgeon and the radiologist was 5.3% while that of negative readings was 6.9%. The concordance rate between radiologist and pathologist assessments was 100%. Intra-operative specimen mammography specificity was 94% (95% CI: 88–97), and sensitivity was 47% (95% CI: 38-56), with PPV found to be 53% (95% CI: 95% 44-62) and NPV determined to be 92% (95% CI: 86–96), when the assessment was made by the surgeon.

Conclusion: Intra-operative specimen mammography is a helpful tool to identify infiltrated margins and to reduce the rate of secondary surgeries by recommending targeted re-excisions of corresponding orientations in order to obtain a final negative margin status. In our experience, not only radiologists but also surgeons could correctly read Faxitron® intra-operative specimen mammography.

> margins are regarded as an essential prerequisite for correct surgical treatment. BCS provides, if combined with adjuvant radiotherapy, the same (or better) overall survival of mastectomy.<sup>16</sup> It is preferable to combine the two treatments rather than just mastectomy, because it is less invasive and better accepted by patients. Post-operative complications rate (e.g. infections), aesthetic outcome, and patient satisfaction are the advantages of BCS compared to mastectomy. Therefore, BCS is an excellent alternative to mastectomy, but re-operation is sometimes required

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because of infiltrated surgical margins (defined as "positive"). In the literature, there is a wide variability in the frequency of re-excision, from less than 10% to more than 50% of lumpectomies.<sup>7-9</sup> Re-operations after breast conserving surgery adversely affect cosmetic outcome and cause additional stress for patients and their families. According to EUSOMA recommendations (European Society of Breast Cancer Specialist), the proportion of patients who received a re-operation for the primary tumor may not exceed 10%.<sup>10</sup> The role of breast imaging is therefore essential not only for a diagnostic purpose, but also for the correct surgical strategy choice in case of non-palpable lesions. Tumor size, its distance from muscular fascia and skin and its precise localization are essential for a complete excision with free surgical margins (pathology report of "no-ink on tumor" for invasive lesions and a clear margin of 2 mm for in situ lesions).

Localization techniques are multiple: metallic hook wire, carbon marking, skin tattoo, clip marker localization and radio-guided localization. During surgery, the surgeon follows the guide (e.i., metallic wire, skin tattoo) to the target area. Nevertheless, margin status assessment of the specimen is mandatory after breast cancer surgery (BCS) of nonpalpable breast cancer (BC). Several methods are available that are already part of standard of care for margin detections.<sup>11</sup> In Italian hospitals, intraoperative pathology analysis (frozen section or touch cytology) and specimen radiography (intraoperative specimen mammography, ISM) are the most common techniques.

Frozen section use should be considered for margin assessment if reoperation rates at an institution are > 15%.<sup>12</sup> Conversely, imaging techniques such as ISM can be used to achieve tumor-free margins in health centers that are specialized in breast cancer treatment. Technological advances have developed a dedicated X-ray equipment for the operating room, such as Faxitron®, which optimize intraoperative specimen analysis times and avoid unnecessary specimen transport to the radiology department.

Faxitron® is a tool that surgeons can use immediately after lumpectomy: the acquired radiographies are read by the surgeon and sent by PACS (Picture Archiving and Communication System) to the radiology department. The exchange of opinion between the surgeon and the radiologist about margins status is a learning opportunity and a moment of professional growth for both medical specialists.

The aim of our study is to evaluate the accuracy of ISM in surgical margins status assessment and highlight the concordance between the interpretations made by the surgeon and the radiologist.

### Methods

Our cross-sectional study included 130 patients with mammography detected early breast cancer (a

disease confined to the breast with or without regional lymph node involvement, and the absence of distant metastatic disease), surgically treated between October 2013 and September 2017 in the multidisciplinary breast center of the A.O.U. City of Health and Science in Turin, Italy. Breast cancer (BC) subtypes were grouped into the following categories: luminal A (ER+/PR+/HER2-, Ki-67<20%), luminal B/HER2+(ER+/HER2+/any Ki-67/any PR), luminal B/HER2- (ER+/HER2and at least one of Ki-67≥20 % or PR-), HER2enriched (ER-/PR-/HER2+), and triple negative (ER-/PR-/HER2-).<sup>13, 14</sup> All recruited patients underwent breast conservative surgery. Preoperatively, non-palpable lesions were marked by wireguided localization (which was performed with a hooked wire through an 18-G spinal needle) or skin tattoo or carbon marking. All surgical procedures were performed by the same specialized breast cancer surgeon. All specimens were directionoriented using metallic stitches. Surgical margins were evaluated intraoperatively, using ISM. A standard compression ISM was obtained by the surgeon using the dedicated radiological equipment (Faxitron®, BioVision). In 100% of the cases, two orthogonal projections of the specimens were acquired (Figure 1).

The surgeon analyzed ISMs and defined the surgical margins as positive (infiltrated) or negative (tumor-free), according to the presence or the absence of the tumor on surgical margins. Faxitron images were sent to PACS. All ISMs images were analyzed by the same specialized radiologist in remote access to confirm the surgeon evaluation, without knowing the surgeon's conclusions. The procedure took 5 minutes and the communication between radiologist and surgeon took place by phone.

Additional tissue was taken if the radiologist indicated positive margins. Finally, the specimen was sent to the pathology department for pathology analysis, which is the gold standard for the assessment of surgical margins.

We collected pre-operative and post-operative data of all the 130 patients using PACS IDS7 (Sectra Medical Systems, Linköping, Sweden), Synapse (Fujifilm Holdings) systems and TrakCare Information System (InterSystems Corporation, Cambridge, MA, USA).

We used kappa formula to report concordance.

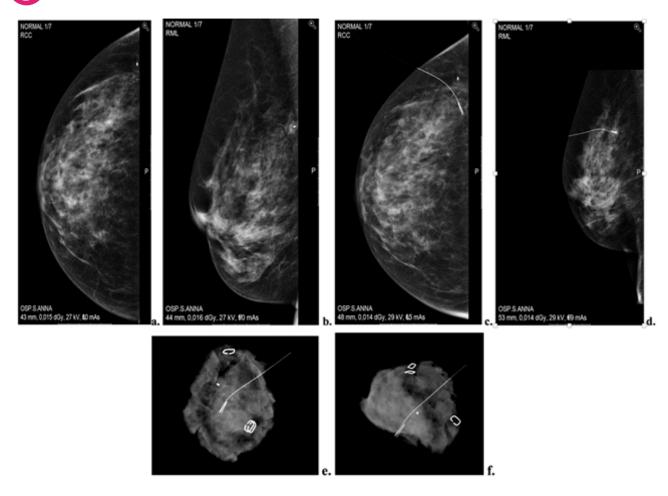
BMI data were collected as well and we used the following classification:

- BMI 25-29 kg/m<sup>2</sup>: overweight
- BMI more than  $30 \text{kg/m}^2$ : obesity

### Results

The mean patients' age was 62 years (range: 27-92 years old.). The mean $\pm$ SD body mass index (BMI) was 25 kg/m<sup>2</sup> (over-weight range). The most frequent

### Margin status by surgeons and radiologists



**Figure 1.** Pre-operative mammography revealed a group of microcalcification in upper-external quadrant of the right breast (a, b, c, d). During VABB (vacuum assisted breast biopsy) a metallic clip was placed. A metallic hook wire was placed for intraoperative localization of the tumor. ISM confirmed the complete removal of the residual microcalcifications (e, f).

mammographic finding was a mass (97 cases out of 130, 74%), following by distortion (11 cases out of 130, 8%), microcalcifications (5 cases out of 130, 4%). Multiple findings were reported in 17 patients out of 130 (13%) and were characterized by the coexistence of radiopacity areas associated with microcalcifications and/or distortions.

Breast lesions were palpable in 84 patients out of 130 (65%). Non-palpable lesions were localized by metallic hook wire (36 lumpectomies out of 46; 78%) or by skin tattoo (10 lumpectomies out of 46; 22%). Fifteen patients were interpreted as having positive margins by the surgeon but only 8 out of 15 patients had a positive margin on pathology (ISM false positive cases). After the surgeon's assessment, 115 lumpectomies were interpreted as having free margins by ISM but 9 lumpectomies out of 115 were histologically involved (ISM false negative cases). The radiologist's assessment agreed with histological results in 130 cases out of 130 (100%), obtaining 17 true positives and 113 true negatives. The concordance rates are shown in Table 1.

The discordance rate of positive readings between the surgeon and the radiologist was 5.3% while that of negative readings was 6.9%.

The concordance rate between radiologist and

pathologist assessments was 100%.

ISM specificity was 94% (CI 95% 88–97), and sensitivity was 47% (CI 95% 38–56), with PPV standing at 53% (CI 95% 44-62) and NPV at 92% (CI 95% 86–96), when the assessment was made by the surgeon.

Pathology reports revealed that 6 cases out of 130 (4%) were ductal in situ carcinomas and 124 cases out of 130 were invasive tumours. An in situ component was observed in 31 cases out of 126 invasive tumours (25%). In our case series, 74 BC were Luminal A (57%), 31 BC were luminal B/HER2 negative (23%), 3 BC were luminal B/HER2 enriched (3%), 15 BC were triple negatives (11%), 1 BC was HER 2 positive without hormonal receptors expression (<0,5%). Therefore, regarding HER2 status, only four patients (3%) were HER2 positives.

Histological results showed that 66 tumors (51%) were Non-Special Type (NST) carcinomas and 64 tumors (49%) were special type carcinomas (lobular, tubular, micropapillary).

The most frequent tumoral staging was pT1c (60 cases out of 130, 46%), followed by pT2 (32 cases out of 130, 24%), pT1b (23 cases out of 130, 18%) and pT1a (9 cases of 130, 7%).

N° Cases (130)	Surgeon	Radiologist	Pathologist
True Negative	106 (81,5%)	113 (87%)	113 (87%)
True Positive	8 (6%)	17 (13%)	17 (13%)
False Negative	9 (7%)	0 (0%)	0 (0%)
False Positive	7 (5%)	0 (0%)	0 (0%)

Table 1. Concordance between surgeon, radiologist and pathologist assessment of surgical margins status

Sixteen patients out of 130 underwenta wider resection because of margins status (12%): targeted re-excision was always performed intraoperative. Additional tumoral cells were found only in 50% of them (8/16).

### Discussion

The principal disadvantages related to BCS include the possibility of re-intervention because of positive margins.<sup>1-3, 5, 6</sup> This eventuality causes discomfort for the patient, higher cost for the health system, an increased risk of poor aesthetic outcomes, and delays adjuvant therapies (radiotherapy or chemotherapy). Efforts are necessary to avoid re-intervention for positive margins.

On the other hand, false positivity may affect aesthetic outcome because of unnecessary wider breast resection. In the literature, some elements are recognized as risk factors for re-intervention after BCS. Most of them are intrinsic factors such as dense breast<sup>15</sup>, young age<sup>16</sup>, low BMI<sup>17</sup>, and HER2 positivity.<sup>18</sup>

In our sample, re-operation rate was 0%, probably because of scarce representation of these risk factors: our patients set was old, overweight, and rarely HER2 positive. These features are related to tumor biology and patient characteristics and can not be changed, but the improvement of intraoperative margin status assessment techniques can reduce reoperation rate. ISM represents a valid tool to identify intra-operatively positive margin after lumpectomy. If tumors are not detectable with mammography, ultrasound could be used for surgical margins assessment instead of mammography. PACS system allows a rapid and effective communication with the radiology department, avoiding the transport of the specimen from the operative room to the radiology department, thereby reducing operatory time. Nevertheless, the possibility that the surgeon may read ISM by himself represents a further advantage in term of costs and operative times. ISM interpretation requires the radiologist to dedicate time and to discontinue other activities that are not related to the operating room (screening mammography, second look mammography, breast ultrasound).

Furthermore, it should be noted that ISM is prone to sources of error such as specimen orientation, resulting in false positive findings and consequently aesthetic and economic disadvantages.<sup>19</sup> When the surgeon reads ISM of the specimen that has been removed and oriented with metallic stitches, the interpretation of the specimen orientation is easy and accurate: this is essential in case of positive margin because the surgeon knows better than anyone where metallic stitches are located and, therefore, can remove the target margin with extreme precision. Thus, among the advantages of Faxitron®, the surgeon's desirable independence in ISM reading must certainly be included.

Discordance rate between surgeon and radiologist evaluations was found to be very low. Only 5% of the surgical margins were considered to be involved by the surgeon but non-involved by the radiologist (and the pathologist). FPs cases were non palpable in the 86% (6/7): six cancers out of seven were NSTs and one case was a special types carcinoma (papillar).

In 7% of the cases, the surgeon false negatives could cause a successive re-intervention for involved margins if not compared with radiologist evaluation. FNs cases were non palpable in the 88% (2/9): five cancers out of nine were NST, three cancers were special types tumors (1 mucinous; 1 papillar, 1 tubular) and one DCIS.

Analyzing the cases of discrepancy between the surgeon and radiologist, the tumors manifestation was calcifications: thus, we could suggest that the surgeon can read alone ISM imagines in case of mass lesion, but surgeons need the radiologist's support in case of calcifications.

In conclusion, ISM is a helpful tool to identify infiltrated margins and to reduce the rate of secondary surgeries by recommending targeted reexcisions of corresponding orientations in order to obtain a final negative margin status. In our experience, not only radiologists but also surgeons could correctly read Faxitron® ISM. Intrahospital refresher courses could increase surgeons' experience and accuracy in surgical margins status assessment by Faxitron ISMs.

#### **Conflict of Interest**

No conflict of interest or funding source to declare.

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# DOI: 10.32768/abc.20218121-28 Patient Throughput Times for Supplemental Breast Cancer Screening Exams

Michael J. Plaza\*<sup>a</sup>, Denzel Cole<sup>b</sup>, Marcos A. Sanchez-Gonzalez<sup>b</sup>, Christopher J. Starr<sup>c</sup>

<sup>a</sup> Department of Radiology, Diagnostic Center for Women, Miami, Florida, USA

<sup>b</sup> Department of Radiology, Larkin Community Hospital, Miami, Florida, USA

<sup>°</sup> Department of Radiology, Kaiser Permanente Downey Medical Center, Miami, Florida, USA

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#### ABSTRACT

**Background:** To optimize screening abbreviated breast MRI (ABMR) operations, patient throughput times of ABMR were compared to breast ultrasound (US) and full protocol breast MRI (FPMR).

**Methods:** Patient throughput times (mean  $\pm$  standard error) and its subcomponents were analyzed for 95 ABMRs, 90 breast US exams, and 50 FPMRs. Total patient throughput was measured from registration time to the time of the last acquired image. Actual exam time was time difference between the first and last acquired images and pre-examination time was the calculated difference between throughput and actual exam times.

**Results:** ABMR total patient throughput time was shorter than FPMR ( $55.7 \pm 1.7 \text{ vs.} 63.1 \pm 2.0 \text{ min}$ ; difference, 7.4 min, 13%; p<0.001), but longer than breast US ( $39.1 \pm 1.3 \text{ min}$ ; difference, 16.6 min, 30%; p<0.001). ABMR had shorter actual scan times than FPMR ( $13.4 \pm 0.14 \text{ vs.} 18.6 \pm 0.25 \text{ min}$ ; p<0.001), but longer than US ( $9.6 \pm 0.46 \text{ minutes}$ ; p<0.001). There was no difference in the pre-examination times between ABMR and FPMR ( $42.3 \pm 1.7 \text{ vs.} 44.6 \pm 1.9 \text{ min}$ ; p = 0.357); pre-examination times were longer for both MR exam types compared to US ( $29.5 \pm 1.3 \text{ minutes}$ ; p<0.001).

**Conclusion:** ABMR patient throughput times are faster than FPMR, but these gains are limited as they have no impact on pre-examination activities which comprise the lengthiest components of the patient flow process. US patient flow currently remains faster than ABMR; however, comparable ABMR times could be achieved by further omitting certain sequences and optimizing pre-examination processes.

Key words: Breast MRI, patient flow, patient throughput, breast cancer screening, breast ultrasound

# Introduction

Patients with heterogeneously dense or extremely dense breast tissue composition are at an increased risk for breast cancer and require supplemental breast cancer screening due to the limited sensitivity of mammography in this population.<sup>1</sup> Since approximately 43% of women have dense breast tissue, this

Address for correspondence: Michael J. Plaza, MD (MJP) Diagnostic Center for Women 7500 SW 87th Ave, Suite 100, Miami, FL, 33173 Tel: +1 305 740 5100, +1 704 779 5604 Fax: +1 305 424 5445 Email: michael.j.plaza@gmail.com represents a large number of patients for which a mammographically occult breast cancer may be detected.<sup>2</sup>

There are many converging lines of evidence to show that breast MRI is superior to ultrasound (US) in cancer detection and in reducing interval cancer rate in patients with dense breasts who may not meet the criteria for additional MRI screening by lifetime risk.<sup>3</sup> The value of MRI is further made apparent by the fact that additional MRI detected cancers are more aggressive; and thus, earlier detection of this cancer subset is tantamount to better survival rates and to allow for management with less morbid systemic medical therapies and surgical management.<sup>4,5</sup> Although breast MRI has been shown to be superior to breast US in cancer detection, most practices that utilize supplemental screening primarily use breast US given its availability, accessibility, and affordability.<sup>6</sup> However, screening breast US can have high inter-operator variability leading to higher number of false positives and additional downstream costs including unnecessary follow-up and biopsies.<sup>7</sup>

Full protocol breast MRI (FPMR), as it stands, faces many challenges in terms of cost, access, and availability to be utilized mainstream for screening average-risk women with dense breasts. Given these limitations, the scope of FPMR use is limited to patients with greater than 20% lifetime risk and even in this population breast MRI remains highly underutilized.<sup>8, 9</sup> Nonetheless, studies have also shown that abbreviated breast MRI (ABMR) demonstrates sensitivity and specificity in cancer detection comparable to FPMR and with improved patient throughput.<sup>10, 11</sup> Improved patient flow in radiology translates to reduced costs and greater patient access.<sup>12</sup> Thus, utilization of ABMR in highrisk patients and those with dense breast tissues have the potential to increase cancer detection in a large number of patients that may not have otherwise been readily detected by screening mammogram or US.<sup>3,11</sup>

However, to date, it is unknown to what degree ABMR improves patient flow compared to FPMR in an outpatient setting, and if gains in operational efficiency are enough such that it would be feasible to employ it in lieu of screening breast US in routine clinical practice. In this study, we examine the operations of three supplemental breast cancer exam types, technologist-performed hand-held whole breast ultrasound, ABMR, and FPMR. The purpose is to optimize screening ABMR operations via a "lean" methodology and through process maps analyses as well as compare ABMR patient throughput times to US and FPMR.

# Methods

This Health Insurance Portability and Accountability Act compliant retrospective observational study was performed as a quality improvement initiative and received IRB exemption. The need to obtain informed consent was waived. We retrospectively reviewed all supplemental breast cancer screening examinations in women with normal screening mammograms from November 2019 through January 2020. This included technologist performed hand-held breast ultrasound (n=90), abbreviated screening breast MRI (n = 95), and full protocol breast MRI (n = 50). We included patients who had a return visit only for the purpose of performing the supplemental breast cancer screening examination. We excluded symptomatic patients (palpable abnormality or nipple discharge), patients with newly diagnosed breast cancer, patients with

history of mastectomy, patients with supplemental exams performed the same day as the screening mammogram, and if any additional diagnostic imaging exam (i.e. DEXA, thyroid ultrasound, pelvic ultrasound, etc.) was performed the same day.

# Facility

The facility is a free standing women's diagnostic imaging center and is part of an over 500-physician multispecialty group. We are a hybrid private practice/teaching facility and American College of Radiology accredited Breast Imaging Center for Excellence. The center performs over 30000 mammograms, 17000 breast ultrasounds, and over 1700 breast MRIs annually.

# Breast Ultrasound

Our institutional policy is to recommend supplemental technologist-performed hand-held screening whole breast ultrasound on all women with dense breasts. Our facility has 13 breast ultrasound technologists ranging in experience from 2-20 years; 12 of 13 are breast specialty certified by American Registry for Diagnostic Medical Sonography. We have six rooms available for performing screening breast ultrasound; five utilize a Samsung RS80A (Samsung Healthcare, San Jose, CA) and one utilizes an Epig 5W (Philips Healthcare; Andover, MA). All technologists are required to undergo a rigorous supervised training for at least 3 weeks with a more experienced technologist prior to scanning alone. Our ultrasound protocol requires taking at least one static image of 2:00, 4:00, 6:00, 8:00, 10:00, 12:00, subareaolar, and the axilla for each breast. All cysts are documented with gray-scale and power Doppler imaging. All solid masses are documented in orthogonal planes, with and without measurement calipers, and with power Doppler imaging.

# Breast MRI

All breast MRIs were performed using a single 1.5T Siemens Magnetom Espree scanner (Siemens Medical Solutions USA, Malvern, PA) with a 16channel bilateral Sentinelle breast coil (Invivo, Gaineville, FL). ABMRs are performed on all highrisk with women with normal prior MRI using the protocol described in a previous publication.<sup>13</sup> FPMRs are performed for all baseline screening examinations in high-risk patients. We have two MRI technologists, both with over 10 years experience performing contrast-enhanced breast MRI. Expected scan time was obtained by summing the time of acquisition for each imaging series under each protocol. The FPMR expected scan time was 13.1 minutes and consists of the following sequences: localizer images, unenhanced non-fat-suppressed T1-weighted imaging, unenhanced fat-suppressed short inversion time inversion recovery (STIR)

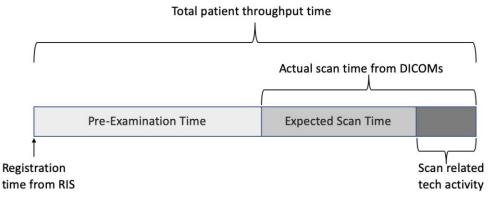


Figure 1. Time elements measured or calculated in the study. Expected scan time is only for the MR examinations. RIS = radiology information system.

imaging, unenhanced fat-suppressed gradient-echo T1-weighted imaging followed by two early phase dynamic contrast-enhanced fat-suppressed gradient-echo sequences and one 6-minute postcontrast late phase sequence. The ABMR expected scan time was 9.5 minutes and consists of the following sequences: localizer images, unenhanced fat-suppressed STIR imaging, unenhanced fat-suppressed gradient-echo T1-weighted imaging followed by two continuously scanned early phase dynamic contrast-enhanced fat-suppressed gradient-echo gradient-echo sequences.

### Patient Throughput Times and Data Analysis

Figure 1 displays the different elements recorded in measuring or calculating patient throughput times. Initial registration time representing the time of arrival was recorded from Intergy (Greenway Health, Tampa, FL), the radiology information system (RIS). We used Infinitt (Infinitt North America, Phillipsburg, NJ), our picture archiving and communication systems (PACS) to record the DICOM times stamps for the first and last acquired images.

Total patient throughput time was defined as the time from registration to the time of the final acquired image. Actual scan time was the difference in time between the first and last acquired images. Pre-examination time was the difference in total patient throughput time and actual scan time. Scan related technologist activity was the difference in actual scan time and expected scan time.

We recorded patient variables that could potentially influence the study activity times, including age, breast volume, and whether or not they have breast implants. Breast volume was summed together for each breast and estimated by using the craniocaudal mammographic projection measuring the width W (distance from medial-to-lateral breast surfaces along the posterior edge), the posterior-toanterior height H (perpendicular distance from posterior edge to the nipple), and the compression thickness C using the follow equation14:

A one-way analysis of variance (ANOVA) test was used compare mean age and mean breast volume between the three exam types. Proportions of patients with and without breast implants were compared between the three exam types using a Fisher's exact test with 3x2 contingency table. For each exam type, a Pearson's correlation test was performed to determine if there was an association between age, breast volume, or implant status that may influence any of the activity times (i.e. preexamination time, actual scan time, patient throughput time.) Mean activity times and standard error were calculated for each exam type and compared controlling for any differences in patient variables using a hierarchical multiple regression analysis. A priori p value of less than 0.05 was considered statistically significant for all calculations. Analyses were performed using statistical software SPSS version 22.0 (IBM; Chicago, IL).

### "Lean" Initiative

After completing the data collection phase, a "lean" approach to improving operations was initiated by engaging supervisors and staff from the front-desk, ultrasound, and MRI departments in a dialogue and qualitative interview.<sup>15</sup> A single researcher served as both interviewer and note taker. The steps involved as a patient moves through the imaging center from the time of registration to the time of the final acquired image were discussed in great detail with staff members. Questions ranged from being broad and open-ended to focused and specific in order to gain insights into inefficiencies, improve understanding of differences unique to each imaging modality, and highlight opportunities for improvement of workflow and performance. The interview guide is displayed in Table 1.

#### Results

#### Patient Characteristics

All patient characteristics are summarized in Table 2. A total of 90 female patients underwent

Staff	General questions	Deep-dive questions
Registration	<ul><li>What is the process when a patient arrives at registration?</li><li>Do you have any suggestions for improving efficiency?</li></ul>	<ul> <li>•Do patients usually have these forms filled out before arriving?</li> <li>•What forms do US (or MRI) patients fill out at registration?</li> <li>•How is the patient transferred to the US (MRI) department?</li> </ul>
Ultrasound	<ul> <li>•What tasks do you need to complete before you pick up a patient from the waiting room?</li> <li>•What are the steps that occur when you take the patient to the exam room?</li> <li>•Do you have any suggestions for improving efficiency?</li> </ul>	<ul> <li>What are sources for delay prior to getting the patient to the exam room?</li> <li>What are the most common reasons for delays during the ultrasound examination?</li> </ul>
MRI	<ul> <li>What tasks do you need to complete before you pick up a patient from registration?</li> <li>What tasks do you perform before positioning the patient in the magnet?</li> <li>What steps occur after patient is positioned in the magnet?</li> <li>Do you have any suggestions for improving efficiency?</li> </ul>	<ul> <li>How are you made aware of the patient arrival?</li> <li>What forms and consents do you review with the patient?</li> <li>How long does it typically take to get IV access? Who do you typically call for assistance with difficult veins and how long does it take that person to arrive?</li> <li>What are the most common questions patient have?</li> <li>What sequences do you usually have to repeat?</li> </ul>

 Table 1. List of questions for qualitative interview of front-desk staff, ultrasound technologists, and MRI technologists.

supplemental screening breast US with mean age 47.4  $\pm$  0.8 (mean  $\pm$  standard error) years. Mean breast volume was 1847  $\pm$  150 cc and 24% (22 of 90) had breast implants. A total of 95 female patients underwent supplemental screening ABMR with a mean age of 50.9  $\pm$  0.78 years. Within this group, mean breast volume was 1779  $\pm$  92 cc and 16% (15 of 95) had breast implants. The FPMR group included 50 female patients with a mean age of 50.5  $\pm$  1.5 years. Mean breast volume was 1838  $\pm$  73 cc and 18% (9 of 50) had breast implants.

The ultrasound group was found to be statistically younger than the ABMR or FPMR groups (p = 0.015). There was no significant difference in ages between the ABMR and FPMR groups. There was no significant difference in the breast volumes (p = 0.723) or proportion of patients with/without implants (p = 0.257) among the

different exam types.

Pearson correlation coefficients were calculated and demonstrated no significant association between age, breast volume, or implant status for any of the study activity times for US, ABMR, and FPMR exams; respectively.

### Patient Throughput Times

All activity time results are summarized in Table 3. The total patient throughput times for US, ABMR, and FPMR were  $39.1 \pm 1.3$  minutes (mean  $\pm$  standard error),  $55.7 \pm 1.7$  minutes, and  $63.1 \pm 2.0$  minutes; respectively. ABMR total patient throughput time was significantly shorter than FPMR (difference, 7.4 minutes, 13%; p<0.001), but significantly longer than breast US (difference, 16.6 minutes, 30%; p<0.001).

Table 2. Summary of patient demographics for each exam type, including age, breast volume, and implant status.

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Demographic	US (n = 90)	ABMR $(n = 95)$	FPMR $(n = 50)$	P value
Mean Age (years)	47.4	50.9	50.5	0.015
Mean Breast Volume (cc)	1847	1779	1838	0.723
Patients with Implants (%)	24	16	18	0.257

US = ultrasound, ABMR = abbreviated breast MRI, FPMR = full protocol breast MRI.

Table 3.	Data table of mean	patient throughput times an	d derived values in i	minutes for each exam type.
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Exam type	Patient Throughput Time	Pre-Examination Time	Actual Scan Time	Expected Scan Time	Scan related Technologist Activity
US	39.1	29.5	9.6	N/A	N/A
ABMR	55.7	42.3	13.4	9.5	3.9
FPMR	63.1	44.6	18.6	13.1	5.5

US = ultrasound, ABMR = abbreviated breast MRI, FPMR = full protocol breast MRI, N/A = not applicable

Actual scan times yielded similar results. ABMR actual scan time was significantly longer than breast US ( $13.4 \pm 0.14$  minutes versus  $9.6 \pm 0.46$  minutes; difference, 3.8 minutes, 28%; p<0.001), but significantly shorter than FPMR ( $13.4 \pm 0.14$  minutes versus  $18.6 \pm 0.25$  minutes; difference, 5.2 minutes, 39%; p<0.001). Scan related technologist activity times were statistically shorter for ABMR versus FPMR (3.9 versus 5.5 minutes; difference, 1.6 minutes; p<0.001).

Pre-examination times were longer for the MRI examinations (ABMR =  $42.3 \pm 1.7$  minutes, FPMR =  $44.6 \pm 1.9$  minutes) compared to breast US ( $29.5 \pm 1.3$  minutes; difference 12.8 minutes, 30%; p < 0.001). However, there was no significant difference in pre-examination times between ABMR and FPMR (p = 0.357).

Hierarchical logistic regression analysis confirmed that differences in study activity times were not impacted by any variations in age, breast volume, or implant status between the different exam types.

# "Lean" Process Qualitative Interview

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Staff interviews performed as part of the "lean" initiative allowed for the development of process maps for each exam type (Figure 2). This then facilitated the identification of sources of variations in the study activity times. It was clear that a patient coming in for supplemental screening breast MRI had several additional steps unique to the modality. At the registration desk, this included MRI specific forms which although patients are asked to fill out before arriving, most do not. US patients are taken by registration staff to the diagnostic waiting area and are already changed into a robe when the technologist calls upon the patient; which is in stark contrast to MRI patients who are taken from registration to the MRI suite by the technologist and then the MRI technologist waits for the patient to change into a robe. Intravenous (IV) access, gadolinium consent, and patient questions/concerns regarding gadolinium deposition are additional steps unique to the pre-examination times for both

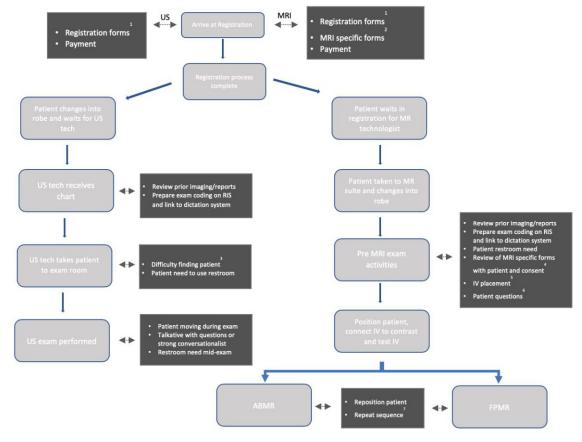


Figure 2. Ultrasound and MRI process maps beginning from registration time to final acquired image. Dark rectangles include elements identified at that time point which may be sources for variations in the activity times. US = ultrasound, RIS = radiology information system, IV = intravenous access, ABMR = abbreviated breast MRI, FPMR = full protocol MRI. <sup>1</sup>Forms include notice of privacy, financial policy, and medical record release.

<sup>2</sup>MRI forms include clinical history and MR safety questionnaire, consent to MRI and use of gadolinium contrast, insurance authorization disclaimer specific to MRI.

<sup>3</sup>Patients go to use restroom, leave to make phone calls, go missing in the center, or fail to answer when called by the technologist due to being distracted.

<sup>4</sup>MRI forms given in registration are reviewed by the technologist with the patient. Forms often incomplete or filled out incorrectly.

<sup>5</sup>IV access performed by MRI technologist sometimes requires multiple sticks or nurse is needed to be called for additional assistance. <sup>6</sup>Majority of questions pertaining to MRI/magnet safety and gadolinium safety/deposition.

7Need to repeat sequences for motion or for suboptimal fat saturation in implants/large breasts.

ABMRs and FPMRs. Difficulty obtaining IV access can result in significant delays if the nurse needs to be called for assistance and is not readily available. There were no difference in the pre-examination processes described for patients having an ABMR or FPMR. MRI technologists subjectively described that the need to repeat sequences most commonly occurred with large almost entirely fatty breasts and implants due to inhomogeneous fat suppression.

# Discussion

Implementing ABMR into our outpatient clinical practice for high risk patients has resulted in faster overall patient throughput times and shorter magnet time compared to FPMR. As one may expect, there was no difference in MRI pre-examination times which is in keeping with the recorded steps of the process map (Figure 2) and with Borthakur and colleagues' prior analysis of ABMRs in an academic hospital setting.<sup>16</sup> Our study demonstrated ABMR to have a 13% faster overall patient flow rate compared to FPMR. This gain in operational efficiency translates to increased capacity, improved patient access, reduced unit cost, and increased potential for revenue growth.<sup>12, 17</sup> ABMR demonstrated a 39% reduction in magnet time compared to FPMR which is postulated to result in better patient care through improved compliance and less image motion artifact especially for patients who suffer from claustrophobia.<sup>10</sup> The operational gains from ABMR at our institution may be even greater at other sites as our FPMR is already under 15 minutes, while other literature describes typical standard full protocol breast MR to be in the range of 20-30 minutes.<sup>10, 16</sup> In addition, although not formally recorded in our analysis, an ABMR inherently results in acquiring fewer images reducing image storage costs, allowing for faster interpretation, and less likelihood of repetitive injury of the interpreting radiologist.<sup>10,18</sup>

Patient throughput times for ultrasound were 30% faster compared to ABMR with an average difference of 16.6 minutes. This difference could be further reduced to 10.2 minutes by eliminating the STIR and second post-contrast sequence which would be expected to have negligible impact on screening metrics. Most published literature on ABMR describe protocols without a T2 weighted sequence or second post contrast sequence yielding similar accuracy to FPMR.<sup>11</sup> The added value of T2-weighted imaging remains controversial, but there is data suggesting its value is only minimal as it may only change the final assessment in as little as 3% of exams.<sup>11,19</sup> Lean interview and process map analysis identified preexamination operational inefficiencies of ABMRs. These include MR technologists waiting on patients to change into a robe, delays in IV access, and delays due heightened patient concerns and questions regarding gadolinium safety. Implementation of changes to address these forms of muda and mura

would only have to improve processes by approximately 10 minutes on average to achieve ABMR throughput times comparable to US screening.<sup>15</sup>

A time-driven activity-based costing analysis is beyond the scope of this manuscript, but it is intuitive that ABMR is less costly than FPMR as the unit cost parameters are no different, while the unit time required is clearly less as demonstrated in our study.<sup>20</sup> Screening breast MRI has been shown to be cost-effective in high-risk populations and implementation of ABMR in this population would only add to its value.<sup>21, 22</sup> As mentioned earlier implementing ABMR also has the side-effect of improving capacity and patient accessibility, which is an important consideration given that screening breast MRI remains significantly underutilized by high-risk population despite its proven benefits.<sup>9</sup>

The question remains if the time gains achievable through ABMR are enough to offset the lower cost of US if implemented in an average risk population with dense breasts. This would require a costeffectiveness analysis as the benefit of detecting 7-16 additional mammographically occult cancers by MRI versus 2-4 additional US-detected cancers per 1000 women screened would need to offset the higher cost of MRI.<sup>3,23-25</sup> Without a CPT code for an ABMR exam, reimbursement and widespread adaptation in an average risk population remains a challenge. The mean cost of a breast US in the United States is \$134 versus \$1,197 for a standard full protocol breast MRI.<sup>26</sup> Our results imply that given the resources and time required to perform ABMR as well as the known greater value provided to the patient in terms of greater sensitivity and fewer false positives compared to US, it is reasonable to charge at price point greater than US, but less than a FPMR.<sup>27</sup> Institutional dependent out-of-pocket prices are in the range of \$250-\$500 which is supported by our results and further corroborated by using other self-pay advanced imaging screening tests such as lung cancer CT screening and cardiac CT scoring as precedents.<sup>3,11</sup>

We had hypothesized that age would reflect how agile a patient is and therefore how quickly they move through the center thus affecting activity times. We also expected that a larger breast volume would require longer US scan times as more breast tissue has to be covered and that implants could results in longer MRI times due to difficulties in positioning or achieving homogeneous fat saturation. However, none of these patient factors impacted activity times in our cohort, although the US group was found to be slightly younger than the MR groups. Perhaps sample size was too small to detect statistically significant differences or there are other demographics worthy of exploration that may be more impactful on throughput times such as Karnofsky or ECOG performance status or selfreported claustrophobia.<sup>28,29</sup>

There were several limitations to our study. This was performed at a community practice in a single outpatient free-standing imaging center where over 90% of the volume is breast imaging. These exact times may not be generalizable to other practice types, but the principle that ABMR is more operationally efficient than FPMR and that throughput times comparable to screening breast US are potentially attainable is universal. Another limitation is that pre-examination times were not separated to record specific time spent during registration prior to being seen by the technologist. Subjectively, interviews of the front-desk staff unanimously agreed that there was no difference in the time take it takes to register an US screening or breast MRI screening patient. Another limitation is that we did not account for variability of patient arriving early or late to their appointment. However, all patients are uniformly asked to arrive 30 minutes prior to their appointment time and we assume that this variability is ingrained into the acquired data and is reported in the standard error of the times.

Furthermore, we do not discuss implemented improvements in the identified inefficiencies of ABMR patient throughput. This was due to the time at which the data was collected which was subsequently followed by the COVID-19 pandemic. Operations and patient volumes have drastically changed in our institution just as it has in others since the pandemic, such that a comparison of patient throughput times pre- and post-pandemic would be fraught with bias.<sup>30, 31</sup> Nevertheless, these results provide insight comparing patient throughput times of different supplemental breast cancer screening exams and in view of the pandemic come at a critical time when healthcare resources are limited and the value of radiology exams are under careful scrutiny. This is an opportune time for greater widespread implementation ABMR in at least the high-risk population to reduce backlogs due to the pandemic and increase accessibility. All stakeholders stand to benefit, but policy makers must recognize that a billable code for ABMR is prerequisite to routine clinical use.

In conclusion, ABMR demonstrates clear operational gains compared to FPMR in the outpatient setting. Despite inherent processes unique to MRI exams, ABMR patient throughput times comparable to US screening are likely attainable by further selecting out unnecessary imaging sequences and identifying and eliminating operational waste at one's institution. A larger multicenter study would be an essential next step to ensure the reproducibility of our results across multiple practice types.

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# **Conflict of Interest**

None of the authors have any conflicts of interest.

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# DOI: 10.32768/abc.20218129-36 COX-2 Expression in Carcinoma of the Breast and Surrounding Non-neoplastic Breast Tissue

Namita Bhutani<sup>\*\*</sup>, Shilpi Moga<sup>b</sup>, Pooja Poswal<sup>b</sup>, Bhanu Sharma<sup>b</sup>, Sunil Arora<sup>b</sup>, Sham Singla<sup>c</sup>

<sup>a</sup> North DMC Medical College and Hindu Rao Hospital, Delhi, India

<sup>b</sup> Department of Pathology, SGT Medical College & University Gurugram, Haryana, India <sup>°</sup>Department of Surgery, SGT Medical College & University Gurugram, Haryana, India

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## ABSTRACT

**Background:** Breast carcinoma is the most common malignant tumor and leading cause of cancer related death in women worldwide. Apart from traditional markers, estrogen receptor, progesterone receptor and Her-2neu, which are important for prognostication and staging purposes, a novel marker cyclooxygenase-2 (COX-2) is being studied extensively. We intend to study the spectrum of COX-2 expression in normal breast tissue, ductal carcinoma in situ (DCIS) adjacent to invasive cancer, and in invasive cancer and compare COX-2 expression with histological prognostic parameters and hormone receptor status.

**Methods:** The present study is a prospective study that was conducted in the department of Pathology, SGT Medical College and Hospital, Gurugram (2019-2020). Fifty patients, aged between 21 and 70, suffering from primary breast cancer constituted the study group. Various histological prognostic parameters were assessed. Immunohistochemical profile of the tumor was assessed. COX-2 score was correlated with various clinicopathologic parameters.

**Results:** Among the total of 50 patients suffering from invasive breast carcinoma, 94 percent (47/50) of cases showed the same COX-2 expression level in normal breast epithelium and corresponding tumor areas and this correlation was statistically significant. The correlation between the level of COX-2 expression in tumor and DCIS was highly significant.

**Conclusion:** Inhibition of COX-2 may represent a potential target for preventing breast cancer oncogenesis and as an adjuvant treatment following surgery to reduce local recurrence.

# paracrine effect Introduction

COX-2 expression,

Key words:

Autocrine effect,

breast Cancer, carcinogenesis,

Breast carcinoma is the most common malignant tumor and leading cause of cancer related death in women worldwide.<sup>1</sup> Its incidence in India is rising and has replaced cancer of cervix as the leading site of cancer.<sup>2</sup> Prognostic factors constitute important tools for the individualization of breast cancer therapy to provide efficient treatment and to spare patients with

\*Address for correspondence: Namita Bhutani, MD Address: G-46, First Floor, East of KAILASH New Delhi, 110065, India Tel:+91 9873101393 Email: <u>namitabhutani89@gmail.com</u> excellent low-risk profiles from unwanted side effects of overtreatment.<sup>3</sup>

Apart from traditional markers, estrogen receptor (ER), progesterone receptor (PR) and Her-2neu, which are important for prognostication and staging purpose, a novel marker called cyclooxygenase-2 (COX-2) is being studied extensively. Two isoforms of COX gene have been described: COX-1, a house keeping gene and COX-2, an inducible gene, stimulated by mitogenic and inflammatory causes. COX-2 can be stimulated by oncogenes, growth factors, cytokines and tumor promoters. COX-2 is upregulated in a number of human malignancies: colon, lung, gastric and oesophageal adenocarcinomas.<sup>4</sup>

The role of COX-2 in breast carcinoma has been linked to increased prostaglandin synthesis which



COX-2 expression in BC

increases aromatase activity in breast and fat tissue leading to increased estradiol synthesis and development of breast cancer.<sup>5</sup> On the basis of various epidemiologic studies on COX-2 expression, strong evidence has been linked to the use of COX-2 inhibitors as chemo-preventive agent in breast cancer and DCIS lesions. We intend to study the spectrum of COX-2 expression in normal breast tissue, ductal carcinoma in situ (DCIS) adjacent to invasive cancer, and in invasive cancer and compare COX-2 expression with histological prognostic parameters and hormone receptor status.

#### Methods

The present study is a prospective study that was conducted in the Department of Pathology, SGT Medical College and University, Gurugram (2019-2020). The ethical approval was waived by institutional review board (SGT IB) as MRM specimens were routinely sent for histopathology and nothing special was done here. Fifty cases of primary breast cancer that underwent radical or modified radical mastectomy constituted the study group. Patients with breast cancer other than primary invasive ductal carcinoma such as lymphoma, sarcoma, stromal tumor, metastases were excluded from the study. Specimens were examined grossly for tumor size, consistency, margin and cut surface along with axillary lymph node status.

Representative blocks were prepared from tumor, normal tissue, area adjacent to tumor, tumor margins, overlying skin, deepest resection margin and axillary lymph nodes. Histopathological diagnosis was established on routine Haematoxylin and Eosin (H&E) stain and various histological prognostic parameters including histologic type, grade and lymph node metastases were assessed. Histologic grading was done by Modified Bloom-Richardson system (MBR) taking into account the scores for tubule formation, nuclear pleomorphism and mitotic count. Histologic grade was assessed by adding up the scores of the three parameters. Lymph node stage in each case was assessed. Using size, MBR histologic grade and lymph node stage, Nottingham prognostic index (NPI) was calculated.

Immunohistochemical profile of the tumor was assessed by subjecting one section each from a representative block of tumor to ER, PR, HER2/neu and COX-2. Immunoquantification was performed using light microscopy at 400X magnification. IHC was performed by peroxidase -antiperoxidase method. Positive and negative controls were run with each batch of IHC stain.

# The Interpretation of Immunohistochemical Stains

### ER/PR staining

Brown diffuse or grainy nuclear staining was taken as positive for ER/PR and assessed by Quick

scoring based on assessment of proportion and intensity.

- Score for proportion (PS): 0 =no staining
- 1 = <1% nuclei stained
- 2 = 1-10% nuclei stained
- 3 = 11-33% nuclei stained
- 4 = 34-66% nuclei stained
- 5 = 67 100% nuclei stained
- Score for intensity (IS): 0 = no staining 1 = weak staining
- 2 =moderate staining
- 3 = strong staining

The scores were summed to give a maximum of 8. Patients with tumors scoring 2 or less were regarded as ER/PR negative.

#### HER2/neu staining

Uniform, intense brown membrane staining of >10% of the tumour cells was taken as positive for HER2/neu.

#### COX-2 Staining

Positive cases showed brown cytoplasmic stain. The IHS (Immunohisto-chemical Score) was calculated by combining an estimate of the percentage of immunoreactive cells (quantity score) with an estimate of the staining intensity (staining intensity score), as follows:

Quantity Score was rated on a scale of 0 to 4, with Score 0: 0-5% of cells stained Score 1: 6-25% of cells stained Score 2: 26-50% of cells stained Score 3: 51-75% of cells stained Score 4: 76-100% of cells stained

Staining intensity was rated on a scale of 0 to 3, as follows:

- 0: Negative
- 1: Weak
- 2: Moderate
- 3: Strong

When there were multifocal immunoreactivity and significant differences in staining intensities between foci, the average of the least intense and most intense staining was recorded. The raw data was converted to the IHS by multiplying the quantity and staining intensity scores. The scores theoretically ranged from 0 to 12.

Interpretation of IHS scoring was as follows:

0 to 3: Negative

4 to 8: Moderate

9 to 12: Strong Brown

Diffuse or grainy nuclear staining was taken as positive for ER/PR and assessed by Quick scoring

based on assessment of proportion and intensity. Brown membranous staining of Her 2 neu was taken as positive. Immunohistochemical analysis showing uniform, intense membrane staining of >10% of the tumour cells was taken as positive.

COX-2 score was correlated with clinicopathologic parameters including age, tumor size, tumor type, histologic tumor grade, axillary lymph node status, DCIS nuclear grade and NPI along with ER, PR and HER2/neu status. The results obtained were interpreted and correlated statistically.

#### Statistical analysis

The results obtained were interpreted and correlated statistically using all the data obtained, analysed statistically using IBM SPSS statistics for windows, version 20.0. (IBM Corp., Armonk, NY). Mean and standard deviations were calculated. When the data was qualitative, a chi-square test was used to assess the association between these parameters. A p-value < 0.05 was taken as significant (S) and p-value < 0.01 was taken as highly significant (HS) whereas the p-value of more than 0.05 was taken as non-significant. Correlation of COX-2 IHS with clinicopathological parameters and different areas (normal breast, DCIS and Invasive Carcinoma) was calculated by Spearman rank correlation  $(r_s)$ . It gave a value of 'r<sub>s</sub>' between -1 and +1. The significance of correlation was evaluated using critical values table for Spearman's coefficient of correlation (statistically significant with a  $P \leq 0.05$ ).

#### Results

A total of 50 patients aged from 21 to 70, suffering

from invasive breast carcinoma participated in the study . Mean age at presentation was 48.22 years. Premenopausal and postmenopausal cases were 38% and 62% respectively. The patients were divided into three groups depending on size (TNM Classification) i.e. < 2cm, 2-5 cm and >5 cm. Seventy eight percent (78%) of cases belonged to 2-5 cm size group.

Histologically, all the patients were infiltrating duct carcinoma (IDC-NOS type) who were graded using Modified Bloom Richardson grading system. Grade II constituted 54% of the cases followed by grade I (32%) and grade III (14%). Lymph node involvement as an important prognostic variable was assessed in all cases and staging was done based on the number of lymph nodes involved. In 42% of the cases, lymph node involvement was not seen (N0), while 30% of the cases were in N1 and 28% of the patients had four or more lymph nodes involvement falling under N2. Fifty six percent of the patients were in moderate prognostic group, 28% in poor and 16% in good prognostic group, respectively.

ER, PR and Her 2 neu status was assessed. Sixty percent of the cases were ER positive and 52% were PR positive. Forty percent of the cases were both ER/PR negative. Only 24% cases had Her 2 neu positivity. COX-2 IHS was separately calculated for normal breast epithelium (10mm away from tumor), DCIS (wherever possible) and tumor tissue. In invasive carcinoma 66% of the cases were moderately positive and 34% were negative for COX-2 expression. None of the cases revealed strong positivity. COX-2 IHS was moderately positive in 72% of normal breast epithelial tissue. DCIS

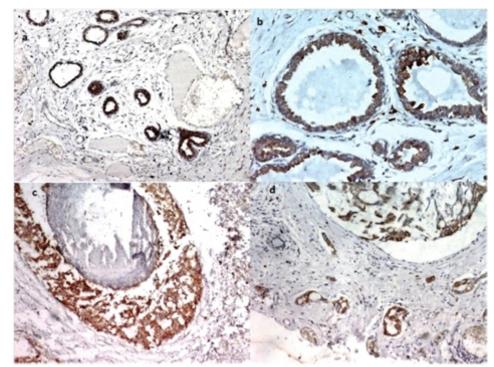


Figure 1. COX-2 expression in normal breast tissue (score-4, intensity-2, IHS-moderate); a) (10X) and; b) (20X);
c) COX-2 expression in DCIS. (Score-4, intensity-3, IHS-strong) (10X); d) COX-2 expression in normal breast tissue (score-4, intensity-2, IHS-moderate) and DCIS. (Score-4, intensity-3, IHS-strong) (10X).

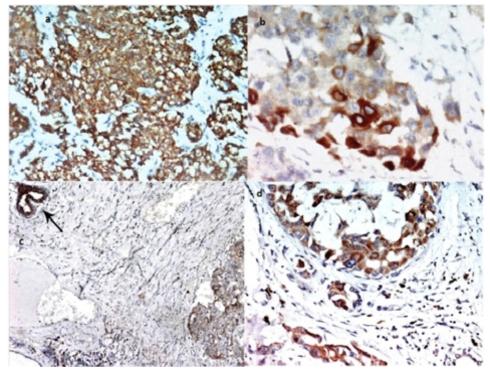


Figure 2. COX-2 expression in invasive breast carcinoma; a) score-4, intensity-1, IHS-moderate (10X) and; b) score-3, intensity-2, IHS-moderate (40X); c) COX-2 expression in invasive breast carcinoma (score-3, intensity-2, IHS-moderate) and normal breast tissue (arrow). (Score-4, intensity-3, IHS-strong) (10X); d) COX-2 expression in invasive breast carcinoma (score-2, intensity-3, IHS-moderate) and DCIS (Score-4, intensity-3, IHS-strong) (20X).

component was seen in 23 cases. Moderate Positive COX-2 IHS score was present in 86% of the DCIS component, while it was negative in 14% of cases.

Ninety four percent (47/50) of cases showed the same COX-2 expression level as normal breast epithelium (Figure 1) and corresponding tumor areas (Figure 2) and this correlation was statistically significant. ( $P<0.001, r_s=0.869$ )

In our study, 23 cases had both DCIS and Invasive Carcinoma. In 3 cases with negative COX-2 expression in DCIS, the paired invasive cancer lesion was also negative. Conversely, 90% (18/20) of DCIS lesions with moderate COX-2 expression were matched by a similar expression level in paired invasive cancer samples. Only 2 cases with moderate COX-2 expression in DCIS showed negative expression in the corresponding tumor area. The correlation between the level of COX-2 expression in tumor and DCIS was highly significant. (rs =0.735, P<0.001). In all 23 cases with a DCIS component, COX-2 IHS between normal tissue and DCIS was similar and this correlation was highly significant. (P<0.01,  $r_s$ =1.0).

COX-2 IHS was compared with different clinicopathological parameters including age, menopausal status, tumor size, histopathological grade, nodal status, NPI scoring and hormonal receptor status (Table 1). COX-2 expression was statistically insignificant in normal, tumor and DCIS area in relation to age groups, menopausal status, lymph nodal status and hormonal status. COX-2 expression was stronger in T2 pathologic stage rather than in T3. No significant correlation was seen between COX-2 expression in tumor and DCIS with size of tumor. Positive COX-2 expression was higher in grade I and II groups in tumor and DCIS area (P=0.098). COX-2 expression was statistically significant in DCIS areas and tumor tissue in relation to histopathological grades. COX-2 expression was higher in good and moderate prognostic groups of NPI (p value=0.045). However, in poor prognostic group, COX-2 expression was poor. COX-2 expression in tumor was statistically significant in various prognostic groups of NPI while it was insignificant in DCIS areas.

# Discussion

Elevated expression of COX-2 has been established to be a feature of breast cancer. There has been inconsistency in literature regarding the precise significance due to paucity of data on COX-2 expression in normal breast tissue and on the changes in COX-2 expression from normal tissue via ductal carcinoma in situ (DCIS) lesion to invasive cancer. Some studies have found no clinicopathological relevance at all, while others have concluded that COX-2 expression is an important biomarker in invasive breast cancer and pre-cancerous lesions, correlating with poor prognostic features.<sup>6-10</sup> The aim of our study, therefore, was to investigate the significance of COX-2 expression in normal breast tissue, DCIS and invasive breast cancer samples from the same patients.

COX-2 was moderately positive in 66% of the

Clinicopathologic	CC	OX-2 Expression		$P(r_s)=T/N/DCIS$
parameters	Tumor(%)	Normal(%)	DCIS(%)	
Tumor size				
<2 cm	12	11	1	
2-5 cm	80	83	85	
>5 cm	28	6	14	0.098(0.237)/0.048(0.281)/0.157(0.305)
Histologic grade				
Grade I	39	38	45	
Grade II	51	48	40	
Grade III	10	14	15	0.098(0.237)/0.048(0.281)/0.011(0.962)
Lymph node status				
NO	45	48	45	
N1	33	30	25	
N2	22	22	30	0.251(0.166)/0.142(0.211)/2.42(0.182)
NPI				
Good	18	20	20	
Moderate	62	60	55	
Poor	20	20	25	0.045(0.285)/0.0245(0.319)/0.239(0.290)
ER				
Positive	63	58	50	
Negative	37	42	50	0.332(-0.140)/0.971(0.005)/0.610(0.112)
PR				
Positive	60	55	50	
Negative	40	45	50	0.051(-0.278)/0.258(-0.163)/0.610 (0.112)
Her 2neu				
Positive	21	22	25	
Negative	79	78	75	0.53(0.091)/0.067(0.645)/0.305(0.157)

cases of tumor, 72% of adjacent normal breast epithelial tissue and 86% of DCIS component. There was no significant difference in COX-2 expression in these groups.

In present study, out of 36 cases with COX-2 positivity in normal tissue, positive COX-2 expression was detected in 33 cases of corresponding tumor areas. Fourteen cases with negative COX-2 expression in normal tissue also showed negative COX-2 expression in corresponding tumor areas. Thus, 94% of cases investigated showed similar COX-2 expression level in normal breast epithelium and the corresponding tumor area in the same patient. The extent of COX-2 expression in normal breast epithelium correlated significantly with that in invasive breast cancer of the same patient. ( $r_s = 0.869$ , P<0.001).

Published data regarding COX-2 expression in normal breast tissue are conflicting.<sup>6,8,11</sup> Consistent with our study, Leo et al. found that in 83% of cases with a negative COX-2 expression in normal breast epithelium, the paired invasive breast cancer lesions were also negative.<sup>8</sup> Conversely, in 95% of cases with a moderate or strong COX-2 expression in normal breast epithelium, this was matched by a moderate or strong COX-2 expression in the invasive breast cancer of the same patient. However, some studies have reported different results. Half et al. found COX-2 expression in 81% of benign adjacent tissue and described it to be of similar or reduced intensity relative to the malignant tissue within the same tissue sections.<sup>11</sup> Half et al. used reverse transcriptase polymerase chain reaction to detect COX-2 messenger RNA (mRNA). Ranger et al. did not find any COX-2 immunoreactivity in normal breast and adjacent non-cancerous tissue (ANCT).<sup>6</sup> This discrepancy can be partly explained by the paucity of ductal units in normal breast tissue as compared with malignant breast tissue or due to different methods used in the evaluation of the results in different studies (RT-PCR, Immunobloting).

In a study by Leo et al., there was a statistically significant correlation between the COX-2 expression in DCIS and invasive breast cancer.<sup>8</sup> In 85% of the cases with a negative COX-2 expression in DCIS, the paired invasive cancer lesions were also negative. Conversely, 94% of DCIS lesions with moderate or strong COX-2 expression were matched by a similar expression level in the paired invasive breast cancer samples.

Half et al. showed that within the same tissue sections, COX-2 expression in invasive breast tumors and adjacent DCIS were highly correlated (p=0.019).<sup>11</sup> Ranger et al. studied 30 patients with



invasive breast cancer and a significant statistical association was observed between invasive carcinoma and concomitant DCIS lesions (p=0.007).<sup>6</sup> Shim et al. studied 64 cases of breast cancer of which 4 cases were composed solely of DCIS, whereas 38 cases of invasive ductal carcinoma contained areas of DCIS.<sup>7</sup> Thirty-two of the 42 cases, including pure DCIS cases and the DCIS component of invasive duct carcinoma (76%), demonstrated COX-2 positivity. Of the cases in which DCIS and invasive carcinoma coexisted, 31 cases showed COX-2 over-expression in both DCIS and invasive components.

Given the high frequency of COX-2 in DCIS area, it can be hypothesized that COX-2 overexpression is involved in the progression to invasive cancer and may be an early event in breast carcinogenesis. But this suggestion needs to be confirmed by further studies.

In the present study, all the cases with a negative COX-2 expression in normal breast epithelium were matched by negative expression in DCIS lesion and all cases with a moderate COX-2 expression in normal breast epithelium coincided with a similar expression in paired DCIS areas. Thus, in all the 23 cases of DCIS, we found a significant correlation between COX-2 expression in DCIS and normal breast epithelium. ( $r_s$ =1.0, p<0.01).

There was a significant correlation between the COX-2 expression levels in normal breast tissue and DCIS lesion of the same patient. This was in concordance with studies done by Leo et al.<sup>8</sup> and Shim et al.<sup>7</sup> and Boland et al.<sup>12</sup> Our observation that COX-2 is up-regulated in the surrounding epithelial tissue raises the strong possibility that the adjacent normal epithelium is part of the disease process in DCIS, which is further supported by the study of Shim et al., who stated that COX-2 intensity in the normal adjacent epithelium is stronger than in the lesion itself and correlated with DCIS nuclear grade.<sup>7</sup>

In the study, COX-2 expression was correlated with various clinicopathologic parameters including age, menopausal status, tumor size, lymph node status, histological grade, NPI and hormone expression. In the present study, correlation of COX-2 expression with patient's age was statistically insignificant and our observation is in line with various other studies in the literature.<sup>8,9,10,11,13</sup>

COX-2 expression in tumor when compared to different tumor sizes was not statistically significant in our study. This could be due to the small sample size in this study. Tumors with a size range of 2-5 cm were associated with higher expression of COX-2 though it was insignificant. Our findings are in agreement with studies by Leo et al.<sup>8</sup> and Ranger et al<sup>6</sup> although Ristimaki et al. reported a statistically significant association between COX-2 expression and tumor size.<sup>9</sup>

In the current study, we did not observe a

statistically significant correlation between COX-2 expression and MBR grade in tumor areas, ( $p_t$ = 0.098), but it was significant in DCIS areas ( $p_{dcis}$ =0.011). Small sample size can also explain the insignificance of COX-2 expression in different grades of invasive carcinoma. Leo et al.<sup>8</sup>, Shim et al.<sup>7</sup> and Ranger et al.<sup>6</sup> did not find any significant association between COX-2 expression and Tumor grade; on the contrary, studies by Ristimaki et al.<sup>9</sup> and Takeshita et al.<sup>10</sup> found a statistically significant correlation between COX-2 expression levels and tumor grades.

The discrepancy in the observation can be partly explained by more cases with a higher grade (grade III) in both studies whereas in our study grade III cases constituted the smallest group. Apart from this, other factors which might have influenced the results could be the number of cases studied and the histological type.

In our study, no correlation was seen between COX-2 expression and lymph node status. This could be because of small sample size in our study. Our observation is supported by Shim et al.<sup>7</sup>, but refuted by Ristimaki et al.<sup>9</sup> and Takeshita et al.<sup>10</sup> who found a statistically significant correlation between COX-2 expression and nodal status among tumor areas. The discrepancy could be partly explained by the small number of cases and different histological types included in the study.

The number of cases with positive COX-2 expression was higher in good and moderate prognostic groups; however, in poor prognostic group, we found less COX-2 expression. COX-2 expression in tumor was statistically significant with prognostic groups (p=0.045). None of the studies used NPI as a parameter for studying its correlation with COX-2 expression.

In addition, positive COX-2 expression was seen in both ER/PR positive/negative group and Her2neu positive/negative groups, which was not dependent on hormonal receptor status. On statistical analysis, COX-2 expression was not found to be significant in relation to hormonal receptor status, which was in line with various studies except for some studies which are tabulated below in Table 2.

Most of the literature<sup>6,7,8,10,11,14</sup> on the correlation of COX-2 expression among the tumor areas and hormonal status show that there is no correlation except for Ristimaki et al.<sup>9</sup>, Boland et al.<sup>12</sup> and Perrone et al.<sup>13</sup> who found a significant correlation. This discrepancy could be partly explained by the selection of high grade cases and with different histological types.

To the best of our knowledge, the present study is the largest study comparing COX-2 expression in paired samples of DCIS, invasive breast cancer and adjacent normal breast and establishing a significant correlation amongst the 3 categories. These findings signify that:

Studies (year) N	o. of cases	CO	X-2 Expression	n in		P value		
		ER Positive (%)	PR Positive(%)	Her neu Positive(%)	ER Positive (%)	PR Positive(%)	Her neu Positive(%)	
Ristimaki et al(2002)	1576	33	31	50	< 0.0001	< 0.0001	< 0.0001	
Half et al (2002)	57	-	-	-	>0.05	>0.05	>0.05	
Shim J et al (2003)	64	67	28	ND	0.273	0.542	ND	
Shim V et al (2003)	46	-	-	-	>0.05	>0.05	>0.05	
Boland et al (2004)	65	50	ND	79	0.005	ND	0.014	
Ranger et al (2004)	30	-	-	-	>0.05	>0.05	>0.05	
Perrone et al (2005)	49	86	55	100	>0.05	>0.05	< 0.0001	
Takeshita et al(2005)	30	55	50	ND	>0.05	>0.05	ND	
Leo et al (2006)	39	-	-	-	>0.05	>0.05	>0.05	
Present Study (2019)	50	70	40	58	>0.05	>0.05	>0.05	

 Table 2. Correlation of cox-2 expression with hormonal receptor status in various studies

ND= Not Done, -= Number not mentioned in the study

1. COX-2 exerts autocrine and paracrine effects, an observation that has been made earlier too by Shim et al, who observed diminishing COX-2 expression with increasing distance from the lesion.<sup>7</sup>

2. Another important and possibly more significant conclusion drawn from our study was that COX-2 intensity in the normal adjacent area was stronger than in the lesion itself and correlated with the DCIS nuclear grade.

These observations support the possibility that adjacent normal epithelium is part of disease process in DCIS and this could be an early event preceding the changes in DCIS and tumor areas.

The limitations which we encountered and which could have affected the final outcome of the study were as follows:

1. The histological types in our study solely comprised infiltrating duct carcinoma (NOS) as per WHO classification whereas other studies included different histological types as their study group.

2. Failure to follow up many of our patients and unavailability of significant clinical details in some cases adversely affected our ability to provide correlative data regarding clinical behavior and survival information.

In conclusion, a statistically significant correlation exists between tumor, adjacent normal epithelium and DCIS, suggesting that COX-2 exerts paracrine effect and is involved in early breast cancer carcinogenesis. Since most infiltrating breast carcinomas are believed to originate from DCIS, the available data suggests that inhibition of COX-2 may represent a potential target for preventing breast cancer oncogenesis and as an adjuvant treatment following surgery to reduce local recurrence. But further studies are mandatory to confirm the findings.

# **Conflict of Interest**

The authors declare no conflict of interest.

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# The Number of Sentinel Lymph Nodes Could be Optimized by Adjusting the Injection Dose

Masujiro Makita\*<sup>a</sup>, Eriko Manabe<sup>a</sup>, Michiko Sato<sup>a</sup>, Hiroyuki Takei<sup>b</sup>

<sup>a</sup> Department of Surgery, Breast Surgery Division, Nippon Medical School Musashikosugi Hospital, Kanagawa, Japan

<sup>b</sup> Department of Breast Oncology, Nippon Medical School Hospital, Tokyo, Japan

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# ABSTRACT

**Background:** The optimal number of sentinel lymph nodes (SLNs) to be removed is controversial based on the false negative rate and prognosis. We investigated factors related to the number of SLNs and the possibility of optimizing the number of SLNs.

**Methods:** We retrospectively reviewed 167 cases in which 0.3 or 0.5 ml of ferucarbotran was sub-dermally injected without massage from July 2016 to November 2018. Sentinel lymph node biopsy (SNB) was conducted using both radioisotope (RI) and superparamagnetic iron oxide (SPIO). The removed nodes with a value of  $\geq$ 0.5 µT on a magnetometer were considered to be SLNs (SPIO nodes). The total SPIO node count in each case was calculated.

**Results:** There was a significant correlation between the number of SPIO nodes and total count of SPIO nodes (rs=0.821, p<0.0001). With RI and SPIO methods, the average number of removed nodes in the age $\geq$ 75 years and BMI $\geq$ 25 subgroups was significantly lower than that in the age<75 years and BMI<25 subgroups. The number of SPIO nodes was significantly influenced by the injected dose. The average number of SPIO nodes in the age  $\geq$ 75 years and BMI $\geq$ 25 subgroups after injection of 0.5 ml was almost the same as that of the age <75 years and BMI<25 subgroups after injection of 0.3 ml.

**Conclusion:** Obesity and old age seemed to be associated with slow lymphatic flow; however, increasing the dose increased the number of SPIO nodes. Thus, optimization of the number of SLNs seems possible.

Introduction

Sentinel lymph node biopsy (SNB) has been established as the standard method for staging clinically node-negative breast cancer.<sup>1, 2</sup> While the radioisotope (RI) and dye-combined method has been considered the standard technique, an SNB technique using superparamagnetic iron oxide nanoparticles

Address: Department of Surgery, Breast Surgery Division,Nippon Medical School Musashikosugi Hospital 1-396 Nakahara-ku, Kosugicho, Kawasaki, Kanagawa, 211-8533, Japan Tel: +81 44 733 5181 Fax: +81 44 711 8875 E-mail: <u>m-makita@nms.ac.jp</u> (SPIO) and a handheld magnetometer has been reported.<sup>3</sup> In a clinical trial of sentinel lymph node biopsy using SPIO, we reported that magnet movement facilitated the arrival of magnetic nanoparticles at the lymph nodes artificially, increasing the count on the skin surface.<sup>4</sup> Several factors, including obesity and age<sup>5-8</sup>, have been reported to affect the outcomes of SNB. Lymphatic flow from the breast to the lymphatic system is expected to be slower in obese or elderly patients in comparison to non-obese and non-elderly individuals. There is a concern that a small number of removed nodes will increase the probability of a false negative result. Another concern is that edema

<sup>\*</sup> Address for correspondence:

Masujiro Makita, MD



of the affected upper limb will occur if more nodes than necessary are collected. Although a sentinel lymph node (SLN) is defined as the first node to receive lymphatic drainage from a primary tumor bed<sup>9, 10</sup>, the optimal number of lymph nodes to be removed is controversial based on the false negative rate and prognosis.<sup>8, 11-17</sup> Since the procedure from drug injection to SLN removal is performed within a certain period of time, the number of lymph nodes identified and removed reflects the speed of lymph flow. Therefore, we investigated the factors related to the number of SLNs and the possibility of optimizing the number of SLNs.

#### Methods

The study was approved by the local ethics committees and was registered in the University hospital Medical Information Network (UMIN) Clinical Registry (UMIN000029475). The participants of the study were primary breast cancer patients of  $\geq 20$  years of age who were diagnosed by a needle biopsy or fine-needle aspiration cytology, without suspected axillary lymph node metastasis on imaging or cytology. We excluded cases with a history of breast and/or axillary surgery (for example, after breast implant insertion), male breast cancer, and ipsilateral breast tumor recurrence after breast-conserving surgery. Patients who met the inclusion criteria were consecutively enrolled in this study. Written, informed consent was obtained from 180 patients who participated in the study from July 2016 to November 2018. We retrospectively reviewed 167 cases in which 0.3 ml or 0.5 ml of ferucarbotran (Resovist® Inj.; FUJIFILM Toyama Chemical Co., Ltd., Tokyo, Japan) was injected subdermally without massage. Thirteen cases were excluded: five cases with intradermal injection, 4 cases with massage, and 4 cases with different injection volumes.

SNB was conducted using both the RI and SPIO methods. Tc-99m phytate was injected on the day before surgery at a dose of 74 MBq, and a dose of 37 MBq was given if the patient was injected on the day of surgery. After the induction of general anesthesia, ferucarbotran was injected subdermally into the subareolar area (for total mastectomy) or peritumorally (for partial mastectomy). A neodymium magnet (Neomag, KOKUYO Co., Ltd., Osaka, Japan) or a magnetometer head (the magnetometer developed by Tokyo University contains a small neodymium magnet in its tip) was moved over the skin from the injection site to the axilla to promote the migration of the magnetic tracer without massage, as reported previously.<sup>4</sup> Regarding the magnet movement procedure, the practitioner stood beside the affected breast of the patient and performed a wiper-like movement with the elbow as the axis at a distance of approximately 40 cm and a speed of approximately

1 second for 1 round trip. The dye method of SNB was not performed in addition to the RI method because of the omission of massage after the administration of the injections in this study. Before the skin incision, the monitoring count on the skin surface was measured by a magnetometer and confirmed twice. After the skin incision, if the removed node had a measurable RI count or a value  $\geq 0.5 \,\mu$ T on the magnetometer, it was considered an SLN. In each SLN removed by the SPIO method (SPIO node), the ex vivo count was measured by a magnetometer. The total count of SPIO nodes was calculated in each case. A histological analysis of frozen sections was performed intraoperatively for all SLNs.

A total of 167 patients were divided into the four groups according to the procedure (injection dose, length of movement and timing of movement as can be seen in Table 1). We investigated the factors affecting the number of SLNs and the total count of SPIO nodes. The detection status of metastatic lymph nodes was also investigated.

For the comparison of the four groups, the  $\chi 2$  test was used for variables presented as numbers of cases, and the Kruskal-Wallis test was used for those presented as average values. When comparing the average values of counts between two groups, the Mann-Whitney test was used. Wilcoxon's signed-rank test was used when comparing the average numbers of the RI and SPIO methods. Spearman's rank correlation coefficient was used to evaluate bivariate correlation. The Stat View for Windows software program (version 4.54, Abacus Concepts, Inc., Berkley, CA, USA) was used to perform all statistical analyses. P values of <0.05 were considered to indicate statistical significance.

### Results

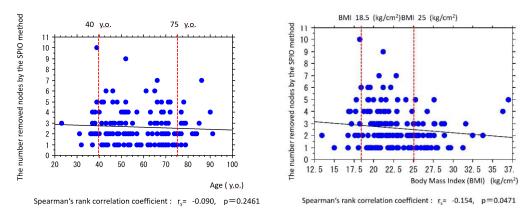
Table 1 shows the characteristics of the cases. The identification rates with the SPIO and RI methods were 100% and 97.0%, respectively. There was a significant correlation between the number of SPIO nodes and total count of SPIO nodes (rs=0.821, p<0.0001). The scatter plot in Figure 1 shows the relationship between the number of SPIO nodes and age or body mass index (BMI). Based on the two scatterplots, the number of SPIO nodes was small for the age  $\geq$ 75 years and BMI $\geq$ 25 subgroups.

Table 2 shows the relationship between four groups and the counts on the skin surface, the number of SPIO nodes, the total count of SPIO nodes, and the number of nodes removed by the RI method. Although the number of nodes removed by the RI method did not differ among 4 the groups, there was a significant difference in the counts on the skin surface, the number of SPIO nodes and total count of SPIO nodes.



Group		А	В	С	D	Total	Р
Cases		95	16	11	45	167	
SPIO method	Injection dose Length of movement Timing of movement	0.3ml <1 min right after injection	0.5ml <1 min right after injection	≥1 min right after injection	≥1 min after interval	Total	χ2 test , *: Kruskal- Wallis test
Age (y)		54.4	52.4	50.0			0 (200*
	(mean)	56.4	53.4	59.8	57.2	56.6	0.6299*
	<u>≤</u> 39	9	3	2	5	19	0.4628
	40-49	27	4	2	11	44	
	50-59	21	1	1	11	34	
	60-69	16	7	2	9	34	
	70-74	12	0	1	4	17	
	≥75	10	1	3	5	19	
Body mass index $(kg/m^2)$	<18.5	13	0	2	4	19	0.7105
	≥18.5 ~ 25>	61	11	6	30	108	
	≥25	21	5	3	11	40	
Tumor location	Lateral/Central	70	11	9	29	119	0.5847
	Medial	25	5	2	16	48	
T Classification	Tis	24	4	3	9	40	0.8410
	T1	55	11	7	29	102	
	T2	16	1	1	6	24	
	T4	0	0	0	1	1	
Assessment preoperative	US/MRI/CT	91	15	11	45	162	0.4241
axillary lymph node	Confirmed by FNA	4	1	0	0	5	
Surgery	Dortial mastasta	50	9	7	27	93	0.8055
Surgery	Partial mastectomy	45	7	4	18	93 74	0.00000
	Mastectomy	45	/	4	10	/4	
Histology	DCIS	14	4	2	11	31	0.7580
	IDC	63	10	6	25	104	
	Others	18	2	3	9	32	

# Table 1. Patient characteristics



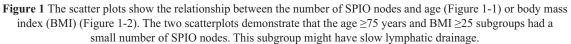


Table 2	. The	results	of	SNB
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Group		А	В	С	D	Kruskal-Wallis test P
Cases		95	16	11	45	Total 167
Count on the skin surface	(mean) (range)	1.5 1 ~ 7	16 2.2	11 2.7	45 3.4	P<0.0001
Nodes removed by SPIO method	(mean) (range)	2.3 1 ~ 7	$1.5 \sim 3.5$ 3.3	$2.5 \sim 3 \\ 3.2$	$2 \sim 6$ 2.9	P=0.0329
Total count of nodes removed by SPIO method (µT)	ved (mean) (range)	3.3 1.2 ~ 11	$1 \sim 6 \\ 5.6$	2~5 6.6	$\begin{array}{c} 1 \sim 10 \\ 6.9 \end{array}$	P<0.0001
Nodes removed by RI method	(mean) (range)	1.8 0 ~ 7	$1.8 \sim 12.2$ 2.4	$\begin{array}{c} 3.5 \sim 11 \\ 1.6 \end{array}$	$\begin{array}{c} 1.5 \sim 22.4 \\ 2.0 \end{array}$	P=0.2818
Metastasis of SLN	None (136) Micrometastasis (6) Macrometastasis (25)	78 3 14	$1 \sim 5$ 15 0 1	$\begin{array}{c} 0 \sim 3\\ 9\\ 0\\ 2\end{array}$	$\begin{array}{c} 0 \sim 9 \\ 34 \\ 3 \\ 8 \end{array}$	P=0.7001*

\*: χ2 test

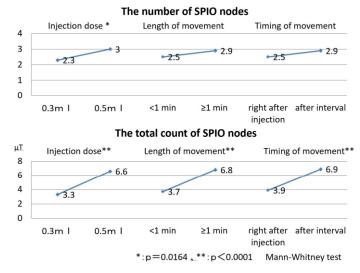


Figure 2. The relationship between the results of SNB and the magnet movement procedure in the SPIO method (injection dose, length of movement and timing of movement)

Table 3. Removed	nodes and	biopsy	<sup>r</sup> method
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	Cases		Removed	P, Wilcoxon's	
	Cases		SPIO method	RI method	signed-rank test
All cases	167	(mean) (range)	2.6 1 ~ 10	1.9 0 ~ 9	P<0.0001
Age ≥75 years or BMI ≥25	52	(mean) (range)	2.3* 1 ~ 7	1.5 # 0 ~ 5	P<0.0001
Age <75 years and BMI <25	115	(mean) (range)	2.8* 1 ~ 10	$\begin{array}{c} 2.1 \ \# \\ 0 \sim 9 \end{array}$	P<0.0001

\*: p=0.0398 Mann-Whitney test

#: p=0.0087 Mann-Whitney test

Figure 2 shows the relationship between the results of SNB and the magnet movement procedure in the SPIO method. The number of SPIO nodes and total count of SPIO nodes were influenced by the procedure (injection dose, length of movement and timing of movement). The only factor to significantly influence the number of SPIO nodes was the injection dose (P=0.0164).

Table 3 shows the difference in the number of removed nodes between the RI method and the SPIO

method. The average number of nodes removed by the SPIO method was significantly greater than that removed by the RI method (2.6 vs. 1.9, p<0.0001). This table also shows average numbers of nodes removed for comparison between 2 subgroups (age  $\geq$ 75 years or BMI  $\geq$ 25 vs. age <75 years and BMI <25). The average number of nodes removed among the age  $\geq$ 75 years or BMI  $\geq$ 25 subgroups was significantly lower in comparison to the age <75 years and BMI <25 subgroup with both methods.

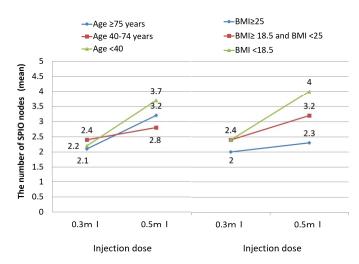


Figure 3. Injection dose and the number of SPIO nodes. The number of SPIO nodes was increased by dose escalation in each subgroup.

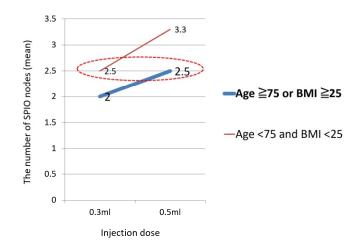


Figure 4. The relationship between the number of SPIO nodes and the injection dose. The average number of SPIO nodes in the age ≥75 years and BMI≥25 subgroups after injection of 0.5 ml was almost the same as that of the age <75 years and BMI<25 subgroups after injection of 0.3 ml.

Figure 3 shows that the number of SPIO nodes was increased by dose escalation in each subgroup. The number of nodes removed by the SPIO method almost doubled with dose escalation in the age <40 and BMI < 18.5 subgroups.

The average number of nodes removed in the age  $\geq$ 75 years and BMI  $\geq$ 25 subgroups after the injection 0.5 ml was almost the same as that of the age <75 years and BMI <25 subgroups after the injection of 0.3 ml (Figure 4).

The number of SLNs removed in this study was 440, and 317 SLNs (72.0%) were counted by both the RI and SPIO methods. On the other hand, the number of SLNS only counted by the RI and SPIO methods was 2 (0.5%) and 121 (27.5%), respectively. Although there were 43 metastatic lymph nodes, 9 (20.9%) metastatic lymph nodes were detected only by the SPIO method.

#### Discussion

During SNB, small injected molecules pass through lymphatic vessels from the injected site and leach into the nodes through the lymphatic flow. The outcome of SNB is affected by several factors, including tracer infiltration into the lymphatic vessels, the flow of lymph, and lodging in the nodes. A longer period from injection to detection<sup>6, 18</sup> and massage after injection have been previously applied as methods to improve tracer infiltration into the lymphatic vessels.<sup>19</sup> While these approaches did result in a small amount of tracer leaching into the nodes, the majority of the tracer failed to do so, and instead spread into the surrounding breast tissue. We reported that magnetic movement accelerated the speed of magnetic tracer flow in lymph vessels and increased the accumulation in lymph nodes.<sup>4</sup> The faster the lymph flow, the greater the amount of drug that arrives in a given amount of time, and the more lymph nodes that are identified because it also flows into the lower-priority lymph nodes. Thus, we focused on the number of removed lymph nodes and the total count of removed nodes. Regarding the physical characteristics of patients with slow lymphatic flow, the average numbers of lymph nodes removed in the age  $\geq$ 75 years and BMI  $\geq$ 25 subgroups were significantly lower in comparison to the age <75 years and BMI <25 subgroups under both methods. In the present study, lymphatic flow from the breast to the sentinel nodes in obese or elderly patients was slower in comparison to nonobese and non-elderly patients, in line with previous reports.<sup>5-8</sup>

There is a concern that a small number of removed nodes may increase the probability of false negatives; however, Schrenk et al. reported that the pathological status of the axilla was independently determined by the removal of the first or first and second SLN in 99% of patients. The false negative rate (FNR) of SNB was reported to be associated with the number of SLNs<sup>14,16</sup>, and the FNR in patients who had one SLN was reported to be higher than that in those who had multiple SLNs.8 Patients for whom only one lymph node was harvested were reported to show poor recurrence-free survival<sup>17</sup>. Bonneau *et al*. reported that in a large series—when patients were compared based on disease-specific survival-the optimal number of harvested SLNs was three.<sup>15</sup> In this study, the number of SLNs differed for the RI and SPIO methods. Although there were 43 metastatic lymph nodes, there were 9 (20.9%) metastatic lymph nodes that were only identified by the SPIO method. Because fast lymphatic flow increased the amount of drug reaching the lymph nodes, lymph nodes with a lower priority were detected by the SPIO method, demonstrating metastasis. Accordingly, adjusting the number of lymph nodes removed by SNB is considered to be a countermeasure to the concern that a small number of removed nodes may increase the probability of false negative results.

We previously reported that magnet movement facilitated the arrival of magnetic nanoparticles at the lymph nodes, increasing the count on the skin surface.<sup>4</sup> In this study, the number of SPIO nodes or the total count of SPIO nodes as well as the count on the skin surface was influenced by the procedural factors (injection dose, length of movement and timing of movement). In the SPIO method, magnetic force can be used to promote lymphatic flow. However, the increase in the amount of drug injected also seemed to have a strong influence on the number of nodes that were removed by the SPIO method. Similar to the relationship between voltage and current, increasing the dose of the drug that is injected will probably accelerate lymphatic flow by increasing the pressure by which the injected drug passes through the lymphatic vessels. Actually, when the injected dose was 0.5 ml, the average number of lymph nodes removed in the age  $\geq 75$ years and BMI  $\geq$ 25 subgroups was almost the same as that of the age <75 years and BMI <25 subgroups when the injected dose was 0.3 ml. On the other hand, the number of nodes removed by the SPIO method almost doubled with dose escalation in the age <40 years and BMI < 18.5 subgroups. If the lymph flow is fast and likely to reach a large number of lymph nodes, the dose of the drug may be reduced. Adjusting the dose may contribute to the optimization of the number of removed lymph nodes.

The present study suffered from several limitations. The number of patients in each group was not set in advance because the method changed while devising new ways to increase the count at the skin surface, as previously reported.<sup>4</sup> Furthermore, because of the consecutive nature of the enrollment and the retrospective design, it was not possible to control various background factors, such as age and obesity. An SNB is an intraoperative examination performed under general anesthesia that ends with the removal of lymph nodes; thus, it was not possible to inject doses of 0.3 ml and 0.5 ml into the same patient. Finally, the study was not a randomized controlled trial.

Obesity and old age seemed to be associated with slow lymphatic flow; however, it was suggested that increasing the injected dose could increase the number of lymph nodes that were removed. Considering SNB as a method of prioritizing lymphatic flow, it seems possible to improve the identification rate and adjust the number of removed nodes by adjusting the dose that is injected. We have begun clinical studies to regulate the number of lymph nodes removed by adjusting the drug infusion dosage for SNB using the indocyanine green (ICG) fluorescence method (Study title: Study to optimize the number of lymph nodes collected by sentinel lymph node biopsy. UMIN000040989).

In conclusion, we investigated the factors that are

associated with the number of SLNs. Regarding the physical characteristics of patients with slow lymphatic flow, the average numbers of nodes removed in the age  $\geq$ 75 years or BMI  $\geq$ 25 subgroups were significant in comparison to the age <75 years and BMI <25 subgroup, with both methods. However, with an injected dose of 0.5 ml, the average number of nodes removed in the age  $\geq 75$ years and BMI  $\geq$ 25 subgroups was almost the same as that of the age <75 years and BMI <25 subgroups after the administration of a dose of 0.3 ml. It was suggested that the number of removed SLNs could be adjusted by adjusting the injected dose. The average number of nodes removed by the SPIO method was significantly greater than that by the RI method. Because fast lymphatic flow increased the amount of drug reaching the lymph nodes, lymph nodes with a lower priority were detected by the SPIO method. There were 9 (20.9%) metastatic lymph nodes that were only detected by the SPIO method. Accordingly, the adjustment of the number of lymph nodes removed by SNB is thought to be a countermeasure to the concern that a small number of removed nodes will increase the probability of false negatives. Considering SNB as a method of prioritizing lymphatic flow, it seems possible to optimize the number of SLNs.

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# **Conflicts of Interest**

The authors declare no conflicts of interest in association with the present study.

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# The Association of Neoadjuvant Systemic Treatment on Hormone Receptor and Her2 Expression in Breast Cancer

William MacFaul<sup>\*\*</sup>, T Michael D Hughesa<sup>a,b,c</sup>, Kerry Hitosd<sup>d,e</sup>, Nirmala Pathmanathane<sup>f,g</sup>, Nicholas K Nguia<sup>a,b,c</sup>

<sup>a</sup> The University of Sydney, Sydney Adventist Hospital Clinical School, Sydney, NSW, Australia

<sup>b</sup> Breast Multidisciplinary Team, Sydney Adventist Hospital, Sydney, NSW, Australia

<sup>c</sup> Division of Surgery, Sydney Adventist Hospital, Sydney, NSW, Australia

<sup>d</sup> Westmead Research Centre for Evaluation of Surgical Outcomes, Department of Surgery, Westmead Hospital, Sydney, NSW, Australia

<sup>®</sup> Westmead Clinical School, The University of Sydney, NSW, Australia

<sup>f</sup> Westmead Breast Cancer Institute, Westmead, NSW, Australia

<sup>9</sup> Douglass Hanly Moir Pathology, Macquarie Park, NSW, Australia

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# ABSTRACT

**Background:** Pathological complete response (pCR) following neoadjuvant systemic treatment(NAST) for breast cancer is associated with improved prognosis; however, a large proportion of patients have residual disease. Oestrogen Receptor (ER) and HER2 status have been shown to affect likelihood of achieving pCR, with ER positive tumors being more treatment resistant. As hormone receptor status is heterogeneous within tumors, we postulated that, following NAST, ER expression would change in patients with residual disease, as the ER negative cells within the tumor are more treatment sensitive.

**Methods:** A retrospective case series of patients treated with NAST prior to surgery at our institution was conducted. Information collected included demographic data, tumor grade, hormone receptor and HER2 status both before and after treatment, and pCR rates.

**Results:** Of the 44 patients included, half achieved pCR. HER2 status (P=0.01), and subtype (P=0.008) were significantly associated with pCR. HER2 positive/ER negative tumors were most likely to undergo pCR. Approximately 80% of residual disease was ER positive. Higher levels of ER expression were also associated with increasing residual cancer burden (RCB) class (P=0.037). There was no trend between change in ER or HER2 expression following NAST. Median change in ER expression was 80% to 90% (P=0.89), HER2 intensity changed from 3.0 to 2.2 (P=0.67) following treatment.

**Conclusion:** Consistent with the literature, we have shown associations between ER and HER2 status and PCR, and between ER expression and residual disease burden. Our study was not able to demonstrate a significant trend in hormone and HER2 expression.

\* Address for correspondence: William MacFaul, MB ChB Address: SAN Clinic, Suite 404, Sydney Adventist Hospital, Sydney, New South Wales 2071, Australia.

Tel: +61 2 9487 9111 Email: <u>willmacfaul@gmail.com</u>

# Introduction

Breast cancer is the second leading cause of death from cancer in women worldwide, with around 18,000 Australians diagnosed with breast cancer each year.<sup>1, 2</sup> Over recent years neoadjuvant systemic therapy (NAST), which entails systemic therapy prior to definitive surgery, has been widely used with

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the aim of downstaging the breast cancer and deescalating the extent of breast and axillary surgery.<sup>3</sup>

Pathological complete response (pCR), defined as absence of residual invasive disease in the breast and in lymph nodes following NAST, has been proposed as a surrogate marker for treatment efficacy, as pCR has been shown to confer both an improved disease-free and overall survival.<sup>4</sup> A pooled analysis of 12 international neoadjuvant therapy trials, found that pCR was associated with increased overall survival, and that patients with ER positive, HER2 negative cancers were least likely to achieve pCR, while triple negative and HER2 positive tumors had the highest pCR rates.<sup>4</sup>

Other trials have further demonstrated the significance of hormone and HER2 status on pCR. A multi-centre study found that pCR rates were lower in ER positive cancer subtypes, luminal A and B cancers.<sup>5</sup> Carey *et al.* assessed response to treatment depending on genomic subtype and found that response to treatment was approximately double in HER2 enriched rather than Luminal A and B subtypes.<sup>6</sup> This study also looked at the residual disease tumor biology following treatment and found lower HER2 enriched cell types in those with residual disease; there was also a shift in intrinsic subtype within tumors which were treatment resistant.<sup>6</sup> They noted shifts towards Luminal A subtypes from Luminal B and HER2 enriched tumors following treatment. This may also reflect tumor heterogeneity with neoadjuvant treatment eliminating the chemo-sensitive cell population but having little or no effect on the chemo-resistant cell population.<sup>6</sup> However, it may also suggest some alteration of tumor biology from the NAST itself.

These studies highlight the significance of the heterogeneity within breast cancers and how this impacts response to treatment and prognosis.<sup>4-6</sup> These trials demonstrate that ER negative and HER2 positive cancers are more likely to achieve pCR.<sup>4-7</sup> Furthermore, given the overall pCR rates in these studies were 56%, 46%, and 18%, this leaves a significant proportion of patients with residual disease found at surgery.<sup>4-6</sup>

Further understanding of the biology of residual disease has important treatment implications. Findings from the multicentre open label, phase II randomised NeoSphere trial evaluated the efficacy of dual anti-HER2 therapy. Results from this study showed that even in patients who did not undergo a pCR, there was a progression free survival benefit in those treated with dual anti-HER2 therapy combined with chemotherapy compared with single agent therapy.<sup>8</sup> The CREATE-X trial investigated the treatment benefit of ongoing adjuvant capecitabine for patients with residual disease following NAST in HER2 negative patients.<sup>9</sup> They randomised patients with residual disease to receive either a further 8 cycles of capecitabine or no further treatment and

found an increased overall survival and disease free survival in the treatment arm with the greatest benefit in the triple negative cohort.<sup>9</sup> To ensure that these findings were not related simply to length of systemic treatment, further studies have given additional chemotherapy to pCR patients and have not demonstrated any further benefit, suggesting that the additional treatment only seems to benefit those with residual disease.<sup>10</sup>

Evidence from neoadjuvant trials shows pCR is an important determinant of disease prognosis in breast cancer and that in those patients who have failed to undergo pCR, there is a reduced duration of survival. ER negative cancers and HER2 positive cancers are much more sensitive to NAST and more likely to undergo pCR.

We postulate that in ER positive cancer the population of cells which have nil or lower oestrogen receptor expression are more responsive to systemic chemotherapy than cells which have a high level of oestrogen receptor expression. If this theory were true, then one would expect that the percentage of ER receptor expressing cells would be lower in the pre-operative biopsy compared to the post NAST surgical specimen. Similarly, the HER2 status may also show changes following NAST- possibly explaining the subtype changes that have been seen in previous studies.

The primary aim of this study was to evaluate whether there is a change in hormonal and HER2 receptor expression between the pre-neoadjuvant core biopsies compared with residual disease following NAST, with a secondary aim of evaluating local pCR rates across subtypes.

### Methods

We conducted a retrospective study of patients treated at the Sydney Adventist Hospital, Sydney, Australia over a 10-year period from 2009 to 2019. The breast cancer database was searched to identify patients who had undergone NAST followed by definitive surgery. The medical records of these patients were reviewed to retrieve clinical details and pathological information.

The inclusion criteria included early and locally advanced breast cancer, treatment with neoadjuvant therapy prior to definitive surgical management. Pre-operative data included patient demographics, histological evidence of lymph node involvement, tumor type and grade, hormone (ER and PR) status (percentage and intensity), and HER2 status by immunohistochemistry or in situ hybridisation (SISH or FISH).

ER and PR status were defined as positive if there were at least 1% positive tumor nuclei stained as per The American Society of Clinical Oncology and the College of American Pathologists Guidelines which classifies Oestrogen receptor as weakly positives if it is between 1-10%.<sup>11</sup>

Type and length of NAST were recorded as was the type of procedure on the breast and axilla. The operative pathology report was examined for presence of residual disease or PCR, tumor grade, ER and PR status with percentage and intensity, HER2 status, lymph node status with the number of positive nodes, and Residual Cancer Burden (RCB) class. pCR rates were defined as no residual invasive cancer in the breast and axillary lymph node(s), including isolated tumor cell in the lymph node after surgery, in keeping with the current 8th edition of the AJCC cancer staging manual.<sup>12</sup>

The residual cancer burden (RCB) index is a standardised calculated score based on pathological data which is usually provided within the pathology report.<sup>13</sup> Five variables are included in the calculation, namely, primary tumor bed area (mm x mm), percentage overall cancer cellularity, percentage of cancer that is in-situ disease, number of positive lymph nodes, and diameter of largest metastasis.

This study was granted approval by Adventist Healthcare Limited Human Research Ethics Committee, in April 2019, and was carried out in accordance with the National Health and Medical Research Council, Medical National Statement on Ethical Conduct in Human Research. Patient consent for research was obtained as part of the consent for treatment.

For the statistical analysis, matched pairs were compared for percentage and intensity across ER, PR and HER2. Chi squared test for trends, or Man-Whitney U test was used to analyse differences in ER expression and HER2 intensity. Subgroups analysed for difference in pCR rates were ER positive/HER negative low grade, ER positive/ HER negative high grade, ER positive/HER positive and triple negative. Low grade incorporated Grade 1 and 2 tumors, high grade referring to Grade 3 tumors. This is similar to grouping conducted by a previous large multicentre pooled analysis.<sup>4</sup> For non-parametric data distribution, the medians and where possible the interquartile range was reported. The interquartile range was defined as the 25<sup>th</sup> to 75<sup>th</sup> percentile (IQR), parametric continuous data were reported with the mean, range and where possible the 95% confidence intervals were included. All tests were two tailed and statistical significance was set at P <0.05. All analyses were performed using IMB SPSS Statistics for Windows, Version 26 (IBM SPSS Statistics for Windows, IBM Corporation, Armonk, NY).

## Results

In total, 62 female patients treated over a 10-year period were identified as suitable from the breast cancer database. Forty-four women met the inclusion criteria and had complete data for analysis. Baseline clinicopathological data is summarised in Table 1.

NAST consisted of the following: 20 patients (45%) received Pertuzumab, Trastuzumab, and Taxane (Paclitaxel), the remainder received differing combinations of chemotherapy, the most common being doxorubicin hydrochloride (Adriamycin), cyclophosphamide, and paclitaxel (AC-T treatment).

In total, six patients (14%) did not complete the full neoadjuvant treatment due to a range of different side effects.

Following NAST, 27 patients (63%) underwent wide local excision as definitive surgical treatment, with the remainder undergoing mastectomy (47%). In terms of axillary management, 26 (60%) of patients had a sentinel lymph node biopsy, 16 (37%) patients had a full axillary dissection, with one patient having no axillary management. Overall, 12 patients (27%) were lymph node positive following treatment.

Pre-Operative Data		Frequency (%)
Age (years)		Median (IQR) 54 (49-64)
Tumor Type	Ductal Lobular Other	39 (88.6) 4 (9.1) 1 (2.3)
Node status	Node positive Node negative	18 (40.9) 26 (59.1)
Grade	1 2 3	19 (43.2) 22 (50) 3 (6.8)
Oestrogen Status	ER -ve ER +ve	15 (34.1) 29 (65.9)
Progesterone Status	PR +ve PR -ve	24 (43.2) 20 (54.5)
HER2 Status	HER2 -ve HER 2 +ve	14 (31.8) 30 (68.2)

<b>Fable 1.</b> Clinicopatholo	ogical Data
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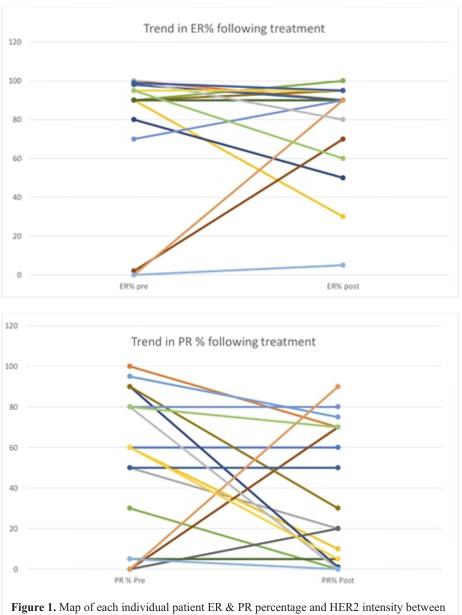
#### Residual Disease

Twenty-two patients had residual disease, ER pos/HER2 neg cancers made up 54.6% of residual disease subtype, with 45.5% being ER pos low grade, and 9.1% being ER pos high grade. HER2 pos/ER pos cancers made up 31.8% of patients with residual disease, with triple negative cancers and HER2 pos/ER neg cancers making up 9.1 and triple negative cancers 4.5% of residual disease.

For the 29 patients with ER positive cancer, the median percentage of oestrogen positive cells prior to treatment was 80% (IQR 1-90), and the median percentage following treatment was 90% (IQR 50-95) (p-value across both medians 0.89).

Treatment was associated with a decrease in positivity in seven of 15 ER positive tumors with residual disease, with treatment associated with an increase in 4/15, and no change in 3/15. One patient's ER expression was associated with a change from





baseline and after neoadjuvant therapy

0% ER expression (ER negative), to 5% ER expression (ER positive) following neoadjuvant treatment. The median percentage of progesterone expression was 45% (IQR: 0.25-75) pre-treatment and 30% following treatment (IQR 0.00-70, P=0.77). HER2 intensity pre-treatment was 3.0 (IQR 2-3) and 2.2 (IQR 1-3) after treatment (P=0.67). The difference in expression for individual patient associated with treatment for oestrogen and progesterone percentage, and HER 2 intensity can be seen in Figure 1. None of these associated changes reached statistical significance.

The range of difference of ER percentage in tumor cells of patients following treatment compared to diagnostic biopsy was between -35 to +90, and the change in PR expression was between -85 to +75.

RCB class was available for 15 out of 22 (68%) of patients with residual disease. RCB class was

associated with oestrogen positivity; the higher the ER percentage prior to treatment, the higher the RCB class (P=0.037).

## Pathological Complete Response

Following treatment, 22 (50%) patients had a pCR, leaving 22 (50%) with residual disease. When pCR rates were evaluated by hormone status, ER negative cancers were three times more likely to undergo pCR than ER positive cancers (OR 2.97; 95% CI 0.8-10.8; P=0.06).

HER2 positive patients were more likely to undergo pCR than HER2 negative, with 19 out of 30 HER2 positive patients achieving pCR compared to 3 out of 14 HER2 negative (P= 0.01). Patients were six times more likely to undergo pCR if they were HER2 positive than HER2 negative (OR 6.33; 95% CI 1.4–27; P=0.01).

When subtype was evaluated by pCR, the



patients most likely to achieve pCR were HER2 pos/ER neg patients, with 80% of these achieving pCR, followed by HER pos/ER pos with 55% and triple negative, with 50% achieving pCR (P=0.008). No HER2 neg/ER pos cancers (n=8) in our study underwent pCR.

# Discussion

In this retrospective study we found that of the 50% of our patients with residual disease, patients were more likely to be ER positive prior to treatment, but this was just short of statistical significance (P= 0.06). When residual disease was broken down by subtype, ER pos/Her neg were the most common, but interestingly despite a high pCR rate of 56% for HER2 pos/ER pos patients, a large proportion of patients with residual disease were HER2 pos/ER pos (31.8%). Overall, of those with residual disease, 86% were made up of ER positive cancers, highlighting the impact of hormone status on residual disease biology. The low proportion of triple negative cancers with residual disease is a positive finding given the generally poor prognosis of these fumors

There was no apparent statistically significant treatment effect on oestrogen or progesterone percentage or intensity (P=: 0.82, P=0.86). HER2 expression was significantly associated with pCR (P=0.01).

Despite the associated differences in individual patients' ER and PR expression in the tumor following neoadjuvant treatment, there was no overall pattern observed in the direction or magnitude of change.

The main aim of this study was to assess the impact of NAST on hormone receptor expression in breast cancer cells. A change in hormone receptor expression has been reported previously in the literature. Van de Ven *et al.* found that of 10 trials that assessed for change in ER expression levels, 4 trials, including a large retrospective study, found a changes in ER expression, with both increases and decreases being observed, as found in this study.<sup>14-17</sup>

Our failure to detect a significant change within ER expression could be related to the high ER expression of >90% on initial biopsy in over 50% of ER positive patients. Our hypothesis that the ER percentage may increase would be difficult to detect in these patients due to such a small proportion of ER negative cells.

There are two outliers that are worthy of discussion. One patient's ER expression was 1-2% on diagnostic biopsy and 90% following NAST. On further examination, it appeared that the patient had a number of possible satellite lesions in addition to the lesion that was biopsied. It may be that this discordance could be explained by a pCR in the original lesion, and the different hormone profile on the surgical histology is from concurrent cancer.

Similarly, a difference in receptor expression across treatment from 2% to 70% following NAST in a different patient appears to be related to a pCR in the breast from the original lesion, with the hormone profile taken from a metastatic deposit within the lymph node. Such outliers may have affected our results given the small number of patients in the study; furthermore, some hormone negative and HER2 negative tumors became positive due to tumor heterogeneity which the small core biopsy sample may not detect.

An interesting finding in our study was that ER percentage prior to treatment was the relationship between ER positivity and RCB class. RCB class has been shown to be prognostic for long term survival, and therefore has been proposed as a surrogacy for treatment efficacy in those patients who do not undergo pCR. The association between an increased ER expression and increased RCB class may be related to the fact that ER positivity is known to impact pCR. However, it is interesting as ER status in regards to pCR was not found to be statistically significant in this study, whilst association with RCB class was. This would suggest that not only does oestrogen positivity predict likelihood of having residual disease, but also the extent of the residual disease. It may also be worth considering comparing scores that combine intensity and expression, such as the Allred score or H score, before and after NAST to see whether combining these factors allows more of a treatment effect to be identified.

The pCR rate in our population of 50% is comparable to other landmark studies. The TRYPHAENA trial, which assessed pCR rates across over 200 patients receiving anti-HER2 therapy in combination with chemotherapy, reported pCR rates of 56 and 55% for the two arms of the trial. The NeoSphere trial, which similarly compared differing anti-HER2 regimens, reported pCR rates of 46% for the combined HER2 therapy arm.<sup>18, 19</sup> The GBG GeparSepto study comparing anti HER2 chemotherapy across over 1000 patients with early breast cancer reported a pCR rate of 56%.<sup>20</sup> Our high pCR rate did limit the number of patients with residual disease, which was unfortunate given that our primary objective was the impact on residual disease.

When our pCR rates were broken down by hormone status, patients were more likely to undergo pCR if they were ER negative, but this was just short of clinical significance (p-value 0.06). This finding is in line with evidence in the literature that ER negative patients are more likely to undergo pCR<sup>21</sup>. Our finding that HER2 positive patients were significantly more likely to undergo pCR, is also in line with the literature.

It appears that there may be interplay between HER2 and ER signalling pathways and this

interaction may impact response to treatment.<sup>22</sup> The 'Cross-talk' hypothesis suggests that oestrogen binding to cytoplasmic oestrogen receptors in breast cancer cells activates signalling pathways that bypass the blockage of HER2 by anti-HER2 chemotherapy agents which may therefore lead to increased resistance to anti-HER2 treatment in HER2 pos/ER pos cancers.<sup>22, 23</sup> PCR rates have even been shown to differ within HER2 pos/ER pos cancers with an inverse relationship between pCR rate and ER percentage.<sup>24</sup> Due to the small sample size in this study, we could not explore this relationship.

This retrospective, single institution study investigating the effect of NAST on early breast cancer produced a number of important findings which we feel add to the current literature. Whilst there have been many studies on pCR, there have been few on residual disease; our study provides further information on the biology of residual disease. Over 80% of patients with residual disease were ER positive. Furthermore, increasing ER percentage in the pre-treatment biopsy was associated with increasing residual cancer burden following treatment, providing implications of hormone status on both likelihood and volume of residual disease.

The study confirms that ER positive tumors are less likely to have pCR, an important observation to acknowledge when advising patients of likely outcomes from NAST.

Additionally, our study provides further information to the literature on pCR. We demonstrated an overall pCR rate of 50%, with HER2 positive and ER negative patients much more likely to undergo pCR. Notably, when these two characteristics were combined, 80% of HER2 positive/ER negative patients achieved pCR. The study was limited by the small numbers, but in spite of this it has shown that ER status and HER2 are not significantly impacted by NAST when there is residual disease to assess.

# **Conflicts of Interest**

There are no conflicts of interest to declare, and there was no external funding for the study.

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# DOI: 10.32768/abc.20218151-56 Similar Prognosis of Patients with Bone-only Metastatic Breast Cancer and Visceral Metastasis

Ryutaro Mori\*<sup>a</sup>, Manabu Futamura<sup>a</sup>, Yoshimi Asano<sup>a</sup>, Akira Nakakami<sup>a</sup>, Kazuhiro Yoshida<sup>a</sup>

<sup>a</sup> Department of Surgical Oncology, Gifu University Graduate School of Medicine, 1-1 Yanagido, 501-1194, Gifu, Japan

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#### ABSTRACT

**Background:** Bone-only metastatic breast cancer is believed to be non-lifethreatening, and mild therapy is frequently selected to avoid adverse events of drug therapy. However, the prognoses of such patients are not well studied.

**Methods:** Patients who received drug therapies for metastatic breast cancer between 2004 and 2016 at our institution were divided into the "Bone-only metastasis", "non-visceral", and "visceral" groups based on the mode of the first metastasis, and the efficacy of the first-line therapy and survival of these patients were compared.

**Results:** There were 131 eligible patients, and the bone-only metastasis, nonvisceral, and visceral groups included 26, 25, and 80 patients, respectively. The median survival time (MST) of the overall survival (OS) in each group was 35.1, 34.9, and 37.4 months, respectively (p=0.71). The clinical benefit rates of first-line therapy in the bone-only metastasis, non-visceral, and visceral groups were 66.7%, 45%, and 69.3%, respectively, and the MST of the time to treatment failure (TTF) in each group was 6.3, 5.5, and 5.8 months, respectively, showing that the efficacy of first-line therapy did not significantly differ among the groups. In the bone-only metastasis group, patients with <5 metastases tended to have a good prognosis, and those with a low nuclear grade and long first-line therapy duration had a significantly better prognosis than others.

Key words: Secondary breast neoplasms, bone metastasis, prognosis

**Conclusion:** The patients with bone-only metastasis had a similar prognosis and treatment response to those with other modes of metastasis, and the patients with a good response to the first-line therapy had a good prognosis.

# Introduction

Although metastatic breast cancer (MBC) is unlikely to be cured,<sup>1</sup> the survival of patients with MBC has been improved by the development of drug therapies, such as chemotherapy and hormone therapy.<sup>2</sup> According to the guidelines for MBC, such as Hortobagyi's algorithm and the National

\* Address for correspondence: Ryutaro Mori, MD Department of Surgical Oncology, Gifu University Graduate School of Medicine, 1-1 Yanagido, Gifu 501-1194, Japan Tel.: +81-58-230-6235 Fax: +81-58-230-6236 E-mail: <u>moriry52@gmail.com</u> Comprehensive Cancer Network (NCCN) guidelines, hormone therapy should be introduced in cases of hormone-receptor (HR)-positive MBC prior to chemotherapy if the metastatic tumor is not life-threatening, as the adverse effect of hormone therapy is mild.<sup>34</sup>

Bone metastases are often treated with hormone therapy because most cases are HR-positive, and bone metastasis is believed to be non-lifethreatening.<sup>5</sup> Indeed, some patients with bone-only metastasis have a very long survival time.<sup>6</sup> However, almost all patients with bone metastasis ultimately develop life-threatening visceral metastases and die due to their MBC, like other modes of metastasis. To our knowledge, the prognoses of the patients with bone-only metastasis have not been well studied, and whether or not bone-only metastasis can be treated with mild therapy, such as hormone therapy alone, is unclear.

In the present study, we retrospectively compared the prognoses of patients with bone-only metastasis as the first site of MBC with those of patients with other modes of metastases to elucidate how best to manage bone metastasis.

#### Methods

This was a retrospective study where the clinical records of breast cancer patients who received drug therapy for advanced or metastatic breast cancer at Gifu University Hospital between 2004 and 2016 were reviewed. The patients were divided into three groups based on the mode of the first metastasis as follows: "Bone-only metastasis" for patients who developed only bone metastasis as the first recurrence, "Non-visceral" for patients with local recurrence or lymph node metastasis with/without bone metastasis as the first recurrence, and "Visceral" for patients with visceral metastasis with/without bone metastasis and/or non-visceral metastasis as the first recurrence.

The efficacy of the first-line drug therapy in each case was evaluated from the perspective of the objective response assessed by the investigators based on the Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 and time to treatment failure (TTF).<sup>1</sup> The objective response was divided into three categories: complete response (CR; all lesions disappeared), non-complete response/non-

Table 1. Patients' of	characteristics
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progressive disease (non-CR/non-PD; lesions were unchanged or diminished), and progressive disease (PD; lesions apparently increased). The clinical benefit rate (CBR) was then calculated as CR + non-CR / non-PD. The TTF was defined as the time from the start of the therapy to the end of the therapy.

#### Ethical approval

This study was approved by the Institutional Ethics Committee of Gifu University, Graduate School of Medicine (Approval number: 29-108) and informed consent was obtained via the opt-out method on the website.

#### Statistical analyses

The TTF and survival of the patients were analyzed using Kaplan-Meier curves and compared by log-rank test. The median survival time (MST) was then calculated. The CBR was analyzed using the chi-squared test. All statistical analyses were conducted using the software EZR software program (version 3.4.1 with R commander 2.4-0).

#### Results

#### Patient characteristics

A total of 139 patients received drug therapy for locally advanced or metastatic breast cancer and eight patients were excluded because of a lack of detailed records. Therefore, 131 patients were included. The patients were a median 61 years of age. Most of the primary tumors were 2-5 cm in size (T2). A total of 94 (74%) patients had N(+) status, 87 (66%) had estrogen-receptor (ER)-positive tumors, and 25

	Bone-only metastasis 26	Non-visceral 25	Visceral 80
Age (N)			
<50	5	1	17
50-59	11	12	17
>59	10	12	46
Menopausal status			
Premenopausal	5	1	17
Postmenopausal	21	24	63
T factor			
T1	3	2	10
T2	12	11	29
Т3		0	6
T4	3 3 5	4	22
Unknown	5	8	13
Nodal status			
N(-)	4	4	13
N(+)	17	17	60
Unknown	5	4	7
Subtype			
Luminal A/B	22	13	42
Luminal HER2	1	2	7
HER2	ī	2 5 5	14
Triple negative	2	5	16
First-line therapy Hormone therapy	21	13	36
Chemotherapy	5	13	44

T factor was defined based on Union for International Cancer Control (UICC) 7th

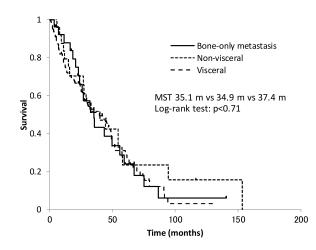


Figure 1. The overall survival of the patients with bone-only metastasis, non-visceral metastasis, and visceral metastasis. Bone-only metastasis means only bone metastasis was detected at first recurrence. Non-visceral metastasis includes local recurrence and lymph node metastasis with/without bone metastasis. Visceral metastasis means visceral metastasis with/without bone metastasis.

(19%) had HER2-positive tumors. The bone-only metastasis, non-visceral, and visceral groups included 26, 25, and 80 patients, respectively. There were significantly more HR-positive cases in the bone-only metastasis group than in the other groups (vs. non-visceral group: p=0.017, vs. visceral group: p=0.012). Seventy (53%) patients received hormone therapy, and 21 (81%) patients in the bone-only metastasis group received hormone therapy as the first-line therapy. The details are shown in Table 1.

# *The survival of the bone-only metastasis group versus other groups*

Kaplan-Meier curves for the overall survival (OS) in each group are shown in Figure 1. The MST values in the bone-only metastasis, non-visceral, and visceral groups were 35.1, 34.9, and 37.4 months, respectively. There were no significant differences in the OS among groups (log-rank test: p=0.71). These

results indicated that the survival time of the patients with bone-only metastasis were not necessarily better than in other groups.

#### *The efficacy of the first-line therapy in the boneonly metastasis group vs. other groups*

We compared the efficacy of the first-line therapy in each group based on the objective response and TTF. The CBR values in the bone-only metastasis, non-visceral, and visceral groups were 67%, 45%, and 69.3%, respectively. There were no significant differences in the CBR among the groups (bone-only metastasis group vs. non-visceral group: p=0.20, bone-only metastasis vs. visceral group: p=0.85; Figure 2a). The MST values of the TTF in the boneonly metastasis, non-visceral, and visceral groups were 6.3, 5.5, and 5.8 months, respectively. There were no significant differences in the TTF among the groups (bone-only metastasis group vs. non-visceral

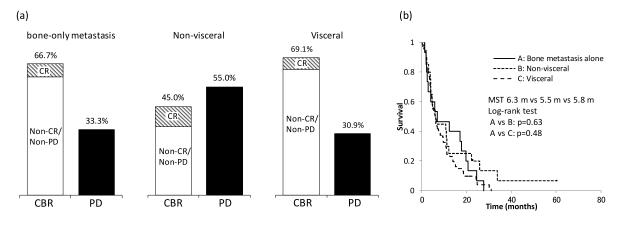


Figure 2. The efficacy of the first-line therapy. (a) The clinical benefit rate (CBR) and progressive disease (PD) of the patients with bone-only metastasis, with non-visceral metastasis, and with visceral metastasis. (b) The time to treatment failure of the first-line therapy among the patients with bone-only metastasis, with non-visceral metastasis, and with visceral metastasis.

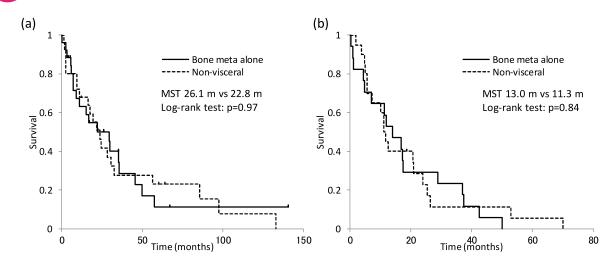


Figure 3. A comparison of the prognoses between the patients with bone-only metastasis and with non-visceral metastasis. (a) The time to the development of visceral metastasis. (b) The survival time after the development of visceral metastasis.

group: p=0.63, bone-only metastasis vs. visceral group: p=0.48; Figure 2b).

These results suggested that the efficacy of the first-line therapy in the patients with bone-only metastasis was similar to that in other groups

# Rate of developing visceral metastasis in the bone-only metastasis group vs. non-visceral group

The development of visceral metastasis is considered to be associated with death from cancer. Therefore, we compared the rate of developing visceral metastasis in the bone-only metastasis group with that in the non-visceral group.

The MST values of the time to the development of visceral metastasis in the bone-only metastasis and non-visceral groups were 26.1 and 22.8 months, respectively, with no significant difference (Figure 3a). Furthermore, the MST values of the survival after the development of visceral metastasis in the bone-only metastasis and non-visceral metastasis groups were 13.0 and 11.3 months, respectively, with no significant difference (Figure 3b).

These results suggested that the patients with bone-only metastasis did not have a better outcome than those with non-visceral metastasis. .Prognostic factors among the patients with bone-only metastasis.

As described above, we found that the prognosis of the patients with bone-only metastasis was not better than that of the patients with other modes of metastasis. However, some patients with bone-only metastasis still had a very good survival. Therefore, we investigated the prognostic factors among the patients with bone-only metastasis.

The survival of patients with <5 metastases tended to be long but not significantly as compared with that of the patients with  $\geq 5$  metastases (MST: 35.3 vs. 22.2 months, p=0.42; Figure 4a). "Nuclear grade" refers to the tumor grading system used in Japan, which consists of a nuclear atypia score and mitotic count score<sup>2</sup>, and the survival of the patients with a low nuclear grade (nuclear grade 1 or 2) was good compared with that of the patients with a high nuclear grade (nuclear grade 3) (MST: 35.1 vs. 16.1 months, p<0.01; Figure 4b). The patients who received first-line therapy for  $\geq 9.6$ months, which was the median duration of first-line therapy, had a good survival compared to those with <9.6 months of first-line therapy (MST: 47.4 vs. 19.8 months, p<0.01; Figure 4c)

These results suggested that the tumor biology

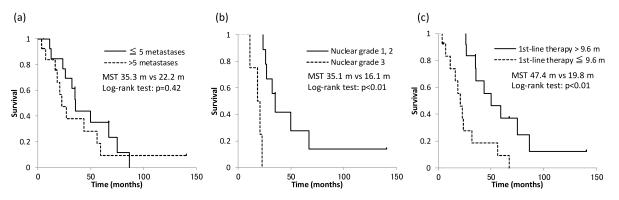


Figure 4. An analysis of the prognostic factors among the patients with bone-only metastasis. The overall survival of the patients with (a) ≤5 metastases or >5 metastases, (b) nuclear grade 1/2 or nuclear grade 3, and (c) a short duration (≤9.6 months) of first-line therapy or long duration (>9.6 month) of the first-line therapy. A duration of 9.6 months is the median duration of first-line therapy.

and treatment efficacy were important to consider when predicting patients' prognoses.

## Discussion

We investigated the prognosis of the patients with bone-only metastasis and found that the survival of such patients was similar to that of the patients with other modes of metastasis. In addition, we found that the nuclear grade and duration of the first-line therapy was more influential than the number of metastases on the prognosis of the patients with bone-only metastasis.

Although there was a study comparing the prognoses of the patients with bone-only metastasis and that with non-bone-only metastasis,<sup>9</sup> to our knowledge, no other study has compared the prognosis of patients with bone-only metastasis with that of patients with non-visceral metastasis or with visceral metastasis. Most physicians believe that patients with bone-only metastases have a good prognosis because bone metastasis is not life-threatening and mild therapy is frequently selected. Therefore, our finding that patients with bone-only metastasis have a good prognosis is not necessarily good is modest and innovative.

Meanwhile, there have been some studies investigating the prognostic factors among patients with bone metastasis. Niikura et al. investigated the prognostic factors for patients with bone-only metastases using the patient records from The University of Texas MD Anderson Cancer Center and reported that a performance status of 0-1, a single metastasis, and asymptomatic bone disease were related to a longer OS.<sup>10</sup> Kai et al. investigated the clinical course of bone-only metastasis in inflammatory breast cancer and non-inflammatory breast cancer and found that the OS did not differ significantly between the two groups.<sup>11</sup> Ahn et al. also investigated the prognostic factors of the patients with bone-only metastasis using the patient records at Gangnam Severance Hospital and found that bisphosphonate treatment, estrogen receptor positivity, and solitary bone metastasis were significantly associated with a longer OS.<sup>12</sup> Taken together, these previous reports indicate that there are two types of prognostic factor: the tumor burden of metastasis and the tumor biology of the primary site. Our results were largely consistent with those of these reports, although the tumor biology—such as a low nuclear grade and long continuation of first-line therapy—was found to be more important than the tumor burden.

The result of the present study that we emphasize particularly was that the patients with bone-only metastasis did not have a better survival than those with other modes of metastasis, even though many physicians believe that bone metastasis is not lifethreatening. This result suggested that treatment for patients with bone-only metastasis should not be weakened, although some patients with bone-only metastasis have a very good survival.

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The limitations of this study are the small population and retrospective nature of our study. However, we believe that our results provide important evidence for clinical practice and that these findings may help prolong the survival of patients with metastatic breast cancer. A larger prospective study investigating how to manage bone metastasis will be required in the future.

In conclusion, we found that patients with boneonly metastasis had a similar prognosis to those with other modes of metastasis. Therefore, treatment for such patients should be selected as for patients with other modes of metastasis.

# **Conflict of Interests**

The authors declare that there is no conflict of interest.

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### Comparing Local Recurrence and Distant Metastasis Between Breast-Conserving Therapy with Radiotherapy and Total Mastectomy in Candidates for PET/CT Assessment

Abbas Yousefi-Koma<sup>a,b</sup>, Mehrdad Bakhshayeshkaram<sup>a,b</sup>, Homa Zamani<sup>a,b</sup>, Yalda Salehi\*<sup>c</sup>,

Farahnaz Aghahosseini<sup>a,b</sup>

<sup>a</sup> Department of Radiology, Shahid Beheshti University of Medical Sciences, Tehran, Iran

<sup>b</sup> Pediatric Respiratory Diseases Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD). Tehran. Iran

<sup>c</sup> Department of Nuclear Medicine, Tehran University of Medical Sciences, Tehran, Iran

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#### ABSTRACT

**Background:** This study aimed to compare the recurrence rate of breast cancer between women treated with breast-conserving therapy (BCT) with/without radiotherapy and those treated with total mastectomy using 18F-fluorodeoxyglucose positron emission tomography/computed tomography<sup>18</sup> (F-FDG PET/CT).

**Methods:** The current study retrospectively included 588 patients suffering from breast cancer who had been referred to the PET/CT department of Masih-e-Daneshvari Hospital in Tehran between April 2013 and September 2019. Data of all female patients with breast cancer were extracted from the recorded hospital files. Based on the treatment plan, patients were divided into two groups: BCT with/without radiotherapy (n=168) and total mastectomy (n=420). Local, locoregional, and distant metabolically active lesions were determined in<sup>18</sup> F-FDG PET/CT and compared between groups.

**Results:** BCT and total mastectomy were comparable regarding local (28.5% vs. 25.7%, P=0.200) and locoregional (21.4% vs. 22.8%, P=0.712) recurrence, while distant recurrence was significantly higher with total mastectomy (88.5% vs. 64.2%, P<0.001). Also, lymph node invasion (42.9% vs. 60%m P<0.001) and positive PET/CT (78.5% vs. 88.5%, P=0.002) were significantly higher with total mastectomy.

According to multivariate analysis, age, clinical stage, and positive margin are independently correlated with the rate of distant metastasis.

**Conclusion:** According to our analysis, breast-conserving therapy could be a suitable choice of surgery in selected patients since local and locoregional recurrence rate did not significantly differ between patients who underwent breast-conserving surgery compared to those who were treated with total mastectomy. Higher rate of distant metastasis in patients with total mastectomy seems to be influenced by many confounding variables such as age, higher stage of diagnosis and positive margin rather than type of surgery.

\* Address for correspondence: Yalda Salehi, MD Address: Imam Khomeini Hospital Complex, Keshavarz Blvd., Gharib St, Tehran, Iran. Postal Code: 1419733141 Tel: +98 21 6119-2403 Fax: +98 21 6694-5118 Email: <u>salehi\_v@sina.tums.ac.ir</u>

#### Introduction

Breast cancer is the most common diagnosed cancer and the most common cause of death from cancer in women.<sup>1</sup> Breast cancer treatment is either local (surgical and radiotherapy) or systemic (chemotherapy, hormonal or biological therapy). The treatment plan is determined based on multiple prognostic factors including tumor histology, clinical and pathologic features of the primary



tumor, axillary lymph node involvement, presence of hormone receptors (ER/PR/ HER2), genetic predisposition, age, comorbidities, and distant metastases.<sup>2</sup> Patients' preference plays a key role in decision making, especially when the existing therapies are not superior to one another. The results of clinical trials in the past two decades have shown that type of surgical procedures in the early stages of breast cancer do not have a significant impact on the survival of patients; therefore, the conservative surgical method has become widespread and is broadly accepted among patients. Death from breast cancer usually occurs following a relapse, which is even possible in the early stages of cancer when the tumor is small and there is no regional lymph node involvement.<sup>3</sup> Approximately 70% of recurrences occur within the first three years with maximal incidence within one or two years of diagnosis.<sup>4</sup> Recent studies have demonstrated that the frequency of recurrence in patients treated conservatively is higher than those undergoing total mastectomy. Furthermore, the interval between surgery and the first recurrence is associated with distant recurrence and therefore has a prognostic value.<sup>5</sup> Studies have shown that the survival of patients who experience local recurrence within five years of surgery is significantly lower than those who develop local recurrence after five years.6 Given the development of diagnostic methods, as well as treatment based on tumor biology (hormone receptor status, genetic mutations, etc.), recurrence rates after different surgical approaches need to be investigated and compared. The purpose of this study was to evaluate breast cancer recurrence rates based on 18F-FDG PET/CT findings in women treated with BCT (with/without radiotherapy) and total mastectomy. The practical aim of this study was to improve the stratification of patients and to select the optimal surgical procedure in patients with breast cancer.

#### Methods

Study population and data extraction

The present study was a cross-sectional study to determine and compare the frequency of recurrence in patients treated with breast-conserving therapy with/without radiotherapy and total mastectomy based on <sup>18</sup>F-FDG PET/CT findings. In this regard, 588 consecutive patients suffering from breast cancer referred to the PET/CT department of Masih-e-Daneshvari Hospital in Tehran between April 2013 and September 2019 were assessed. Data of all female patients with breast cancer were extracted from the recorded hospital files. Based on the treatment plan, patients were divided into two groups: BCT with/without radiotherapy (n=168) and total mastectomy (n=420). Patients with a history of breastfeeding, recent breast manipulation, as well as patients referred for staging were excluded. Then, by reviewing the picture archiving and communication system (PACS),<sup>18</sup>F-FDG PET/CT images of the

remaining patients were retrieved and the presence of local recurrence (metabolically active lesions in breast tissue or site of the previous surgery between the skin and chest wall), locoregional recurrence (presence of metabolically active lymphadenopathy in ipsilateral axillary, infraclavicular, supraclavicular, internal mammary lymph nodes), and the presence and number of distant recurrences by viscera and location of lymph nodes (metabolically active lesions in distant viscera or lymph nodes) were determined by a nuclear medicine physician and a radiologist side by side and in consensus.

#### <sup>18</sup>*F*-*FDG PET/CT protocol*

<sup>18</sup>F-FDG PET/CT imaging were performed on all patients using the following protocol: with 2.1MBq/kg F-FDG injections after at least 6-hour fasting and observing a 24-hour carbohydrate-free diet containing protein and fat and resting in a supine position with minimal light and sound for an hour. One hour after the injection, patients underwent <sup>18</sup>F-FDG PET/CT using a PET/CT scanner (TOF, Discovery 690 GE). First, craniocaudal CT images were obtained using the following parameters: auto mAs (adults: 50-120), 120 kV, noise factor 19, 2.5 mm thickness. Immediately after CT imaging, craniocaudal PET imaging was performed. The time for each bed position was three minutes and the imaging field was vertex to mid-thigh. At the end of the imaging, PET images were iteratively reconstructed using HD technique.

#### PET/CT interpretation

Images of all selected patients were retrieved from PACS. PET (AC and non-AC) and CT images in all three axial, coronal, and sagittal sections were reviewed using the AW VolumeShare 4.5 software by a team consisting of a radiologist and a nuclear medicine specialist. Local, locoregional, and distant metabolically active lesions were categorized qualitatively as definitely positive (moderate to severe uptake, higher than liver with or without the corresponding anatomy), probably positive (uptake similar to or slightly higher than liver with or without the corresponding anatomy), and equivocal (uptake lower than liver and higher than the background) or semi-quantitatively based on the SUVmax index. SUVmax measurement was semi-automatically done using the VolumeShare AW 4.5 software PET disc scanner (GE discovery 690) by inserting a spherical contour around the lesion at the point where maximal absorption was visualized. Imaging findings were recorded based on location and the number of lesions. Frequency, location, and number of tumor recurrences were compared between the two groups.

#### Statistical analysis

For statistical analysis, results were presented as mean  $\pm$  standard deviation (SD) for quantitative variables and were summarized by frequency

(percentage) for qualitative variables. Quantitative variables were compared using the Student's t-test or the Mann-Whitney test based on the normality of data distribution by the Kolmogorov-Smirnov test. Qualitative variables were, on the other hand, compared using the chi-square test. P values  $\leq 0.05$  were considered statistically significant. For statistical analysis, the Statistical Package for the Social Sciences (SPSS) software (version 25.0, Armonk, NY: IBM Corp.) was used.

#### Results

In total, 168 patients underwent BCT and 420 underwent total mastectomy. Comparison of baseline characteristics between the two groups (Table 1) showed a significantly higher mean age (P=0.001) but lower breast cancer-related genetic mutations in the BCT group compared to the total mastectomy group.

There was also a significant difference regarding the TNM classification as well as tumor staging between the two groups (Table 1). With regard to the tumor receptor status, ER positivity was revealed in 71.4% of the BCT group and 51.4% of the mastectomy group (P<0.001), PR positivity in 71.4% and 51.4% (P<0.001), HER2 positivity in 35.7% and 65.7% (P<0.001), and triple negative in 21.4% and 14.2% (P=0.030), respectively. In the PET/CT report, positive scans were found in 78.5% of patients in the BCT group and 88.5% of patients in the total mastectomy group (P=0.002). Regarding recurrence following intervention, there was no difference between the two groups with regard to local recurrence (P=0.200) and locoregional recurrence (P=0.712), while distant recurrence was significantly higher in the total mastectomy group compared to patients in the BCT group (88.5% vs. 64.2%, P<0.001) (Table 2).

Table 1. Baseline characteristics in the two intervention groups

Variables		Total	BCS+/-radiation	Total mastectomy	Р
			(n=168, 28.6%)	(n=420, 71.4%)	
Age	<40 ≥40	51.6 ±11.8 (32-79) 108 (18.4%) 480 (81.6%)	54.35±13.8 (34-79) 24 (22.2%) 144 (30%)	50.51±10.77 (32-78) 84 (77.8%) 336 (70%)	0.001 0.12
Genetic mutation	n (of 576 tested)	420 (72.9%)	96 (22.9%)	324 (77.1%)	0.000
Clinical stage	I II IIIA	60 300 228	60 (100%) 96 (32%) 12 (5.3%)	0 204 (68%) 216 (94.7%)	0.000
Т	T1 T2 T3	216 (36.7%) 312 (53.1%) 60 (10.2%)	132 (61.1%) 24 (7.7%) 12 (20%)	84 (38.9%) 288 (92.3%) 84 (80%)	0.000
Ν	N0 N1 N2 N3	96 (16.3%) 276 (65.7%) 132 (24.4%) 84 (14.3%)	24 (25%) 144 (34,3%) 0 0	72 (75%) 132 (55%) 132 (100%) 84 (100%)	0.000
Histopathology	IDC ILC Medullary Others	336 (57.1%) 168 (28.6%) 72 (12.2%) 12 (2%)	96 (28.6%) 36 (21.4%) 36 (50%) 0	240 (71.4%) 132 (78.6%) 36 (50%) 12 (100%)	0.000
Highest tumor grade	x I II III	12 (2%) 96 (16.3%) 348 (59.2%) 132 (22.4%)	0 72 (75%) 84 (24.1%) 12 (9.1%)	12 (100%) 24 (25%) 264 (75.9%) 120 (90.9%)	
Receptor status	ER+ PR+ HER2+ Triple negative	336 (57.1%) 336 (57.1%) 336 (57.1%) 96 (16.3%)	120 (35.7%) 120 (35.7%) 60 (17.9%) 36 (37.5%)	216 (64.3%) 216 (64.3%) 276 (82.1%) 60 (62.5%)	$\begin{array}{c} 0.000 \\ 0.000 \\ 0.000 \\ 0.03 \end{array}$
Positive Margin		432 (73.5%)	144 (33.3%)	288 (66.7%)	0.000
Positive lymphovascular invasion		324 (55.1%)	72 (22.2%)	252 (77.8%)	0.000

#### **Table 2.** PET/CT findings and recurrence rates in the two intervention groups

Variables		Total	BCS	Total mastectomy	Р
PET/CT findings	Positive scan Negative scan	504 (85.7%) 84 (14.3%)	132 (26.2%) 36 (42.9%)	372 (73.8%) 48 (57.1%)	0.002
Local recurrence	Yes No	156 (28.9%) 384 (71.1%)	48 (30.8%) 96 (25%)	108 (69.2%) 288 (75%)	0.2
Locoregional recurrence	Yes No	132 (23.9%) 420 (76.1%)	36 (27.3%) 108 (25.7%)	96 (72.7%) 312 (74.3%)	0.7
Distant metastasis	Yes No	480 (87%) 72 (13%)	108 (22.5%) 36 (50%)	372 (77.5%) 36 (50%)	0.000

Variables		PET/CT negative	PET/CT positive	Р
Age		50 ±13.5 (35-78)	51.9±11.5 (32-79)	NS
Age	<40 ≥40	24 (22.2%) 60 (12.5%)	84 (77.8%) 420 (87.5%)	0.009
Genetic mutation (of 576 tested	l) YES NO	48 (11.4%) 24 (15.4%)	372 (88.6%) 132 (84.6%)	0.000
Clinical T stage	IA IB IIA IIB IIIA IIIB IIIC	$\begin{array}{c} 0\\ 12 \ (33.3\%)\\ 24 \ (11.1\%)\\ 24 \ (28.6\%)\\ 0\\ 0\\ 24 \ (28.6\%)\end{array}$	$\begin{array}{c} 24 \ (100\%) \\ 24 \ (66.7\%) \\ 192 \ (88.9\%) \\ 60 \ (71.4\%) \\ 108 \ (100\%) \\ 36 \ (100\%) \\ 60 \ (71.4\%) \end{array}$	0.000
T stage	T1 T2 T3	36 (16.7%) 36 (11.5%) 12 (20%)	180 (83.3%) 276 (88.5%) 48 (80%)	NS
N staging	N0 N1mi N1 N2 N3	$\begin{array}{c} 0\\ 12 \ (33.3\%)\\ 48 \ (20\%)\\ 0\\ 24 \ (28.6\%)\end{array}$	96 (100%) 24 (66.7%) 192 (80%) 132 (100%) 60 (71.4%)	0.000
Histopathology	IDC ILC medullary others	24 (7.1%) 36 (21.4%) 24 (33.3%) 0	312 (92.9%) 132 (78.6%) 48 (66.7%) 12 (100%)	0.000
Highest tumor grade	x I II III	0 24 (25%) 48 (13.8%) 12 (9.1%)	12 (100%) 72 (75%0 300 (86.2%) 120 (90.9%)	0.003
Receptor status	ER+ PR+ HER2+ Friple negative	36 (10.7%) 36 (10.7%0 48 (14.3%0 24 (25%)	300 (89.3%) 300 (89.3%) 288 (85.7%) 72 (75%)	0.004 0.004 NS 0.001
Margin	Negative Positive	36 (8.3%) 48 (30.8%)	396 (91.7%) 108 (69.2%)	0.000
LV invasion	LVI + LVI -	36 (11.1%) 48 (18.2%)	288 (88.9%) 216 (81.8%)	0.01
Type of surgery	BCS Total	36 (21.4%) 48 (11.4%)	132 (78.6%) 372 (88.6%)	0.002

Comparison of baseline variables (Table 3) showed that except for age, tumor staging, and status of HER2 receptor, there was a significant difference between the groups with respect to other variables.

According to multivariate analysis, age, clinical stage, and positive margin are independently correlated with the rate of distant metastasis.

#### Discussion

The initial management of breast cancer has considerably changed within the recent decade which has influenced the incidence of local, regional, and even distant recurrences. Advancement in new therapeutic approaches such as partial mastectomy followed by radiotherapy has led to lower distant metastases as well as better long-term survival.<sup>7</sup> In other words, BCT consisting of breast-conserving surgery (BCS) followed by radiation therapy has led to a considerable reduction in recurrence compared to routine total mastectomy regardless of baseline characteristics such as tumor grading and staging as well as the presence of specific hormonal receptors.<sup>8</sup> Overall, the long-term locoregional recurrence after concurrent BCS and radiotherapy has notably decreased in recent years.<sup>9</sup> However, it has been shown that individuals who undergo BCS alone without radiation have generally higher local recurrence rates compared with those who also receive radiotherapy.<sup>10, 11</sup> In this regard, the findings of the present study with respect to lower distant metastasis following BCT compared to total mastectomy are predictable.

Some large population-based studies have compared the effectiveness of BCT and total mastectomy. Contrary to our findings, Van der Sangen et al.<sup>12</sup> showed that for a median follow-up of 7.4 years, the local recurrence risk for total mastectomy patients was 4.4%, while in the BCT cohort, the 5-year local recurrence risk was 8.3%, indicating a significant difference. In a study by Mahmood et al.<sup>13</sup>, within a median follow-up of 5.7 vears, no difference was found in the 5-, 10-, and 15year rates of cause-specific survival between the two groups receiving total mastectomy or BCT. Subset analyses confirmed that there were no differences in outcomes for local treatment when stratified by age quartiles. Data from the literature, including randomized trials, have shown that locoregional recurrence occurs at a rate of 5% to 15% after conservative surgery or mastectomy plus adjuvant radiotherapy.<sup>14-18</sup> The 10-year recurrence rate after conservative treatment was about 10% to 20% in patients with early stages of invasive breast cancer.<sup>19</sup> The median time to recurrence, after the end of systemic adjuvant treatment, may be short (2-4 years) or significantly prolonged (5-8 years).<sup>19-21</sup> However, many recent publications have shown that these delays may depend on prognostic factors, tumor biology, and molecular subtypes. Thus, the difference in the rates of local, locoregional or distant metastasis in both interventional approaches (conservative treatment or total mastectomy) may be correlated with several factors, especially biological behaviors of the tumor and histological features.

The higher rate of distant metastasis in patients with total mastectomy seems to be influenced by many confounding variables such as age, higher stage of diagnosis and positive surgical margin rather than the type of surgery.

In the current study, Radiotherapy was used for all patients undergoing breast-conservative surgery as a major complementary treatment and had an important role in local control of disease since there was no significant different in local recurrence between the two groups.

There are major drawbacks in the current study. Regarding the retrospective nature of the study, patients' population may not potentially be a real representative of breast cancer patients and hence the results may be validated with caution.

According to our analysis, breast-conserving therapy could be a suitable choice of surgery in selected patients since local and locoregional recurrence rate did not significantly differ between patients who underwent breast-conserving surgery compared to those who were treated with total mastectomy. The higher rate of distant metastasis in patients with total mastectomy seems to be influenced by many confounding variables such as age, higher stage of diagnosis and positive margin rather than the type of surgery.

#### **Conflict of Interest**

None.

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### DOI: 10.32768/abc.20218263-64 Global Breast Cancer Initiative: an Integrative Approach to Thinking Globally, Acting Locally

Mojgan Karbakhsh\*<sup>a</sup>

<sup>a</sup> Department of Community and Preventive Medicine, Tehran University of Medical Sciences, Tehran, Iran

On International Women's Day this year (8 March 2021), Global Breast Cancer Initiative (GBCI) was launched by WHO and intersectoral partners as a renewed commitment to tackle disparities in breast cancer survival around the world. The specific objective of this initiative is to reduce global breast cancer mortality by 2.5% each year until 2040, through increasing access to early detection, timely diagnosis and comprehensive management.<sup>1</sup> To avert an estimated 2.5 million annual deaths, the GBCI will focus on promoting sustainable capacity building, and supporting innovation and monitoring systems that use data for evidence-based decision making.<sup>2</sup>

This initiative is a high priority collaborative effort, as the global cancer landscape is changing: breast cancer has now surpassed lung cancer as the world's most commonly diagnosed cancer, according to the latest estimates released by the International Agency for Research on Cancer (IARC).<sup>3</sup> This increase is partly explained by the longstanding higher prevalence of reproductive, hormonal and lifestyle risk factors, as well as organized mammographic screening in high-income countries. Increasing rates in countries undergoing dramatic socio-cultural and economic shifts-such as obesity epidemic, physical inactivity, postponement of childbearing and having fewer children-should be added to the picture. Examples of this category include countries in South America, Japan, the Republic of Korea<sup>3</sup>, and Iran.<sup>4,5</sup> The most rapid

\* Address for correspondence:

increase is happening in sub-Saharan Africa, with the highest mortality rate throughout the world. This high death rate is mainly attributed to the younger age profile and diagnosis at late stages<sup>6</sup>, as only 17% of the countries in Africa (AFRO) have breast cancer screening programs.<sup>7</sup>

Between 1989 and 2017, deaths from breast cancer mortality dropped by 40% in high-income countries, but little progress has been made in low and middle-income countries. Although the incidence rates are still higher in high income countries, women living in low and middle income countries have higher mortality.<sup>3</sup> Comparing case fatality rate (CFR) between very developed and less developed countries will shed more light on this huge inequity: there is more than a four-fold difference in CFR between low Human Development Index (HDI) countries versus very high HDI (47.0% vs. 10.8%) and nearly a three¬fold difference (56.2% vs 20.8%,) for pre- and postmenopausal breast cancer, respectively.<sup>8</sup>

The first pillar of the GBCI is health promotion and early detection. In a study conducted in Iran, about 31.7% of breast cancer patients had delayed more than one month after the first symptom to visit a health care provider. Women of lower socioeconomic status and those residing in rural areas or small cities with limited access to health resources had more commonly delayed seeking medical advice for their symptoms. Nearly 70 percent of patients who delayed visiting a doctor had assumed the symptoms to be minor and not important<sup>9</sup>, a finding which highlights the importance of promoting awareness about breast cancer symptoms among women.<sup>10</sup> Other barriers for timely diagnosis were fear of being diagnosed with cancer<sup>11</sup> embarrassment for having the breasts examined<sup>12</sup> and other cultural and social beliefs<sup>13</sup>, further emphasizing the urgent need for orchestrated public education interventions to address the stigma and

Mojgan Karbakhsh, MD Address: Department of Community and Preventive Medicine, School of Medicine, Tehran University of Medical Sciences, PoorSina St, Qods St, Enqelab Ave, Tehran, Iran Tel/fax: +98 21 88962357 Email: <u>mkarbakhsh@tums.ac.ir</u>

other psycho-social barriers.

The second pillar of the GBCI is timely breast cancer diagnosis.<sup>2</sup> One contributing factor in "diagnosis delay" has been lack of access to mammography or ultrasonography in the area of residence. Another important issue in transition countries has been non-adherence to the physician's advice, especially when structured referral protocols were not in place. Sadly enough, some women delayed following the recommended diagnostic procedures, as they perceived some "reassurance" from their health care providers.9 In other words, while the doctor attempted to disclose the cancer diagnosis without overwhelming the patient, the main message regarding the importance of immediate and tight adherence to follow-up procedures had not been communicated explicitly to the patient.<sup>14</sup>

The third pillar of the GBCI is comprehensive breast cancer management.<sup>2</sup> Treatment delay is also common is low and middle income countries and occurs due to unclear referral protocols, long waiting lists for admission and surgery, lack of access to equipped care facilities and financial barriers.<sup>9</sup>

While breast cancer accounts for 1 in 4 cancer cases and for 1 in 6 cancer deaths among women<sup>3</sup>, GBCI brings about new aspirations for support and engagement of countries in evidence-based planning and care and provides a roadmap to decrease death and tackle disparities in survival.

#### **Conflict of Interest**

None.

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#### DOI: 10.32768/abc.20218265-67 COVID-19-free Surgical Pathways for Breast Cancer Patients

Sara Grendele<sup>a</sup>, Alice Pellegrini<sup>\*</sup><sup>a</sup>, Matteo Bardelli<sup>a</sup>, Giovanni Cisternino<sup>a</sup>, Marica Melina<sup>a</sup>, Margherita Serra<sup>a</sup>, Mario Taffurelli<sup>a</sup>, Simone Zanotti<sup>a</sup>

<sup>a</sup> Breast Center-General Surgery, Department of Health of Woman, Child and Urological Diseases, University of Bologna, Policlinico S. Orsola-Malpighi, Bologna, Italy

COVID-19 pandemic declared by the WHO on March 11th, 2020 had a strong impact on all aspect of the health system.<sup>1</sup> Under these unprecedented circumstances, special attention must be given to the impact of the pandemic on cancer patients. Cancer patients are fragile and at higher risk of complications due to the underlying disease.<sup>2</sup> Recent findings suggest that they are at higher risk of COVID-19 infection compared to the general population due to several factors such as immunocompromised state and poor functional status.<sup>3</sup> Despite the infective risk, they require effective and timely oncological therapy.

During this pandemic, our big challenge as breast surgeons is to minimize interruption of breast cancer treatment and balance it with patients' risk of infection. Although delays in surgical intervention in early-stage breast cancer of maximum sixty days seems not to be associated with worst prognosis, the big problem of delaying oncological surgery is the unpredictability of the end of the COVID-19 pandemic.<sup>4</sup>

The aim of this report is to describe the measure taken in our surgical department. We also developed a short questionnaire to explore the impacts of COVID-19 on our patients, their perception of the disease and treatment satisfaction.

#### Hospital management reorganization

At S. Orsola Malpighi Hospital in Bologna, Breast Surgery and Gynecology and Obstetrics Departments were concentrated in a COVID-free pavilion. In the operating theatre, three operating

\* Address for correspondence:

Alice Pellegrini, MD Address: Breast Unit, S.Orsola-Malpighi Hospital, Viale Albertoni 15, 40126 Bologna, Italy Tel: 0512143596 Email: <u>alicepellegrini81@gmail.com</u> rooms (OR) were COVID-free and we had one OR dedicated to emergency in COVID-suspicious patients.

Depending on the limited availability of anesthesiologists, ventilators and hospital beds, priority for surgical procedures was given to oncological patients. After discussion with the crisis management team, we selected patients diagnosed with invasive carcinoma or with high-risk breast lesion of uncertain malignant potential and we postponed all elective non-oncological procedures. To reduce the risk of exposure and transmissions of COVID-19 during hospitalization, we adopted several measures following international recommendations and internal health management directives.<sup>5-10</sup>

#### Pre-admission

Before planning preoperative examination, a standard telephone investigation was conducted to screen patients for:

- travels to high-risk areas in the previous 14 days.

- close contact with COVID-19 patients or with people with a SARS-CoV2 positive swab in the previous 14 days.

- signs and symptoms associated with COVID-19 such as cough, fever (>37,5°C), shortness of breath and anosmia.

- recent attendance in a COVID-19 hospital.

In case of any symptoms or suspicious anamnestic factors, patients were referred to the family doctor to assess the need for a nasopharyngeal swab. For those patients, surgical procedures were postponed until they were screened or tested negative for COVID-19.

As part of routine preoperative investigations, all patients underwent chest radiography and standards laboratory tests. Moreover, all patients were tested for COVID-19 using nasopharyngeal swabs 24 hours before surgery or 48 hours before when



patients had to undergo pre-operative tumor localization procedures or lymphoscintigraphy. Only patients with a negative result could enter the ward and they were advised to self-isolation until admission.

#### During admission

All patients were educated about the importance of standard risk reducing protocols such as hand hygiene and the use of personal protection equipment (PPE). Every patient received a surgical face mask upon admittance and every patient bed was equipped with a disinfectant dispenser. Social distancing measures were observed, and beds were spaced two meters apart in each room. All patients reporting symptoms associated with COVID-19 during the hospitalization were promptly isolated in dedicated single rooms until the negative result of the naso-pharingeal swab came out. According to Ministerial Decree, all visits to admitted patients were interrupted, and patients could only be accompanied outside the ward. With the patient's consent, phone calls were used to update family members after surgery and every afternoon after patient's examination.

All general preventive measures were also followed by all the healthcare workers and nasopharingeal swabs were performed biweekly. Standard operating room PPE included hand gloves, surgical goggles, standard protective surgical gown,

#### Table 1. Questions and Answers of the 54 responders

Questions	Answers	%
As an oncological patient do you think to be more vulnerable/at risk to get coronavirus infection?	Definitely Moderately Slightly Not at all	11 33 22 33
During this emergency period due to coronavirus have you been afraid not to receive adequate oncological care?	Definitely Moderately Slightly Not at all	2 17 28 54
During this emergency period due to coronavirus did you feel overlooked by your medical team (surgeon, oncologist) regarding your oncological disease?	Definitely Moderately Slightly Not at all	0 4 85 11
With respect to the coronavirus emergency did you receive exhaustive information on your hospitalization and post-operative outpatient checks?	Definitely Moderately Slightly Not at all	46 39 9 6
During the lockdown (from March 9th to May 3rd) have you been afraid of getting coronavirus in the hospital setting?	Definitely Moderately Slightly Not at all	9 26 3 26
During phase 2 (since May 4th until now) have you been afraid of getting coronavirus in the hospital setting?	Definitely Moderately Slightly Not at all	4 24 43 30
During the surgical hospitalization did you feel safe from getting coronavirus?	Definitely Moderately Slightly Not at all	37 50 9 4
Did the impossibility of receiving any visits from relatives made your hospitalization harder?	Definitely Moderately Slightly Not at all	19 19 33 30
Did you agree with banning visits in order to limit your own and others risk getting coronavirus?	Definitely Moderately Slightly Not at all	78 15 4 4
Overall are you satisfied with the service provided despite the coronavirus emergency?	Definitely Moderately Slightly Not at all	72 24 4 0

shoe covers and FFP2 mask covered by a surgical mask, due to FFP2 shortage. Surgical goggles were reutilized after cleansing with 70% ethanol solution. The unessential personnel were not allowed to enter the OR. Staff communication and case discussion were conducted through telematic meetings to encourage social distancing and decrease the risk of exposure.

#### Questionnaire

For breast cancer patients, in addition to the oncological aspects, importance must be given also to the psychological aspect. With our multidisciplinary team, we developed a qualitative questionnaire using Google Form to investigate the impact of COVID-19 pandemic on our breast cancer patients, their concerns about contracting the virus and about how the virus may affect their treatment. The questionnaire was administered to all the 76 patients treated during the first lockdown at S. Orsola-Malpighi Hospital in Bologna and consisted of 18 questions about sociodemographic data and patients' perceptions of COVID-19 pandemic including specific questions regarding the hospitalization. We decided to contact all patients by phone before sending them the questionnaire to check their health conditions and explain the aim of the survey. The questionnaire was sent by e-mail between May 18th and 22nd and completed anonymously by the patients. All patients responding to the questionnaire gave an informed consent to their involvement in the survey. A total of 54 questionnaires were collected with a response rate of 71%. The main questions and answers are reported in Table 1.

The results emerging from the questionnaire showed that our patients are only moderately aware of their increased risk of infection probably due to their young age and few comorbidities. Although they reported to be afraid of getting COVID-19, risk reducing measures taken made them feel safe during the hospitalization.

None of the patients reported symptoms associated with COVID-19 after dismissing from our department. However, managing oncological elective procedures during COVID-19 pandemic remains a significant challenge for surgeons.

According to our experience, oncological breast surgery can be safely performed in a COVID-19-free pavilion.

Both healthcare workers and patients should carefully be educated on personal hygiene and the correct use of protection equipment, since we can minimize the risk of infection only with collaboration and cooperation between patients and staff. Following COVID-19-free surgical pathways, it was possible not to postpone oncological surgical activity, minimize perioperative viral transmission rates and make patients feel safe during the hospitalization.

#### **Conflict of Interest**

The authors report no proprietary or commercial interest in any concept discussed in this article.

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### Putting Together the Pieces of the Mosaic - What Do We Know About Potential Environmental and Social Risk Factors for Breast Cancer?

Kate Rygiel\*<sup>a</sup>

<sup>a</sup> Department of Family Practice, Medical University of Silesia (SUM), Zabrze, Poland

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ABSTRACT

**Background:** The human breast undergoes processes of proliferation and involution, especially during puberty, pregnancy, and menopause. During these dynamic phases, some specific environmental factors play important roles, including pro-carcinogenic effects. In addition to the usual breast tumor, its pathologic characteristics, and genetic variants, different environmental and social factors create additional challenges to the accurate comprehension of breast cancer (BC) risk, diagnosis, prophylaxis, and treatment.

**Methods:** This mini-review is based on Medline database search for recent clinical studies on BC risk factors, development, and prevention, particularly at the time of puberty, pregnancy, and menopause.

**Results:** Based on the medical literature review, some insights were provided into how external environmental factors influence BC risk, incidence, and mortality. Also, in an attempt to answer this key question, the selected chemical and physical components of the environment, as well as the large spectrum of social and behavioral elements, were analyzed.

**Conclusion:** It has been suggested that a broad spectrum of established and potential environmental elements have been related to BC etiology. Furthermore, a modern transdisciplinary approach to research studies, including epidemiological, biological, toxicological, pathological, genetic, social, and behavioral factors should help provide the "whole picture" of the environmental risk factors and causes for BC. This is particularly valid to medical personnel involved in women's health to facilitate preventive efforts, especially for those who are at the highest risk for this common and devastating malignancy.

Key words: Breast cancer (BC), environment, endocrine-disrupting chemicals (EDC), transdisciplinary research, prevention.

#### Introduction

Breast cancer (BC), as the most frequent neoplasm and the largest mortality cause among malignancies in females around the globe, usually attack during the most vital periods of women's personal and professional life.<sup>1</sup> As a consequence, BC profoundly influences the women's general health status and quality of life, and can affect many aspects of their family dynamics. Despite unquestionable preventive, diagnostic, and therapeutic advances, the BC incidence has increased in all ethnic groups (except whites).<sup>2</sup> It is very challenging to distinguish numerous factors that can be responsible for BC. A puzzling difference in the BC incidence, across various countries, cannot be simply attributed to genetic reasons. Therefore, further research needs to focus on various environmental factors.<sup>3</sup> For instance, it has been reported that many female immigrants, mostly from the underdeveloped countries, with low BC incidence, have subsequently experienced higher BC incidence rates, typical for the highly industrialized

<sup>\*</sup> Address for correspondence: Katarzyna (Kate) Rygiel, M.D., Ph.D. Department of Family Practice, Medical University of Silesia (SUM), Zabrze, Poland; 3 Maja St.; 41-800 Zabrze, Poland Tel: +48 692 576 729

Email: kasiaalpha@yahoo.co.uk

countries (e.g., the U.S.), where they had migrated. Such data suggest that certain environmental factors may augment the BC rates in genetically connected women (such as mothers and daughters from immigrant families).<sup>4</sup> Unquestionably, reproductive factors play some role in this scenario, due to the cyclic estrogen (E) and progesterone (P) stimulations of the breast tissue.<sup>5</sup>

In addition, other possible causal factors, particularly from the external environment (e.g., toxic chemical agents), represent one of the research priorities.<sup>6</sup> To explore these topics in detail, it is mandatory to evaluate the cumulative environmental risk, simultaneously from several sources, using various, precise instruments, and biomarkers (e.g., in areas of molecular biology, genetics, toxicology, endocrinology, and epidemiology).' It is very challenging to find causality between BC and potentially toxic environmental exposures. For instance, with regard to case-control studies, a recall bias is a common inconvenience, and for many longitudinal studies, the unavailability of the toxic chemicals in tissue specimens, represent frequent disadvantages. In an attempt to overcome such methodological difficulties, mapping cumulative toxic effects from the environment, and monitoring the burden of disease, are reasonable approaches. As a consequence, these strategies can help women with BC or at high risk for BC, make individual lifestyle choices and BC management-related decisions.<sup>8</sup>

#### Methods

This mini-review is based on Medline database search for clinical studies on BC risk factors, development, and prevention, particularly in the periods of puberty, pregnancy, and menopause. The main timeframe for this search was set for the last 25 years. Also, the search was supplemented with some information from the relevant cross-references. Publications focused on clinical trials, investigating puberty, pregnancy, and menopause, and target populations of women exposed on specific BC risk factors, often derived from social, built, chemical, and physical environment were analyzed.

#### **Results and Discussion**

Based on the medical literature review, some insights have been provided into how external environmental factors influence BC risk, incidence, and mortality. Also, in an attempt to answer this key question, the selected chemical and physical components of the environment, as well as the large spectrum of social and behavioral elements, have been analyzed.

This narrative review outlines several environmental factors for BC, which have been frequently correlated with BC, or could have caused it. Such factors can exert some direct or indirect effects on BC, particularly, when acting through certain mediating circumstances, like early onset of puberty, obesity, and endocrine or metabolic derangements.

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Moreover, a comprehensive evaluation of cumulative environmental effects should be useful in a deeper understanding of the interconnected causes of BC, in real-life dynamic scenarios. In this way, a transdisciplinary approach (e.g., epidemiological, biological, toxicological, pathological, genetic, social, and behavioral) to research on BC that incorporates a balanced constellation of environmental risk factors and causes should be considered for prevention and management of this common and devastating female cancer. The interpretation of current findings in this area of BC research has been outlined in the consecutive sections of this article, and summarized accordingly.

## *Why the traditional models of BC causation are insufficient?*

Several BC risk factors typically interact with each other, and their combination, after some period of time, can lead to BC in susceptible women.<sup>9</sup> In this situation, a transdisciplinary study on the impact of the environmental exposures on BC etiology is of utmost importance.9 This offers a potential to disentangle a conglomerate of risk factors and pinpoint the most important causal components for BC. In general, the American Cancer Society (ACS) recommendations for BC prevention emphasize reducing alcohol intake, maintaining a healthy body mass, performing regular physical activity, and avoiding post-menopausal hormone therapy (HT).<sup>10</sup> However, for many women, who follow the ACS guidelines, several BC risk factors still remain unclear, and the ACS recommendations appear insufficient.<sup>1</sup>

At this point, observations of female immigrants (e.g., arriving from the underdeveloped to industrialized Western countries), research on nuclear bomb survivors and epidemiological studies can shed some light on the idea of those toxic exposures, at some specific periods in a women's life course, that can be crucial to the later BC risk.<sup>12, 13</sup> Moreover, prenatal exposures to carcinogens (e.g., synthetic estrogens) can have detrimental effects on adult women's health, several years later.<sup>14</sup> These socalled windows of susceptibility (WOS), involving the prenatal, pubertal, pregnancy, and menopausal transition periods, correspond with certain "milestones", during which the mammary glands undergo anatomical and functional transformations.<sup>15</sup> In fact, BC etiology is related to ongoing changes in the breast tissue and alterations of the mammary gland environment. Therefore, when some toxic chemical agents, derived from the environment, such as endocrine-disrupting chemicals (EDC), as well as certain therapeutics (e.g., applied for the coexisting medical conditions) can influence the BC risk, development, course, and prognosis.<sup>15</sup>

# What are the key non-genetic risk factors or causes of BC?

To address the main non-genetic causes of BC, a universal term: "population attributable risk" (PAR) (representing the percentage of excess cases, which may be related to a particular exposure) can be helpful with regard to the BC.<sup>12</sup> However, PAR is often difficult to accurately determine, with regard to many BC risk factors, both internal (e.g., genetic or biological)<sup>16</sup> and external (e.g., occupational, residential, social, or cultural).<sup>17</sup> At this point, it is useful to introduce a working definition of the environment, which means "anything that is not genetic" and includes the social (socioeconomic/sociocultural), built, and toxicological/chemical/physical components.<sup>12</sup>

Interestingly, the BC risk is often elevated in women with higher socioeconomic status (SES) (measured by the education level, residential standard, employment rate, and income level).<sup>18</sup> This phenomenon may by explained, to some degree, by certain reproductive patterns, often linked to typical sociocultural values.<sup>19</sup> Moreover, the neighborhood SES has also been related to a higher BC risk for women with the highest SES, compared to the ones with the lowest SES, across all ethnic groups.<sup>20</sup>

The built environment encompasses the purposeful human actions to create and affect the physical surroundings (e.g., spaces for living, working, shopping, eating, exercising, relaxing, and entertaining), which can be beneficial (e.g., availability of fresh food, clean air/water, safe recreation/sports facilities) or dangerous to health (e.g., the overwhelming presence of fast food, alcoholic beverages, tobacco products, and lack of safe areas for physical exercises).<sup>20</sup> Both the social and built environment characteristics of community neighborhoods appear to influence the BC risk. Hopefully, an analysis of this impact, across various populations, will help explain the relevant ethnic discrepancies in BC risk, incidence, prevalence, or management.<sup>20</sup> Moreover, it should be noted that the multiethnic communities with lower SES and unhealthy features of the built environment are often more obesogenic, prone to diabetes mellitus type 2, metabolic syndrome, or cardiovascular diseases, as well as to the higher risk of postmenopausal BC.<sup>20</sup> Also, it is conceivable that the dangerous combination of highly processed, poor nutritional value food and beverages, with predominant sedentary activities can be associated with excessive energy intake among young girls, and a subsequent adverse impact on their development, including endocrine and reproductive functions.<sup>21</sup> In contrast, there is an inverse correlation between the physical activity (usually associated with good access to safe recreational areas in the neighborhood, and healthy

nutrition) and BC incidence.22

Over the last few decades, relations between exposures to common chemical agents, which represent a large part of toxicological and chemical/physical environment, and the BC risk and incidence (or some intermediate outcomes related to BC, such as an age at the onset of menarche) have been intensely studied.23 In practical terms, environmental exposures can be categorized as modifiable lifestyle factors (or consequences of individual or societal behaviors), and EDC (mostly found in many industrial and agricultural chemical agents, as well as in numerous commercial and personal care products). It should be noted that many behavioral factors are driven by both personal choices and dominant surrounding societal trends. Such pressures are often beyond individual control, and thus, only the well-designed interventions, at the community and public health levels are urgently needed. In fact, promptly addressing the specific, hazardous environmental exposures, and rectifying the situation for the endangered populations, are necessary to counterbalance the possible BC risk, incidence, and adverse outcomes.

# What are the modifiable lifestyle risk factors for BC? - How can women apply personal choices for BC prevention more effectively?

*A.* Obesity - a new look at energy intake and expenditure rather than traditional approaches to diet and physical exercises

Obesity shares some common factors with the built environment.<sup>24</sup> Excessive body mass, including being overweight and obesity, has traditionally been measured via body mass index (BMI). Elevated BMI (e.g., above 25) has been associated with an increased risk of postmenopausal BC, a decreased risk of premenopausal BC, and an earlier age at menarche.<sup>25</sup> However, BMI is not the most accurate parameter to assess body fat content. In fact, abdominal (central) fat is metabolically important, with regard to insulin resistance and potential malignancy risk. The paradoxical relationship of obesity (e.g., BMI above 30) in pre- versus postmenopausal women can be explained by the differential frequency of estrogen receptor-positive/progestin receptor-positive (ER+/PR+) tumors, which can occur in these two age groups.<sup>26</sup> ER+/PR+ tumors, which are more frequent among postmenopausal women, are more sensitive to estrogen (E) that is produced by the adipose tissue. In contrast, ER-/PR- tumors are more common in the premenopausal population, and can be related to some other risk factors. In addition, adipose tissue can serve as a reservoir for EDCs (which are lipophilic and can be stored in the body for prolonged periods of time), playing an obesogenic role.27,28

It is very difficult to demonstrate definite associations between BC and dietary factors in females with excessive body weight. In fact, BC incidence, in various countries worldwide, can be related to high energy intake (e.g., mostly due to the quantity and quality of fats and carbohydrates consumption) and low energy expenditure (e.g., secondary to sedentary behaviors and lack of physical exercises). Such a combination often contributes to prepubertal obesity and weight gain among midlife women.<sup>29</sup> In general, physical activity (regardless of its kind) as a modifiable environmental factor in favor of BC prevention, has protective effects, predominantly for postmenopausal BC, mainly due to decreasing the body adiposity and E levels.<sup>30</sup>

#### B. Alcohol

Alcohol use is a causal factor in BC that acts via the formation of genotoxins (e.g., acetaldehyde) or by the alteration of hormones (e.g., E) and hormone receptors (e.g., ER).<sup>31</sup>

#### C. Tobacco

Tobacco smoke includes over twenty carcinogenic components.<sup>32</sup> These toxins can be detected in the breast tissue of women who smoke (in an active and passive manner).<sup>33</sup> Based on international epidemiologic studies, there is a causal relationship between BC and active tobacco smoking, especially in women, who started smoking before their first full-term pregnancy. Moreover, such a relationship exists also in females, who have a genetic trait, N-acetyltransferase 2 (NAT2) slow acetylator (slowing the metabolism and detoxification of carcinogens).<sup>34,35</sup> Exposure to secondhand smoke has also been related to increased risk of BC in never smokers (e.g., especially among premenopausal women).<sup>36</sup>

# D. Exogenous female hormones - oral contraception (OC), hormone replacement therapy (HT), and diethylstilbestrol (DES)

In general, BC as a hormonally dependent malignancy is sensitive to oral contraceptives (OC) and hormone replacement therapy (HT). Certainly, individual variability and clinical context always should be considered before the possible use of OC or HT.

Oral contraceptives (OCs) are applied for birth control or some other medical reasons (e.g., irregular menstrual cycles or dysmenorrhea) by approximately 16% of women within the age range 15–44 years, in the U.S.<sup>37</sup> OCs have carcinogenic properties, but the risk decreases after 4 years from the termination of their use.<sup>38</sup> Since OCs are mostly being used by young females, in whom the risk of BC is low, and this risk also remains low at the population level. A combined estrogen-progestin hormone therapy (HT) for menopausal women had been used, until the Women's Health Initiative (WHI) study revealed an augmented risk of BC, which was not offset by other medical advantages (related to cardiovascular disease, osteoporosis, or cognitive functions) in

postmenopausal population.<sup>39</sup> At present, HT is used to control menopausal symptoms with great caution, under constant medical supervision, for a short time, depending on an individual clinical context.

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Diethylstilbestrol (DES) is an estrogenic agent, which had been applied for miscarriages prevention. Unfortunately, it was found that daughters of women who took DES during pregnancy, subsequently developed adenocarcinomas of the vagina<sup>40</sup>, and thus, the use of DES was terminated. In addition, it was noted that as the females who took DES were getting older, they experienced higher BC incidence.<sup>41</sup>

#### E. Light at night

Shift work that has been related to elevated BC rates is typically combined with exposure to light at night.<sup>42</sup> In this way, the suppression of melatonin, a hormone which physiologically increases in the darkness of night and has anti-estrogenic actions contributes to elevated BC risk.43 In addition to necessary shift work in certain areas (e.g., health care, emergency or military services, communication, transport, and industrial infrastructure), a light at night may be overused by students, workers using electronic equipment, or persons who excessively watch TV or engage in entertainment at night time. Therefore, a reasonable (e.g., very limited) use of electric and electronic devices, for unnecessary reasons, especially during the late hours should be encouraged.

#### F. Ionizing radiation

Ionizing radiation has demonstrated carcinogenicity for BC, which was reported in survivors of the atomic bomb explosions.<sup>44</sup> Currently, diagnostic radiological imaging (e.g., radiography, fluoroscopy, and computed tomography CT)) represents a common source of ionizing radiation.<sup>12</sup> Therefore, the most reasonable and cautious use of the diagnostic radiation should be promoted to reduce the unnecessary risk related to this exposure.<sup>45</sup>

*G.* Endocrine disrupting chemicals (EDC) – How can we improve conscious control over personal care products, commercial, industrial, and agricultural chemical agents?

In addition to endogenous and exogenous hormones, which influence the BC risk, multiple synthetic chemical agents often mimic or disrupt the endocrine actions (e.g., estrogen signaling). At present, such agents with estrogenic activity, known as EDCs, are commonly used in a plethora of industrial, commercial, and agricultural compounds (or their byproducts), as well as in personal care products.<sup>6</sup> It should be emphasized that the most dangerous EDCs frequently encountered by women at their home or neighborhood and workplace include the following toxic substances: organochlorines (such as polychlorinated biphenyls (PCBs), dioxins (e.g., 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), and dichlorodiphenyltrichloroethane (DDT) (a pesticide), organohalogenated compounds (e.g., polybrominated diphenyl ethers (PBDE)), per-and poly-fluoroalkyl substances (PFAS), perfluorooctanoic acid (PFOA), phenols (e.g., bisphenol A (BPA)), parabens, phthalates (e.g., butyl benzyl phthalate (BBP)), polycyclic aromatic hydrocarbons (PAH), benzene, ethylene oxide, and certain metals (e.g., cadmium).<sup>6</sup> Brief characteristics of the selected EDCs, and the key messages from the most relevant studies in this area are outlined below.

Organochlorines are lipophilic compounds, which are resistant to biodegradation. Although their use was prohibited in the 1970s due to their toxicity, organochlorines can still be present in the environment and biological samples.

Polychlorinated biphenyls (PCBs) are organochlorine compounds, characterized by various biological effects, which had been used as industrial coolants, insulators, and lubricants, until their use was banned, in 1979, in the U.S. PCBs have been related to BC in some epidemiologic studies.<sup>46</sup> In addition, gene-environment interactions with CYP1A1 that have been reported in some studies (e.g., elevated levels of PCB and increased expression of CYP1A1) support an increased BC risk related to the PCB exposure <sup>47,48</sup> For instance, in a trial, which assessed serum PCB levels, among females during early postpartum period, in 1959 -1967 (a time of culmination of the PCB use), women with a higher proportion of PCB 203 (the most toxic PCB type) to the sum of PCBs 167 and 187 (the less toxic PCB types) had a higher probability to be diagnosed with BC by the age of 50.49

Dioxins, including 2,3,7,8-Tetrachlorodibenzo-pdioxin (TCDD), are very toxic organochlorines, obtained during a process of combustion or metal processing, and various chemical technologies. Similar to PCBs, they remain for a long term in the environment, contributing to neoplastic, reproductive and endocrine disorders.<sup>50</sup> The Environmental Protection Agency (EPA) considers TCDD to be a carcinogen for several malignancies, including BC. In particular, in the Seveso Women's Health Study, the relationship between dioxin exposure and BC risk has been investigated in a large population of women living around and working in a chemical plant which was a source of exposure to TCDD. After over thirty years of follow-up, thirty three BC cases were diagnosed, and the BC risk was elevated among females with higher blood levels of TCDD.<sup>51</sup> Also, relations of organochlorines with earlier age at menarche have been observed in some studies.<sup>52,</sup>

Dichlorodiphenyltrichloroethane (DDT) is another toxic organochlorine used in the past as a pesticide. It should be noted that the p,p'-DDT and its metabolite p,p'-DDE were addressed in research. For instance, in a study using serum samples from women participating in the Child Health and Development Studies (CHDS), at the time of their childbirth, it was noted that 129 of such females subsequently developed BC before menopause (e.g., before 50 years of age).<sup>54</sup> Interestingly, those who were in the highest tertile of p,p'-DDT exposure were almost three times as likely to develop BC as were those in the lowest tertile. In addition, this relation was stronger among females who were younger than 14 years at the time of their exposure. These data are convergent with the idea that the timing of exposure, such as an early development period, can be critical for EDC (e.g., p,p'-DDT) to contribute to the carcinogenic action in the breast.<sup>55</sup> Similarly, there are some supportive results derived from the Sister Study, in which it was reported that young girls (e.g., before age 18 years), who were exposed to fogger truck or plane spraying of DDT were at an increased risk for premenopausal BC.<sup>56</sup>

Polybrominated diphenyl ethers (PBDEs) (also known as polybrominated biphenyls (PBBs)) are commonly used flame retardants, which can persist in the environment for a long time (e.g., in the house dust).<sup>57</sup> Interestingly, in the Breast Cancer and the Environment Research Program (BCERP) study of PBDEs, the elevated serum levels of PBDE were detected in 70% of girls from California and Ohio, in the U.S., and such levels were higher for participants from California.<sup>58</sup>

Perfluoroalkyl substances (PFASs) represent perand polyfluorinated agents that have EDC properties, and have been used in many industrial and commercial products (e.g., perfluoroactanoic acid (PFOA) - in Teflon and Gore-Tex materials).<sup>59</sup> The Danish National Birth Cohort study has reported an increased probability of BC among the participants in the highest compared to the lowest quintile of perfluorooctanesulfonamide (PFOSA) (which is metabolized to perfluorooctane sulfate (PFOS)), and the results of this follow-up study have been pending.<sup>60</sup>

Phenols, such as a bisphenol A (BPA), are weakly estrogenic agents, often used industrially in polycarbonate plastic and epoxy resins manufacturing. Hazardous exposure takes place when BPA is leached from plastic-lined food and beverage cans.<sup>61</sup> BPA can exert some abnormal effects on body mass (e.g., weight gain), puberty, and reproductive functions, in both females and males.<sup>62</sup> For instance, according to a study of prepubertal 6-7-year-old girls, participating in the BCERP in the U.S., 94% of the tested urinary samples contained elevated BPA levels.<sup>63</sup>

Parabens act like weak estrogens (Es) that bind to the estrogen receptors (Ers). Parabens can serve as antimicrobial preservatives, often used in personal care products (e.g., underarm cosmetics, deodorants, etc.). They have been found in urine samples and in BC tissue specimens<sup>64</sup>, and can also stimulate BC cell proliferation in vitro.<sup>65</sup> According to a large study, in which the urine paraben levels were measured in a

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group of 1,151 6-8-year-old girls, it was revealed that paraben levels (that often occurred together with benzophenome-3 (BP-3), a phenol present in sunscreens) were higher in the summer time, and among a white group of girls.<sup>66</sup>

Phthalates are widespread, hormonally active pollutants that can alter pubertal timing. Exposures to phthalates may either accelerate or delay pubertal development depending on the age of exposure and some other factors (e.g., obesity).<sup>66</sup> Phthalates can be contained in different personal care products, such as cosmetics, together with parabens and organic solvents.<sup>67</sup> For instance, butyl benzyl phthalate (BBP) is an estrogenic agent and a partial agonist for the ER. BBP is frequently used in food wraps, cosmetic formulations, and plastics. Studies of pubertal timing in 30 Taiwanese girls with premature the larche (breast development) were compared to 26 girls with central precocious puberty, and 33 normal controls. The girls with premature thelarche were found to have higher levels of monomethyl phthalate (MMP) than the control group.<sup>68</sup> Similarly, a study from the BCERP assessed a panel of nine phthalate metabolites. In a group of 1,149 girls, the investigators noted a relationship between the phthalate metabolites and the pubertal onset (measured by either breast or pubic hair development).<sup>66</sup> According to this study, highmolecular-weight phthalates (HMWP) levels were inversely associated with pubic hair development.<sup>66</sup>In addition, increased low-molecular-weight phthalates (LMWP) levels were associated with BMI and waist circumference (WC) gain, among girls in another study.<sup>69</sup> Therefore, it appears that exposure to phthalates may be indirectly related to the BC risk, especially in early stages of the female developmental process.

#### *H. Hazards of Air Pollution and Polycyclic Aromatic Hydrocarbons (PAHs)*

Many genotoxins and estrogenic or antiestrogenic agents contribute to widespread air pollutions that are cancerogenic. Among them, polycyclic aromatic hydrocarbons (PAHs) have been related to BC.<sup>6</sup> PAHs are carcinogenic chemical compounds that are produced during the incomplete combustion of different kinds of fuel (e.g., coal, oil, and gas). In addition, exposure to dietary products (e.g., grilled meats or fish) and contaminated ambient air (e.g., by tobacco smoke, active or passive) poses BC risk, due to the genotoxic properties.<sup>70</sup> Some PAHs have weakly estrogenic properties, and thus can influence BC risk.<sup>71</sup> Since air pollution is different in various neighborhoods, there can be an interaction between the PAH exposure and some social environmental components that may create particular hazards in more disadvantaged local communities.<sup>72</sup> In the ESCAPE Project that included 15 European groups of postmenopausal females, elevated BC risk related to nitrogen oxides

(NO2) (a marker of air pollution) and nickel (a marker of oil combustion and various industrial procedures) was documented.<sup>73</sup> Similarly, findings of the U.S. Sister Study revealed an increased risk of ER+/PR+ breast tumors, correlated with NO2 exposure.<sup>74</sup> Moreover, the results of the California Teachers Study found associations between ER+/PR+ breast tumors and certain carcinogens, as well as between ER-/PR- breast tumors and benzene, cadmium, and arsenic.<sup>75, 76</sup> Furthermore, research on work and residential settings-related exposures to PAHs indicates possible relations between ambient air pollutions and BC. For instance, in a study from New York, women with increased exposure to total suspended particulates (TSP) at their birth location had an elevated BC risk later in life. In addition, the TSP levels were related to the 2.4-fold increased BC risk in these women.<sup>77</sup> Another study from the same group, examined exposure to traffic emissions, in females with BC, in which the residence was considered to be an indirect exposure parameter. This study examined exposures at different times in the life cycle of the participating females (e.g., the age at menarche). In particular, an increased BC risk was noted for exposures at the time of menarche and the age at first childbirth among females with postmenopausal BC.<sup>77</sup>

Convergent with these findings, a recent study from the longitudinal BCERP has shown an association between andrenarche and living in the proximity to traffic-related air pollution exposure.<sup>79</sup> In agreement with that result, a case-control study has revealed that women with detectable PAH levels had a twofold increase in their BC risk compared to the ones without detectable PAH levels. In addition, a doseresponse relationship was reported among women with elevated PAH levels who had over fourfold increase in the BC risk.<sup>80</sup> Also, a study of female employers in Canada reported that the increased risk of BC was related to a longer employment period in some facilities, which were exposed to a higher vehicle exhaust (e.g., especially, if such exposure began when these women were younger than 36 years of age).<sup>8</sup> Interestingly, in the Long Island Breast Cancer Study that explored interactions with more than a dozen gene variants, an association between a higher BC risk and PAH–DNA adducts was observed.<sup>6</sup> This elevated BC risk was observed particularly in the case of higher levels of PAH-DNA adducts, in women with gene variants related to poor cell repair abilities.<sup>6</sup> Although the Long Island Breast Cancer Study linked the elevated PAH-DNA adducts levels with the increased BC risk, the exact role of PAHs in BC etiology still requires an intense research investigation.<sup>8</sup>

Notably, benzene, a very common industrial agent, is an established carcinogen for BC.<sup>83</sup> For instance, as a combustion product of gasoline and natural gas, benzene is widely present in the environment, and exposure to it should be reduced

due to hazardous consequences.<sup>12</sup> Similarly, 1,3butadiene, a gas present in petroleum products and cigarette smoke, is a carcinogen. According to some occupational studies, 1,3-butadiene has been connected to various hematopoietic malignancies. Even though no human studies of its effects on BC in women are available, some animal studies (like in the case of benzene) have revealed elevated rates of mammary tumors and genotoxic damages.<sup>85</sup> Likewise, ethylene oxide, a compound that has been used mostly for medical equipment sterilization, presents the biggest BC hazard to women working in the relevant hospital facilities.<sup>85</sup> For instance, a large U.S. epidemiologic study, conducted among women in the hospital occupational setting, reported an increased risk of BC which remained elevated, even after adjustment for the number of childbirths and family history of BC.81

#### F. Metals

Metals derived from natural and industrial sources are practically unavoidable environmental components. For instance, cadmium, arsenic, beryllium, chromium, and nickel have been considered carcinogenic.<sup>87</sup> In particular, cadmium has revealed estrogenic properties and has been related to elevated BC risk.<sup>88</sup>

#### How to advance BC prevention? - Directions for future research projects examining possible correlations between common environmental factors and BC

It should be pointed out that integrating various factors from the biological, behavioral, social, and physical domains, in order to expand practical knowledge about risk, prevention and, management of BC is superior to analyzing them in isolation. In fact, a recent comprehensive, transdisciplinary approach proposes the multilevel etiology of postmenopausal BC.<sup>89</sup> For instance, in this design, the main parameters included the patient's age, ethnicity, age at the onset of menarche, the birth of the first child, menopause, as well as obesity (assessed by BMI), alcohol or tobacco use, financial income, hormone therapy (HT), and BRCA1/2 genotype. This study has shown that the decrease in HT and BMI, as well as the increased age at menarche, were beneficial for BC prevention. On the one hand, modification of these factors can only modestly affect the BC risk estimates. However, on the other hand, such hormonal and anthropometric modifications (HT and BMI) may influence the absolute number of women affected by BC. In addition, this approach emphasizes the complex BC etiology, shows methodological challenges, and indicates some directions for further studies. Consequently, after completing a pilot project called "Race and Ethnicity in Stage-specific Breast Cancer Survival" (2008), future BC studies are planned to

explore the impact of contextual factors (e.g., body size, physical activity, and various co-morbidities) on ethnic differences in BC survival in detail.9 Furthermore, with regard to the urgent need for advancing the primary BC prevention, the novel structured approach has been developed by the Breast Cancer Prevention Partners (BCPP).<sup>91</sup> In short, the BCPP is a strategic plan that integrates scientific data with community perspectives, focusing on measurable objectives to reduce the incidence of BC in the future.<sup>91</sup> Unfortunately. despite increases in screening and advances in treatment, BC continues to be the most common cancer and cause of cancer mortality among women worldwide. Since BC rates have remained steady for several years, special efforts need to be re-directed and concentrated on the population-level primary prevention. For instance, to address the complexity of the BC at this level, the California Breast Cancer Research Program (CBCRP) has promoted some innovative BC preventive concepts for research studies in many high-priority areas.

In order to develop practical recommendations for the safe use of multiple chemical substances, in the context of BC (and other hormonally-influenced cancers), some important aspects of the commercial agents' testing as well as epidemiology and toxicology investigations need to be applied.<sup>92</sup> Moreover, in an analysis of the environmental exposures, novel statistical approaches will be used to explain the roles of multiple interacting factors, which can influence BC risk and development. In this way, many variables (e.g., demographics) could be investigated simultaneously to better understand disparities between certain ethnic groups of women with BC. For instance, an analysis of the immigrant experiences and BC risk among Asian women in the U.S. may elucidate the patterns, in which some social factors (e.g., discrimination) may influence the BC risk, and particular patterns, which can show how this risk oscillates during the lifespan of these women in the community (Table 1).<sup>92</sup>

Considering insufficient progress in BC prevention, the CBCRP intends to apply current scientific knowledge about BC into primary prevention, at the population level, whenever feasible.<sup>93</sup> In particular, involvement in the community-based participatory research (CBPR), as well as dissemination, and implementation of research findings are needed. If such efforts succeed, the next challenge will be to translate the CBCRP interventions, targeted at the specific BC risk factors and relevant, protective strategies, into evidenceinformed interventions (EIIs). Furthermore, the Californians Linking Action with Science for Prevention of Breast Cancer (CLASP-BC) is planned to detect, spread, and implement population-based prevention strategies, aimed at reducing the risk of BC (or other malignancies and

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or personal) - perspe	ectives for ongoing and future resea	arch studies <sup>92, 93</sup>
Geographical Migration	Women born and raised in the U.S. are more likely to acquire BC than newly arrived immigrant women (except the ones from Northern and Western Europe)	Detect what the particular differences are between the immigrant women from various ethnic groups, which make their BC rates lower. Determine why the chances of immigrant women to acquire BC increase after living in the U.S. Explore the ways in which adopting of the U.S./Western culture influences survival after a diagnosis of BC in various ethnic groups of women.
Urban Living	Women living in cities are more likely to get BC than women living in rural areas	Establish efficient collaboration between medical personnel, researchers, social and environmental scientists, community leaders, public health policy makers, and urban planners, to study the local neighborhood environments. Apply models of multiple stressors and cumulative BC risk to answer the research question.
Socioeconomic Status Income Level Education Level Neighborhood Status	BC is a rare health problem that is more commonly found in well-educated or affluent women than in uneducated/ poor ones; BC is more often seen in high-income neighborhoods than low-income ones	Examine what is different about high socioeconomic status women and high socioeconomic status neighborhoods that correlate with higher BC rates. Explore the relative and joint roles of individual and neighborhood socioeconomic status. Investigate how individual and neighborhood socioeconomic status influences the BC risk of women from various ethnic groups
Occupation	BC risk varies by occupation	Examine long-term BC risk of the women exposed to pesticides, industrial chemicals, solvents, and heavy metals at work. Explore why women performing some professions (e.g., teachers or nurses) have a higher-than-average incidence of BC. Examine BC rates in women performing certain types of work (e.g., cleaning, agriculture, electronics, and cosmetic services), related to exposure to environmental toxins
Ionizing Radiation	Ionizing radiation is a proven cause of BC	Evaluate the use of radiation in mammograms for populations of women, who may be particularly susceptible to its harmful effects (e.g., flying air-lines personnel, especially during long intercontinental flights). Examine how genes affect radiation-related BC risk
Light at Night	Working at night raises a woman's chance of getting BC, probably due to the increased exposure to light at night	Study sleep behaviors (e.g., timing, number of hours, and amount of light in the bedroom) that can influence a woman's hormones in ways which can contribute to or help prevent BC. Examine the link of night-time light exposure with the BC risk. Explore whether exposure to light at night during a mother's pregnancy affects her daughter's risk of BC
Disabilities	Research addressing questions about the BC risk in women with disabilities should be expanded	Investigate the BC-related experiences of women with different disabilities. Explore barriers to BC prevention, detection, and treatment, as well as strategies for overcoming these barriers among women with various disabilities
Sexual Orientation	Research on the BC risk and sexual orientation is needed	Select study populations of sexual minority women for participation in BC research. Study the BC rate of transgender individuals, who use long-term hormonal therapies (e.g., estrogen preparations)
Timing of Exposure	Exposure to toxic chemicals at critical time periods in reproductive life (e.g., prenatal, puberty, and pregnancy) may increase a woman's BC risk many years later, when she is an adult	Investigate exposure to chemicals that act similarly to the female hormone estrogen. Find out if exposure to such chemicals at levels actually found in babies' blood can increase the risk that the laboratory animals will get mammary cancer. Develop better methods for measuring toxic exposures to environmental causes of BC through the woman's life course. Investigate exposures to real-life mixtures of pesticides/other toxins, at critical points in the life course, when these exposures are most likely to increase BC risk (e.g., during development in the womb, at puberty, and before childbearing)
Childbearing Breastfeeding	Having children at younger ages and breastfeeding are protective against BC; after a full-term pregnancy, breast cells are less sensitive to carcinogenesis with the lifetime risk of BC decreased in a half	Investigate multiple aspects of culture and tradition that influence childbearing practices (e.g., age of having children & breastfeeding). Conduct pilot projects to test policies to encourage breastfeeding in low income communities
Vitamin D	Higher levels of vitamin D in the blood are protective against BC	Directly measure vitamin D levels in women's blood and find out how these levels affect BC risk. Investigate whether vitamin D from sun exposure, in conjunction with dietary supply, can reduce the risk of getting BC and can increase BC survival

**Table 1.** Exemplary etiologic risk factors for breast cancer (demographic, social, occupational, behavioral, or personal) - perspectives for ongoing and future research studies<sup>92,93</sup>

chronic illnesses, with similar risk factors) especially among local ethnic minorities or vulnerable populations of women.<sup>93</sup>

In conclusion, promoting research that not only increases knowledge but also points towards practical solutions will facilitate future BC prevention and therapeutic management. At this point, gathering detailed information about certain groups of women, who carry a greater burden of BC, as well as detecting the connections between BC and environmental or social circumstances are certainly merited. Steering future BC research in such directions will hopefully lead to decreasing BC mortality among the most vulnerable ethnic groups of women with high death rates of BC.

In summary, the practical goal for both the researchers and clinicians is to apply pertinent clues from the basic or clinical sciences and public health studies to design the most reasonable "action plans", in various medical and personal contexts for women at the risk for BC or suffering from BC.

#### **Conflict of Interests**

None.

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### Breast Reconstruction and Post Mastectomy Radiotherapy: A Primer for Members of a Multidisciplinary Team

Aska Arnautovic<sup>a</sup>, Sigurast Olafsson<sup>a</sup>, Julia Wong<sup>b</sup>, Shailesh Agarwal<sup>a</sup>, Justin M. Broyles<sup>\*a</sup>

<sup>a</sup> Division of Plastic and Reconstructive Surgery, Brigham and Women's Hospital, Boston, MA, USA <sup>b</sup> Department of Radiation Oncology, Dana Farber Cancer Center, Boston, MA, USA

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#### Introduction

Post-mastectomy radiation therapy (PMRT) is a key component in treating breast cancer patients at high risk for locoregional recurrence. As more patients undergoing mastectomy desire reconstruction, PMRT becomes an increasingly important consideration when deciding the type and timing of reconstruction. PMRT may cause multiple adverse effects that may influence patient satisfaction; therefore, careful

\* Address for correspondence: Justin M. Broyles, MD Associate Surgeon. Brigham and Women's Hospital. Member of the Faculty. Harvard Medical School. Boston, MA. USA Email: jbroyles@bwh.harvard.edu

#### ABSTRACT

**Background:** Post-mastectomy radiation therapy (PMRT) is a key component in managing breast cancer with increased potential for locoregional recurrence. Breast reconstruction has evolved to include various techniques that can be categorized according to the type of reconstruction (implant-based versus autologous reconstruction), and the timing of reconstruction (one versus two-step techniques).

**Methods/Results:** This review article aims to provide a digestible summary of PMRT in the context of breast reconstruction by summarizing salient existing literature with a focus on considerations of the plastic surgeon. The main findings summarized in this review include the technique and timing of breast reconstruction, how breast reconstruction can affect radiation delivery, and the type of reconstruction. Within implant-based reconstruction, existing data on the location of the implant in the context of PMRT and PMRT delivery to the tissue expander versus permanent implant are reviewed. Each consideration may alter the probability of successful reconstruction and patient satisfaction.

**Conclusion:** It is essential for the multidisciplinary breast cancer team to have knowledge of the various reconstructive options, and to understand the safety and comparative effectiveness of staged reconstruction in the setting of PMRT. Additionally, one must consider that reconstructive procedures may have implications on the timely administration of PMRT. This review serves as a reference for members of the oncologic care team when discussing reconstructive options with patients who will receive PMRT as part of their treatment plan.

consideration must be given to the interplay between these two factors.

Breast reconstruction has become increasingly popular after the Women's Health and Cancer Rights Act of 1998 which mandated that insurance providers cover all post-mastectomy reconstructive and symmetrizing procedures. Breast reconstruction enhances aesthetic outcomes after mastectomy and has been consistently shown to improve patient quality of life and decrease psychosocial harms after mastectomy.<sup>1</sup> As different reconstructive techniques emerge, it is necessary for the entire oncologic team to understand the role of reconstructive surgery within the context of concurrent oncologic treatment.

When caring for reconstructive patients receiving PMRT, it is important to acknowledge that there is no well-defined, evidence-based algorithm for choosing the

appropriate reconstructive plan. Often, these decisions are made based on surgeon preference and institutional bias. The interactions between PMRT and breast reconstruction play a crucial role in complication profiles and patient satisfaction. Therefore, it is critical for the entire oncologic team to have a thorough understanding of breast reconstruction in order to assist the patient in making individualized decisions regarding the optimal cancer and reconstructive care.

#### Methods

#### Search strategy

We performed a literature review utilizing PubMed to find articles about breast reconstruction utilizing either autologous or implant-based reconstruction. The articles selected for this review included retrospective chart reviews, descriptive studies, case reports, and cohort studies. Our timeframe included studies published between 2000-2020. We specifically focused on finding papers that analyzed timing of breast reconstruction after PMRT, impact of PMRT on implants, whether implant location affects PMRT, and the effects of PMRT on autologous reconstruction. We chose to include studies that we felt best addressed the most important considerations faced by plastic surgeons when approaching reconstruction after PMRT. Each of these considerations regarding breast reconstruction after PMRT was then summarized and reported in its own subsection in the 'results' section.

#### Results

#### Breast Reconstruction Techniques

Breast reconstruction can be performed with various techniques such as using the patient's own tissue to reconstruct the breast (autologous reconstruction), or with prosthetic implants. These procedures can be performed as single or two-stage reconstruction. In the two-stage reconstruction technique, a temporary tissue expander is initially placed at the time of mastectomy, and later replaced with a permanent implant or autologous tissue flap. Choosing the appropriate reconstructive technique and its timing are crucial considerations when evaluating a patient for breast reconstruction.

The Michigan Breast Reconstruction Outcomes Study demonstrated that patients undergoing reconstruction of any type, even without radiation, had a notable risk of 31.6% of a major complication such as hospitalization and/or reoperation within two years of surgery. Furthermore, this study demonstrated a higher rate of overall complications when reconstruction was performed in conjunction with PMRT.<sup>2</sup>

## Patient Factors to Consider During Pre-Operative Planning

Need for PMRT is just one of the many considerations that play a role in selecting the appropriate type and timing of breast reconstruction.

This is a complex, collaborative discussion that must be made among the patient, plastic surgeon, and oncologists (radiation, medical, and surgical). Patient goals of reconstruction, prior abdominal wall/breast surgical history, anatomy including adequate donor/recipient vessels, and comorbidities (including tobacco use) are additional key patient factors to consider during the pre-operative planning period. Comorbidities that affect a patient's cardiac, pulmonary, and/or renal systems and physiologic ability to tolerate a longer, more complex autologous procedure are also important to recognize. All of these consideration and patient-specific factors should be discussed on an individualized basis to optimize reconstructive outcomes.

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#### Breast Reconstruction Timing

Breast reconstruction can be performed at the time of mastectomy (immediate reconstruction), after mastectomy (delayed reconstruction), or with immediate placement of a tissue expander followed by definitive reconstruction at a later date (delayedimmediate reconstruction) (Table 1). Compared to delayed reconstruction, immediate breast reconstruction is associated with improved aesthetic results, enhanced ability to utilize the native mastectomy tissue envelope, and decreased overall cost.<sup>3</sup> By reducing the number of required surgeries, the patient not only benefits from cost savings but also from the immediate psychological benefits associated with reconstruction which may decrease the number of patients resistant to undergo total mastectomy. Large, multicenter prospective studies have found no significant difference in patient reported outcomes in immediate versus delayed breast reconstruction at two-year follow up.<sup>3,4</sup>

Over 70% of breast reconstructions in the USA are performed at the time of mastectomy in the immediate or delayed-immediate fashion.<sup>5</sup> Despite this trend, there is a growing volume of literature that advocates for delayed reconstruction in certain patient groups receiving PMRT, as it is associated with fewer postoperative complications.<sup>6-12</sup> The Mastectomy Reconstruction Outcomes Consortium (MROC) compared immediate to delayed reconstruction and found that immediate or delayed-immediate reconstruction resulted in significantly higher failure rates compared to delayed reconstruction (6% vs. 1.3%). Furthermore, there were no significant differences in patient-reported outcomes between immediate and delayed reconstruction cohorts.<sup>6</sup>

As the controversy surrounding reconstructive timing deepens, high quality evidence remains limited. Currently there are no randomized trials evaluating the impact of PMRT on reconstruction timing, complications, or cosmetic outcomes. Additionally, potential selection bias may exist as patients and/or surgeons can have strong personal preferences for either immediate, delayed, or delayed immediate reconstruction.(Figure 1)

#### Table 1. Advantages and disadvantages of variable timing of reconstruction.

#### Timing of Reconstruction

Immediate: Reconstruction at time of mastectomy

Advantages:

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- -Obtaining a symmetric reconstruction is more obtainable with immediate reconstruction vs delayed
- -Decreased overall cost via reduction in number of procedures
- -No difference in rates of locoregional recurrence or ability to detect/diagnose recurrence
- -Patients can immediately experience the psychological benefits of reconstruction and have been found to have superior post-operative QOL as compared to those waiting for delayed reconstruction after their mastectomy.
- Disadvantages:
- -Higher failure rate compared to delayed
- -Greater length of hospital-stay compared to mastectomy alone

Delayed: No reconstruction at time of mastectomy with reconstruction at later date

Advantages:

-Fewer post-operative complications in patients receiving PMRT

-Provides patient time to consider reconstructive options

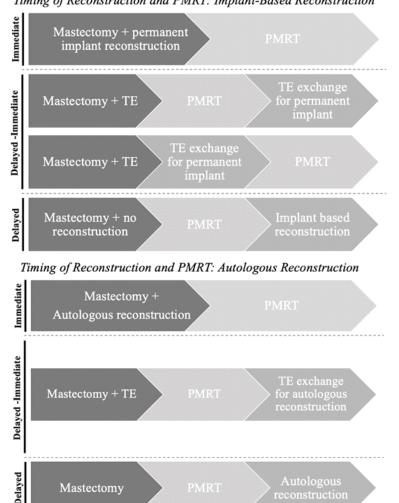
Disadvantages:

-May augment psychosocial harm or decrease patient quality of life following mastectomy

- -Increased number of surgeries required
- -Fibrotic tissue may be difficult to operate on

Delayed-Immediate: Tissue expander placed at mastectomy, definitive reconstruction at later date Advantages:

- -Avoids radiation of definitive reconstruction
- -Provides patient time to consider reconstructive options
- -Can increase size of overall reconstruction
- Disadvantages:
- -Propensity for surgical complications
- -Increased number of surgeries required
- -Fibrotic tissue may be difficult to operate on



Timing of Reconstruction and PMRT: Implant-Based Reconstruction

Figure 1. Timing of implant based reconstruction and autologous reconstruction as it relates to post mastectomy radiation.

#### *The Effect of Breast Reconstruction on Radiation Therapy*

In addition to the shared concerns of many plastic surgeons regarding the outcomes of reconstruction as they relate to the timing of PMRT, there is also the possibility that reconstruction can negatively impact the timing and quality of PMRT. Kahila *et al.* demonstrated that patients who underwent PMRT after immediate reconstruction had no compromise in the quality of PMRT administered.<sup>13</sup> Furthermore, there was no significant difference in target volume coverage or mean lung/heart dosages between reconstructed and non-reconstructed patients.

An additional concern shared by plastic surgeons and radiation oncologists is the risk of post-operative complications and their ability to delay PMRT. Shammas et al. performed a review of the National Cancer Database to identify women undergoing mastectomy with immediate reconstruction and time to PMRT.<sup>14</sup> A delay in radiation therapy was defined as PMRT initiation 12 weeks or more after mastectomy. Patients who underwent immediate breast reconstruction had an increased time to initiating radiation therapy (154 days versus 132 days, p < 0.001), and were more likely to have a delay in initiating PMRT (OR: 1.25). However, despite this delay, there was no significant reduction in survival supporting the collective assertion that immediate reconstruction is well tolerated and does not negatively influence overall survival, even in cases when PMRT is delayed.

# *Types of Breast Reconstruction: Implant Based Reconstruction*

Currently, implant-based reconstruction (IBR)is the most common form of breast reconstruction in the United States, accounting for 82% of all reconstructions.<sup>5</sup> Implants can be placed in the prepectoral plane or the subpectoral plane, either at the time of mastectomy or in a delayed fashion after tissue expander placement. Immediate, direct IBR rates at the time of mastectomy have increased by over 200% in the past 10 years.<sup>15</sup> The decision for single or two-stage IBR depends on a variety of factors including viability of mastectomy flaps, final implant size, potential for PMRT, and surgeon preference. Patients who receive PMRT with IBR have increased rates of corrective surgery and overall poorer aesthetic outcomes when compared to autologous reconstruction.6 Up to 47.5% of immediate IBR patients may require revision after undergoing radiation therapy.<sup>16</sup>

# Impact of Radiation on the Location of Implant Placement

Subpectoral implants have been historically favored in the setting of PMRT as it was thought that the overlying pectoralis muscle protects the implant from mastectomy flap loss, superior pole implant rippling, and capsular contracture. However, this approach can be associated with animation deformities of the implant, increased pain, and muscle spasms.<sup>17,18</sup>

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Pre-pectoral IBR has been shown to result in less pain, improved aesthetic outcomes by mitigating animation deformities, and shorter operative time.<sup>19</sup> However, concerns regarding the safety of PMRT in the setting of pre-pectoral implant placement exist. These include the potential for capsular contracture and the radiation oncologist's ability to properly provide radiation to the chest wall. As pre-pectoral IBR increases in popularity, preliminary studies have demonstrated favorable capsular contracture rates which are comparable to subpectoral IBR.<sup>20,21</sup> Future prospective studies with larger sample sizes and longer follow-up periods are needed to better address the optimal plane for implant placement.

#### Impact of PMRT on Tissue Expander vs Permanent Implant in Two-Stage Reconstruction

In the United States, two-stage IBR is the most common method for IBR constituting 67% of breast reconstructions in 2019.<sup>5</sup> For patients who will require PMRT, a common concern shared by plastic surgeons surrounds the timing of PMRT in relation to the final implant placement. Accordingly, there is no clear consensus indicating whether superior outcomes are obtained by applying radiation to the tissue expander or the permanent implant.

Cordeiro *et al.* demonstrated the acceptability of radiating the permanent implant with regard to aesthetic outcomes and patient satisfaction and was among the first studies to describe standardized radiation timing after immediate two-stage implant reconstruction.<sup>22</sup> Expansion began 10-14 days after placement with exchange to permanent implant four weeks after the completion of chemotherapy. Radiation began one month after implant placement. Sixty-eight percent of radiated patients developed significant capsular contracture compared to 40% of non-radiated patients. Sixty-seven percent of the radiated patients were satisfied with their reconstructions compared to 88% of the non-radiated patients. Ten years later, the same group analyzed 2,133 two-stage implant reconstructions. Grade IV capsular contracture was present in 6.9% of radiated and .5% of non-radiated implants.<sup>23</sup>

In contrast, some surgeons prefer to radiate the tissue expander and later replace it with a permanent implant, typically 3-6 months after the completion of PMRT. This process has several theoretical advantages: it allows the surgeon to inflate or deflate the device in the event of potential compromise of the mastectomy skin flap and it allows the surgeon to perform capsulectomies after the deleterious effects of radiation are apparent. Cordeiro *et al.* analyzed surgical and patient-reported outcomes from 94 and 210 women who had PMRT to their tissue expander

and implant, respectively. These experimental cohorts were compared to a control group of 1,486 women who had two-stage implant reconstruction without PMRT.<sup>24</sup> Both cohorts had tissue expanders placed at the time of mastectomy and expansion was started within 10-14 days and completed by 6 weeks. Tissue expanders were exchanged approximately six months after completion of PMRT. The authors demonstrated higher odds of reconstructive failure when PMRT was applied to the tissue expander (32%) vs. 16.4%). Furthermore, Grade III and IV capsular contracture was significantly lower in the tissue expander group than the implant radiation group. Aesthetic outcomes were significantly poorer in radiated patients; however, there was no significant difference in aesthetic outcomes whether PMRT was administered to the expander or implant. While patients who did not receive PMRT were significantly more satisfied with their reconstruction, there was no difference in patient satisfaction scores between the tissue expander and implant groups.

Overall, these seminal studies demonstrate that PMRT results in increased complications, regardless of whether the implant or tissue expander was radiated. Failure rates tend to be significantly higher when PMRT is applied to the tissue expander in twostage reconstruction. However, when PMRT is applied to the tissue expander, this may result in less capsular contracture, likely due to the ability to perform capsulectomies and/or pocket adjustments at the time of the implant exchange procedure.

## *Types of Breast Reconstruction: Autologous Reconstruction*

Autologous reconstruction describes the process of reconstructing the breast using the patient's own tissue, thereby obviating the need for implants. In 2019, autologous reconstruction represented approximately 14% of breast reconstruction procedures performed.<sup>5</sup> In the United States, the Deep Inferior Epigastric Flap (DIEP) from the abdomen comprises 54% of all autologous reconstructions. The type of autologous reconstruction is limited by the patient's body habitus, prior surgery, medical comorbidities, and patient preference. In general, the type and frequency of complications varies based on the type of flap utilized.

Autologous reconstruction has several distinct advantages when compared with implant-based reconstruction. In many patients, autologous reconstruction can overcome the need for the multiple surgeries required for implant replacement and capsular contracture correction by providing a life-long, durable reconstruction. Autologous reconstruction is associated with improved patient reported satisfaction, psychosocial well-being, and overall sexual well-being when compared with implant-based reconstruction.<sup>25</sup> Additionally, autologous reconstruction is associated with lower odds of surgical site infection and reconstructive failure in patients who undergo PMRT.<sup>26,27</sup> Despite these advantages, autologous reconstruction is a larger, longer operation with the potential for additional scarring and/or injury to the area of flap harvest.

Timing for autologous reconstruction in patients requiring PMRT has a similar algorithm to that of implant reconstruction. Reconstruction can be performed at the time of mastectomy followed by PMRT, in a delayed-immediate fashion with irradiation of the tissue expander, or in a delayed fashion and performed after PMRT completion. Immediate reconstruction with autologous tissue can be advantageous as it is possible to perform the entire reconstruction at the time of mastectomy. However, if PMRT is required, there is the possibility that the radiation may cause unpredictable flap necrosis, discoloration, contracture, displacement, volume loss, or other complications that require additional revision procedures and/or hospitalizations.<sup>28</sup> To illustrate this,



Immediate

Delayed-Immediate

Delayed

Figure 2. Differential skin paddle designs and the impact of timing on autologous reconstruction.

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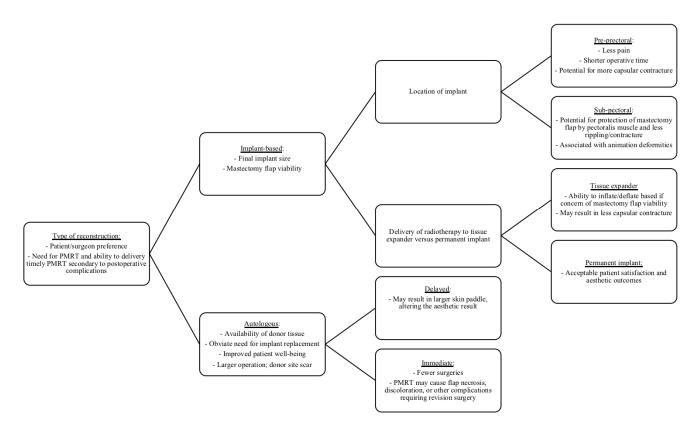


Figure 3. A summary of key findings for various considerations of available breast reconstructive methods.

Williams et al. compared the effects of radiation on immediate and delayed abdominal flaps and found that 31.6% of immediate flaps developed fibrosis with 0% in the delayed reconstruction group.<sup>29</sup> Fifty-two percent of immediate flaps experienced some deleterious post-radiation changes such as fat necrosis, volume loss, or contracture and 32% required revision surgery. These findings led the authors to recommend delaying autologous reconstruction until after radiation completion

The timing of reconstruction has the ability to change the final result of autologous reconstruction. If the mastectomy skin flap is able to be preserved as is the case in immediate reconstruction and delayed immediate reconstruction, the skin paddle of the flap can be tailored to be a small island that can later be used to create the nipple-areola complex. When performing a delayed reconstruction, the autologous flap is larger, as it must be used to reconstruct the entire breast mound. This difference in flap size results in different aesthetic results, although it has been shown that patients have high satisfaction ratings of aesthetic outcomes with both types of autologous reconstruction.<sup>30</sup> (Figure 2)

#### Discussion

Post-mastectomy radiation therapy is a necessary component of breast cancer care for an increasing number of patients as it has been shown to reduce rates of locoregional recurrence and improve overall survival.<sup>31,32,33</sup> Unfortunately, there are deleterious effects associated with PMRT that can negatively impact reconstructive outcomes. Currently, there is no high-level evidence that identifies an optimal treatment algorithm for combining PMRT and breast reconstruction and the vast majority of studies are retrospective, non-randomized, and include small patient samples. However, when many of these studies are analyzed, trends emerge. For example, in cases of autologous reconstruction, delaying definite reconstruction until after PMRT has been associated with better aesthetic outcomes and decreased need for revision surgery. With regard to implant reconstruction, decreased failure rates may sway providers towards providing radiation to the implant instead of the tissue expander. This review provides an overview of various reconstructive techniques and how they are impacted by PMRT. (Figure 3) With this information, oncologic care teams can help provide more comprehensive information to patients in order to optimize their satisfaction with their breast cancer treatment and reconstruction.

#### **Conflict of Interest**

Justin Broyles serves as a consultant for AHRQ and the medical advisory board for Healshape Inc.

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### Role of F-18 FDG PET/CT in Patients with Suspected Recurrent Breast Cancer: Additional Value over Conventional Imaging Modalities

Mehrdad Bakhshayeshkaram<sup>a, b</sup>, Farahnaz Aghahosseini<sup>a, b</sup>, Sara Alavinejad<sup>a, b</sup>, Yalda Salehi\*<sup>c</sup>, Sharareh Seifi<sup>a, b</sup>, Maryam Hassanzad<sup>a, b</sup>

<sup>a</sup> Department of Radiology, Shahid Beheshti University of Medical Sciences, Tehran, Iran

<sup>b</sup> Pediatric Respiratory Diseases Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Tehran, Iran

Lung Diseases (INRTILD), Tenran, Iran

<sup>°</sup> Department of Nuclear Medicine, Tehran University of Medical Sciences, Tehran, Iran

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#### ABSTRACT

**Background:** The aim of the present study was to investigate the added value of F-18 fludeoxyglucose (FDG) positron-emission tomography (PET)/computed tomography (CT) compared with conventional imaging modalities for the evaluation of locoregional and distant sites of recurrence in breast cancer patients.

Archives Of

**Methods:** From May 2013 to September 2016, 109 patients with suspected recurrent breast cancer who underwent conventional imaging and F-18 FDG PET/CT with an interval of 6 weeks were consecutively enrolled (mean age: 52.66 years; range: 29-79). Histopathologic results and clinical follow up based on the gold-standard imaging modality or serial imaging were considered as the reference for verification of F-18 FDG PET/CT findings.

**Results:** Of 109 patients, 81 were found to have at least one site of recurrence (74.31%). Local recurrence was correctly identified in 32/32 patients following PET/CT, which was higher than that on conventional imaging (20/32, 62.5%). PET/CT detected 27 additional nodal metastases compared with conventional imaging (59 vs. 32, 45.76%), most frequently in the hilar/mediastinal region (n=27), followed by the supraclavicular lymph nodes (n=20, 62.5%), internal mammary lymph nodes (n=6, 18.77%), and axillary basin (n=6, 18.77%). Additional sites of distant metastasis were identified in 41 patients (37.61%) following F-18 FDG PET/CT imaging, 48.78% of which were localized in the skeletal system (n=20), 21.95% in the liver (n=9), 12.19% in the lungs (n=5), 12.19% in the brain (n=5), and 4.87% in the adrenal glands (n=2).

**Conclusion:** F-18 FDG PET/CT serves as a useful supplement to conventional imaging techniques by identifying additional sites of disease recurrence in patients with breast cancer, which may change the preferred treatment strategy, particularly in regions that are not routinely evaluated by conventional imaging.

Introduction

F-18 FDG PET/CT,

Key words:

recurrence,

breast cancer, conventional imaging,

Breast cancer recurrence occurs in 10-75% of patients depending on the extent of disease at the time

\* Address for correspondence: Yalda Salehi, MD Department of Nuclear Medicine, Imam Khomeini Hospital, Tehran University of Medical Sciences, Tehran, Iran. Tel: +98 21 6119-2403 Fax: +98 21 6694-5118 Email: <u>salehi\_y@sina.tums.ac.ir</u> of initial diagnosis.<sup>1</sup> Treatment approaches can be stratified based on the location of disease recurrence (locoregional vs. distant) and extension (oligofocal vs. multifocal metastasis). Locoregional recurrence in the primary site, chest wall, axillary basin, internal mammary lymph nodes, and supraclavicular lymph nodes comprises up to 20% of all recurrences<sup>2</sup> and may be treated with curative therapy.<sup>3</sup> The vast majority of disease relapse, however, occurs more frequently at distant sites such as in the skeletal

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system (20-60%), lungs (15-20%), liver (5-15%), and brain (5-10%).<sup>4</sup> Patients with disease relapse have a poor prognosis and generally undergo palliative treatment.<sup>3</sup>

According to current guidelines, patients suspected for breast cancer recurrence should undergo clinical examination and multimodality imaging, including X-ray mammography, breast and axillary ultrasound, liver ultrasound, chest X-ray, and bone scintigraphy.<sup>5, 6</sup> However, there are some major limitations regarding their diagnostic accuracy; postsurgical structural changes, limited field of view and interobserver variability may negatively influence the diagnostic accuracy of X-ray mammography and breast ultrasound for the early detection of local recurrences.<sup>5, 7</sup> Small malignant supraclavicular and mediastinal lymph nodes may be missed by ultrasound or computed tomography (CT), based only on morphologic criteria. Bone scintigraphy has limited sensitivity for the detection of osteolytic metastasis and may potentially underestimate the extent of skeletal disease.<sup>8, 9</sup> In addition, unifocal or oligofocal lesions suspected as bone metastases detected by bone scan must be verified by correlative imaging due to limited specificity.9,10

F-18 fludeoxyglucose (FDG) positron-emission tomography (PET)/CT has recently gained widespread acceptance in oncologic imaging as a highly-sensitive, whole body imaging modality. PET/CT can detect malignant lesions before any morphological changes develop by identifying accelerated metabolism in cancerous cells; however, due to high cost and limited resolution in small lesions, the benefits over conventional imaging must be verified in individual clinical settings.

On the basis of current recommendations, PET/CT imaging has limited application in patients with suspected recurrent breast cancer.<sup>11</sup> However, a growing body of evidence suggests a complementary role for F-18 FDG PET/CT in such patients as well as the potential for F-18 FDG PET/CT to replace conventional imaging modalities based on its high sensitivity and large field of view.<sup>12-15</sup> Thus, the aim of the present study was to investigate the potential added value of F-18 FDG PET/CT over conventional imaging modalities in detecting breast cancer recurrence.

#### Methods

The Review Board of Shahid Beheshti University of Medical Sciences approved this retrospective study and waived the need for informed consent.

#### Patients

This retrospective study included 443 patients with suspected recurrent breast cancer who were referred to the PET/CT division of Masih Daneshvari Hospital between May 2013 and September 2016. Results were available for 109 patients upon final verification. Breast cancer recurrence was suspected for the following reasons: increased tumor markers (n=41, 37.61%); equivocal findings on conventional imaging (n=15, 13.76%); biopsy-proven local recurrence (n=20, 18.35%); disease extension in known cases of distant metastasis (n=33, 30.27%).

#### Conventional imaging

Conventional diagnostic work up was performed as follows: chest CT, abdominopelvic CT, bone scintigraphy, and liver ultrasound (n=32); chest CT, liver ultrasound, and bone scintigraphy (n=21); bone scintigraphy, chest X-ray, and liver ultrasound (n=43); bone scintigraphy and chest CT scan (n=13). Breast and axillary ultrasound investigations were performed in all 109 patients.

#### F-18 FDG PET/CT acquisition protocol

An integrated PET/CT device (GE 690 Discovery, 64 Slice, Time of Flight) was used. The fasting period was maintained for at least 8 hours. The level of blood glucose at the time of radiotracer injection was <150 mg/dL. Sixty minutes ( $\pm 10\%$ ) after the intravenous (IV) administration of 4.6 MBq/kg F-18 FDG (0.12 mCi/kg), craniocaudal CT acquisition was initiated from the vertex to the mid-thigh (or to the toe as indicated) in the supine position. A multidetector CT scanner was used at 50-120 auto mAs with a tube current of 120 kV, a noise factor of 19, and 2.5 mm thickness under tidal breathing. Thirty minutes before imaging acquisition, 40 cc of 76% meglumine (containing 370 mg iodine/cc) in 1500 cc water was administered as an oral contrast solution. The PET data were collected in the reverse direction for 3 minutes per bed position immediately after CT acquisition. Corrections were made to the raw data in terms of attenuation, dead time, random and scatter coincidence. Images were subsequently reconstructed using an iterative method and high-definition (HD) technique.

#### Diagnostic criteria

The PET (attenuation corrected [AC] and non-AC), CT and fused PET/CT images of the eligible cohort were retrieved and reviewed using a workstation (Advantage Window, 4.5, Volumeshare software, GE 690) by a team consisting of an experienced radiologist and a nuclear physician, who reached a consensus regarding disease status.

#### Metabolic criteria

The criteria for malignancy were defined as follows: 1) foci of abnormal increased F-18 FDG uptake markedly greater than liver activity for local recurrence in the chest wall, lymph nodes, adrenal and skeletal system, and more than the surrounding background activity in the lungs and brain, with or without corresponding CT abnormalities; 2) multiple foci of increased F-18 FDG uptake randomly



Variables		
Age		
	Mean	52.6
	range	29-79
Gender		
	Male	3
	Female	106
Histopathologic subtype		
	Invasive Ductal Carcinoma	98 (89.9%)
	Invasive Lobular Carcinoma	11 patients (10.09%)
Reason for recurrence		I man ( man)
	Tumor marker rise	(n=41, 37.61%);
	equivocal findings on conventional imaging	(n=15, 13.76%);
	biopsy-proven local recurrence	(n=20, 18.35%);
	disease extension in known cases of distant metastasis	(n=33, 30.27%).
Baseline Conventional Imaging Work up		
	Breast and axillary ultrasound	N= 109 patients
	Chest/abdominopelvic CT plus bone scintigraphy plus liver ultrasound	(n=32);
	chest CT plus liver ultrasound plus bone scintigraphy	(n=21);
	bone scintigraphy plus chest X-ray plus liver ultrasound	(n=43);
	bone scintigraphy plus chest CT scan	(n=13).

distributed in distant organs with or without corresponding CT abnormalities; 3) highly suspicious morphological abnormalities with or without discernible associated metabolic activity.

#### Standard of reference

All of the included lesions were verified as benign or malignant according to their histopathology (n=32, 29.36%) or clinical follow up, using the goldstandard imaging modality or serial imaging (n=77, 70.64%).

#### Results

#### Patients' Characteristics

Of 109 cases, 106 were women and 3 were men. The mean age of the study cohort was 52.66 years (range: 29-79). In total, 98 patients (89.9%) had invasive ductal carcinoma and 11 patients (10.09%) had invasive lobular carcinoma (Table 1).

#### Lesion detection

Local recurrences confirmed by biopsy were identified in 20 patients, located within the region of the previous surgery (n=8), axillary basin (n=3) or both (n=9) (Table 2).

#### Conventional imaging

Conventional imaging correctly identified 12

nodal metastases in the supraclavicular lymph nodes, 3 in the internal mammary lymph nodes, and 17 in the hilar/mediastinal stations. Distant metastasis was detected in 40 patients with the following distribution pattern: skeletal system only (n=15); lung and bone (n=12); lung only (n=5); bone and liver (n=6); bone, liver and lung (n=2). The most common site of distant metastasis was the skeletal system (n=35), followed by the lungs (n=28) and liver (n=11).

#### F-18 FDG PET/CT

F-18 FDG PET/CT correctly identified 12 additional sites of local recurrence within the region of the previous surgery, which were verified as malignant (n=32). Additional locoregional nodal metastasis was detected in the supraclavicular lymph nodes (n=20), axillary basin (n=6), internal mammary lymph nodes (n=6) and hilar/mediastinal lymph nodes (n=27) with an overall additional detection rate of 45.76% (n=59).

Distant metastasis was detected in 81 patients with the following distribution pattern: skeletal system (n=26); lung, liver and bone (n=11); lung only (n=10); lung and bone (n=9); abdominal cavity (including liver, adrenal glands, lymph nodes, and peritoneum) (n=8). Additional sites of distant metastasis (n=41) were identified mostly in bone

Table 2. Local Recurrence: Comparison between Conventional work up and PET/CT

Local Recurrence	Conventional Imaging	PET/CT
Detection Rate	32	20
Location		
Site of previous sur	gery 12	8
Axillary Basin	6	3
Both	14	9

Distant N	Aetastasis Location		Conventional Imaging	PET/CT
Lymph node			32	53
		Supraclavicular	12	20
		Internal mammary	3	6
		Hilar/mediastinal	17	27
Organ Distribution			40	
	Distribution	Bone (only)	15	26
		Lung+ bone	12	9
		Lung (only)	5	10
		Bone + liver	6	18
		Bone + liver + lung	2	11
		Brain		5
		Adrenal		1
		Peritoneum		1

Table 3. Distant Metastasis: Comparison between Conventional work up and PET/CT

(n=20, 48.78%), liver (n=9, 21.95%), lungs (n=5, 12.19%), brain (n=5, 12.19%), adrenal glands (n=1, 2.43%), and the peritoneum (n=1, 2.43%).

Previously unidentified brain metastases were detected in 5 patients; brain and lung metastases (n=2); brain and bone metastases (n=2); brain and liver metastases (n=1) (Table 3).

#### Discussion

The present study revealed that F-18 FDG PET/CT is superior to conventional imaging techniques for the detection of locoregional and distant metastases in patients with suspected breast cancer recurrence and may play a major complementary role in the accurate assessment of disease extension, which is considered the main prerequisite for treatment decision making.

Locally advanced breast cancer may be treated with curative therapy. One study showed that PET/CT successfully detected 21 additional intrathoracic and 5 additional cervical lymph node metastases in patients suspected to have breast cancer recurrence.<sup>12</sup> Another study revealed that the mediastinal, supraclavicular, axillary and internal mammary lymph nodes were frequent sites for tumor recurrence, as detected by PET.<sup>13</sup> In line with the literature, the present study revealed that F-18 FDG PET/CT correctly identified 45.76% additional sites of regional nodal involvement, mainly in the extraaxillary basin. F-18 FDG PET/CT imaging can identify accelerated metabolic changes in small lymph nodes before the development of morphological changes, and thus may play an important role in the early detection of malignant lymph nodes.

Several studies have compared the diagnostic accuracy of PET/CT imaging with bone scintigraphy, as the imaging modality of choice for the detection of skeletal metastases. PET/CT demonstrated a significantly higher accuracy for the detection of both osteolytic and osteoblastic skeletal metastases.<sup>14,15</sup> The results of our study demonstrated that the most frequent site of additional distant metastasis detected by PET/CT was the skeletal system with a high level of diagnostic confidence and no subsequent need for further verification.

Most hepatic metastases, from different sites of primary origin, have been shown to be highly amenable to F-18 FDG detection. Several studies have shown that F-18 FDG PET/CT may serve as the most sensitive imaging modality for the identification of liver metastases and may have a significant impact on stratification for surgical resection.<sup>16-18</sup> The results of the current study revealed that in patients with suspected breast cancer recurrence, F-18 FDG PET/CT identified 6 additional hepatic metastases, 50% of which were not identified by conventional imaging.

Brain metastasis occurs in 10-16% of patients with breast cancer.<sup>19</sup> The incidence of brain metastasis in patients with breast cancer is increasing due to higher detection rates and improved survival. Brain metastases have a significant negative impact on patients' quality of life and prognosis. Brain magnetic resonance imaging (MRI) is considered the imaging modality of choice to detect brain metastases; however, routine screening of brain metastases is not recommended in asymptomatic patients. Due to a high level of F-18 FDG uptake in normal cerebral parenchyma, F-18 PET/CT has limited sensitivity for the detection of brain metastases.<sup>20</sup> Consequently, most PET/CT centers define the standard field of view for PET/CT acquisition from the skull base to the mid-thigh. One study demonstrated that F-18 FDG PET/CT imaging of the brain may help to stratify lung cancer patients prior to further evaluation of cerebral metastases by MRI.<sup>21</sup> The present study revealed that in patients with suspected breast cancer recurrence, PET/CT correctly identified previously unidentified cerebral metastases in 12% of patients.

There are some major limitations to the present study. The clinical impact of the detection of additional lesions on the optimal treatment strategy and subsequent survival is an important issue that was not evaluated in this study and should be addressed in the future. In addition, the significance of F-18 FDG PET/CT in terms of decreased cost and time intervals from the beginning of diagnostic work up until the initiation of treatment was not evaluated. Furthermore, clinical follow up was not performed in most patients; therefore, a considerable number of potentially eligible patients who underwent neither biopsy nor gold-standard imaging did not undergo further investigation.

In conclusion, F-18 FDG PET/CT serves as a useful supplementary technique to conventional imaging that can detect additional sites of disease recurrence in the supraclavicular lymph nodes and axillary basin in addition to the skeletal system, liver, and brain, which may have a significant impact on the treatment strategy. Large-scale prospective studies are required to investigate the influence of these additional lesions on the optimal treatment strategy and overall survival.

# **Conflict of Interest** None.

None.

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# A New Imaging Workup of The Male Breast: Single-View Mammography and Ultrasonography

Gulten Sezgin\*<sup>a</sup>, Yeliz Yilmaz<sup>b</sup>, Melda Apaydin<sup>a</sup>, Makbule Varer<sup>a</sup>, Seyran Yigit<sup>c</sup>

ABSTRACT

<sup>a</sup> Department of Radiology, Izmir Katip Celebi University Ataturk Training and Research Hospital, Izmir, Turkey

<sup>b</sup> Department of General Surgery, Izmir Katip Celebi University Ataturk Training and Research Hospital, Izmir, Turkey

<sup>°</sup> Department of Pathology, Izmir Katip Celebi University Ataturk Training and Research Hospital, Izmir, Turkey

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#### Introduction

The male breast is anatomically rudimentary and non-functional, but it may involve many benign and malignant pathologies. Most breast diseases in males are benign and the most common is gynecomastia.<sup>1</sup> Male breast cancer (MBC) is a rare disease that

Gulten SEZGIN, MD,

Address: Izmir Katip Celebi University Ataturk Training and Research Hospital, Department of Radiology, 35360, Turkey Tel: +90 232 244 44 44/ 2603 Email: <u>sezgingulten@gmail.com</u>

**Background:** There is no generally accepted standard radiological approach to male breast diseases. Ultrasonography and mammography are the most preferred methods for imaging. The purpose of this study is to investigate the diagnostic performances of only single-view (mediolateral oblique) mammography and routine two-view mammography in the male breast diseases and also to evaluate the contribution of ultrasonography to the diagnosis and the management of the disease.

**Methods:** Three hundred-twenty male patients' breast imaging findings were evaluated retrospectively. Only mediolateral oblique and routine two-view mammograms were re-evaluated four months apart by two breast radiologists. The kappa value was calculated to measure consistency between two groups. The sensitivity, specificity, positive and negative predictive value, and detection accuracy of malignancy were calculated for both mammographic examinations and ultrasonography.

**Results:** The accuracy of detection of malignant breast masses according to mediolateral oblique and two-view mammograms, and ultrasonography were 96.3 %, 97.5 %, and 99.5 %, respectively. Consistency between single-view and two-view mammograms was excellent ( $\kappa = 0.967$ ) and statistically significant (p=0.000). Two-view mammograms did not make any contribution to single-view mammograms.

**Conclusion:** Only mediolateral oblique mammogram is sufficient in terms of characterization, spread and localization of the lesion, as long as mastectomy is preferred and the findings are supported by ultrasonography. Since there is no diagnostic performance difference, only mediolateral oblique view would protect from the potential adverse effects of extra radiation exposure and this is an important advantage.

accounts for less than 1% of all breast cancer cases and about 0.1% of breast cancer-related mortality and typically presents with associated symptoms.<sup>2</sup> With the increasing use of radiological methods, the diagnosis of breast cancer and benign conditions has increased in men.<sup>3</sup> But there is no generally accepted standard radiological approach to breast diseases in the males.<sup>4</sup> If no clinical suspicious finding is present in men, no imaging is usually recommended.

However, if the differentiation between benign or malignant pathologies cannot be made with the clinical findings, imaging is indicated. Ultrasonography (US)

<sup>\*</sup> Address for correspondence:

and mammography (MM) are the most preferred methods for the imaging of the male breast.<sup>5</sup> Rarely, magnetic resonance imaging can be used to evaluate tumor extension such as pectoral muscle invasion.<sup>6</sup>

Although National Comprehensive Cancer Network has advised the use of MM as the initial imaging technique for men at and over the age of 25 who have indeterminate breast mass, some authorities state that US may be used as the first diagnostic tool of choice in palpable male breast abnormalities.<sup>7</sup> The utility of MM in male patients with breast symptoms is not clear and MM adds little diagnostic contribution to the clinical evaluation.<sup>8, 9</sup> In a study published recently, diagnostic performance of unilateral and bilateral MM was compared.<sup>10</sup> In this study it was reported that imaging only the symptomatic breast would be adequate and doing this would provide less radiation exposure.

We investigate the diagnostic performances of

only single-view MM and routine two-view MM in men and evaluate the contribution of US in the diagnosis of the disease and patient management. To the best of our knowledge to date, such a study for male breast patients has not been published yet.

#### Methods

The retrospective study was approved by the institutional ethics committee of our hospital (09.01.2020/532). Since the study was retrospective, informed consent by patients was not required. Between January 2013 and March 2018, 320 male patients presenting to our breast imaging center were reviewed. Symptomatic patients were included in the study. The patients excluded from the study are summarized in the Table 1. A total of 218 patients were included in this study (Figure 1).

Sonographic assessment and US- guided

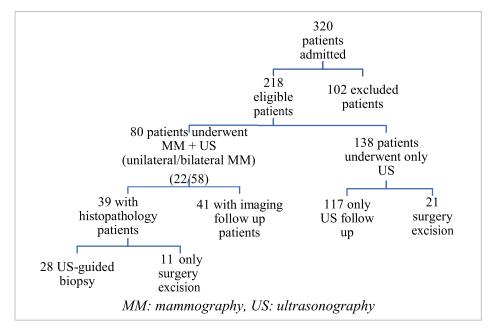


Figure 1. Flow chart of patient selection

Table 1. The patients excluded from the study.

Patients	Ν
Reactive axillary lymph nodes	4
Metastasis to axillary lymph nodes from extramammary malignancy	3
Desmoid tumor	2
Survelliance of the recurring patients	22
Who did not undergone a biopsy or a surgery operation	34
Absence of US- follow-up for at least 24 months	37
Total	102

#### biopsies

First, physical and US examination was done on male patients regardless of age in our department. Gray scale US examinations of all patients were performed by the radiologist with ten years of experience using a 13 MHz superficial probe (Hitachi Ezu-MT28-S1 model, Hitachi Inc. Japan). The reports were summarized according to the Breast Imaging Reporting and Data System (BI-RADS) lesion as 1, 2, 3, 4, and 5. The operator was not blinded to the clinical information. The US-guided biopsies were performed by the same radiologist using the full- automatic 16-gauge biopsyneedles (Bard Magnum, Covington, Georgia, USA).

Histopathological examination and immunohistochemical analyses were carried out by pathologists experienced in breast diseases. Patients without biopsy or surgical operation were followed-up with US for a duration of 24-33 months.

#### Mammographic assessment

Mammography was used when the patients were defined as BI-RADS 4, 5 by US and using physical examination finding of the clinician. Mammograms were obtained by a direct digital device in the mediolateral oblique (MLO) and craniocaudal (CC) positions for each breast (IMS Giotto, Italy). All patients had histopathology or had follow-up of more than 24 months.

Two readings were made four months apart by two dedicated breast radiologists (with 8 and 10 years of experience in breast imaging) working in consensus. In the first reading, only bilateral or unilateral breast single view MMs (MLO) were reviewed. In the second reading, bilateral or unilateral two-view MMs (MLO and CC) were reviewed. The radiologists were aware that the patient was symptomatic, but they were blinded to the other imaging results, clinical findings, and pathologic results. The findings were scored between 1-5 according to BI-RADS.

## Statistical Analyses

The specificity, positive predictive value (PPV), sensitivity and the negative predictive values (NPV), and accuracy were evaluated by binomial tests. We considered the BI-RADS categories 4 or 5 as malignant and 1, 2, 3 as benign for MM and US examinations. The pathological results of the biopsy and surgery specimen or undergoing at least 24 months of sonographic follow-up were accepted as the 'gold standard'<sup>3</sup>

We calculated the kappa value to measure the consistency between single-view and two-view MM groups. A p value lower than 0.05 was accepted as statistically significant.

# Results

The mean age of the patients was  $45\pm19$  years (range, 6-90 years). The symptoms were bilateral or unilateral enlargement in the breast (142, 65.1%), palpable mass (37, 17%), mastalgia (36, 16.5%), and nipple discharge and/or retraction (3, 1.4 %) in the patients.

Cancer detection rate was 11 % (24/218) in our study population. The primary breast cancer (n = 22) and metastasis to the breast from extra mammary

malignancy (lung adenocarcinoma and rectum mucinous adenocarcinoma) (n = 2) were detected. The rate of gynecomastia was 80.7 % (176 /218). Different types of gynecomastia according to sonographic patterns are presented in table 2. Other patients showed lipoma (n = 2), mastitis (n = 2), and lipomastia (n = 16). Primary breast cancer patients underwent MM and US while two metastatic patients were detected by only US examination.

Mean age of the malignant patients was  $60.9\pm11.4$  (range 39-86). Tumor size ranged between 12-45 mm (mean 24.1 mm, SD  $\pm$  10.2 mm) in the malignant lesions. Primary breast tumors were defined as invasive ductal carcinoma (IDC) (n=18, 81.8 %), invasive lobular carcinoma (ILC) (n=1, 4.5%), invasive papillary carcinoma (IPC) (n=1, 4.5%), and Paget's disease and concomitant IDC (n=1, 4.5%). There was no pure ductal carcinoma in situ. All malignant lesions were mass lesions; there was accompanying calcification in one, and no structural distortion was detected.

Twenty-eight patients (11.9 %) underwent USguided biopsy (n = 23) and fine needle biopsy (n = 5). Surgery operations were performed on all of the malignant patients and 32 cases (13 cases underwent MM and 19 cases underwent only US) of gynecomastia (18.2 %, 32/176).

Modified radical mastectomy and axillary dissection were performed on nine malignant diagnosed patients (9/22, 40.9%), and simple mastectomy and sentinel lymph node biopsy were performed for 13 patients (13/22, 59.1%), two of whom received this after neoadjuvant chemotherapy. Six patients (6/22, 27.3 %) received radiotherapy after simple mastectomy. Local excision was applied in a case of rectum mucinous adenocarcinoma metastasis. In another metastatic patient, the lesion was not excised; systemic lung adenocarcinoma treatment was continued.

The sensitivity, specificity, PPV, NPV and accuracy of detection of malignant breast masses according to US are 100, 99.5, 95.8, 100, 99.5, respectively and the values according to MLO and two-view MMs are given in Table 3. The consistency between MLO-view and two-view MM is excellent ( $\kappa = 0.967$ ) and statistically significant (p=0.000). The core biopsy result of one false positive patient in US was gynecomastia. False positive and false negative patients on MMs are shown in Table 4. False negative results were reported in the both groups.

**Table 2.** The distribution of the sonographic patterns of gynecomastia and the laterality of involvement (n=176).

	Nodular N=38	Dendritic N=61	Diffuse N=77
Unilateral N=81	10.1 %	14.2 %	22.7 %
Bilateral N=95	11.5 %	20.4 %	21.1 %

	Single-view (MLO)	Two- views (MLO + CC)	Kappa value	P value
Sensitivity	90.9	90.9	0.967	0.000
Specifity	98.2	100		01000
Positive predictive value Negative predictive value Accuracy	95.2 96.6 96.3	100 96.6 97.5		

Table 3. Diagnostic performance of single-view and two-views mammography for male patients

MLO: Mediolateral oblique, CC: craniocaudal

 Table 4. Discordant assessments in single-view and two-view mammography

Single-view MM	Two-views MM	Diagnosis
BI-RADS 4	BI-RADS 3	Gynecomastia
BI-RADS 3	BI-RADS 3	Invasive cancer
BI-RADS 2	BI-RADS 2	Invasive cancer

BI-RADS: Breast Imaging Reporting and Data System, MM: Mammography

# Discussion

A standard diagnostic algorithm is not available yet for MBC because of male breast anatomy and MBC cancer rarity. Mastectomy is the main treatment. In this study, we showed that the diagnostic value of single (MLO) and two-view MM is high in men with breast symptoms and the compatibility between them is excellent, with no additional significant data obtained by two-view MM compared to single-view MM. At the same time, US, which we applied in all male patients regardless of age, made important contributions to the diagnosis and management of the disease.

Unlike female patients, healthy male breast has a predominantly fatty tissue with few ducts and stroma.<sup>10</sup> Therefore, mammographic sensitivity for cancer is excellent in men<sup>3</sup>. In a study of male patients comparing unilateral and bilateral MM, it was found that there was no difference in diagnostic value and no pathology was detected in the asymptomatic breast, except benign conditions such as gynecomastia.<sup>10</sup> It has been reported that radiation exposure would also be reduced as a result of evaluating only the symptomatic breast by MM.

As is known, there are potential side effects of ionizing radiation, and also radiation has stochastic (dose-independent) effects, so every dose taken matters.<sup>11, 12</sup> The right and left breasts are compared when interpreting MM, and asymmetries can be important for abnormality. In our study, bilateral MLO shots are recommended, which are obtained by reduced radiation allowing the visualization of both breasts. It is known that MM demonstrates the most breast tissue in the MLO position and one of the reasons for obtaining mammograms in two positions is to determine the localization of the lesion.<sup>10,13</sup>

Calcification and structural distortion are mostly associated with breast cancer in women, and are very well demonstrated by MM<sup>4</sup>. However, when the case

groups and articles including men were reviewed, we found a few cases of MBC demonstrating structural distortion.<sup>4, 14, 15</sup> In addition, calcification has been reported to be uncommon in MBC.<sup>15-17</sup> All of the cases were symptomatic in these studies. Calcification is rarely asymptomatic in MBC, usually accompanied by clinically and sonographically detectable lesions and the patients are high-risk.<sup>3, 18, 19</sup> Mammographic screening of 271 high-risk asymptomatic men revealed only three cancers (1.1%) in which the only finding was calcification.<sup>3</sup> In our study, there was no structural distortion and there was one finding of calcification.

The sensitivity, specificity, PPV, and NPV values of MM were reported at 92-100%, 90-95%, 32-55%, and 99-100% in various studies.<sup>3,20,21</sup> Our NPV values were also higher for single and two-view MM groups compared to these studies. Caruso has reported the NPV similar to our study, but by using the combination of US and MM.<sup>21</sup>

According to Chen, there is no statistically significant difference between the sensitivity and specificity of MM and US in the diagnosis of male breast diseases and US does not detect any malignancy in mammographically-negative cases.<sup>4</sup> The opposite of this was observed in our study; we did not have a case that could not be detected by US but detected by MM. Our study did not aim to compare the diagnostic performance of US and MM.

Ultrasound is advantageous as it can demonstrate axillary lymphadenopathy and pectoralis muscle involvement, it guides biopsies, it is easy to apply, and no radiation exposure is present. Also, no masking takes place on US examination. However, in MM, when the malignant mass and gynecomastia coexist in the same breast, the mass may not be diagnosed efficiently.<sup>16</sup> In single and two-view MM groups, in a case of false negative, dendritic gynecomastia masked the millimetric malignant lesion (Figure 1 a, b).

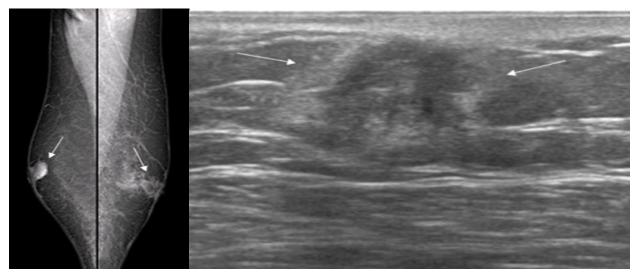


Figure 1 a, b: A 48- year-old male patient with invasive ductal carcinoma. Noduler retroareoler on the right side and flame shaped density on the left side (BI-RADS 2 –gynecomastia) on mediolateral oblique mammograms (arrows) (a). However, a hypoechoic lesion with irregular border (malignant) is observed in the ultrasound image in the left breast (arrows) (b).

Our cancer incidence (11 %) was significantly higher than that reported by some other studies.<sup>8, 22</sup> However, we included patients who were symptomatic and had long-term US follow-up, and those with biopsy or surgical results. Lawson et al. reported a high incidence of 10.1% in their study including patients with metastasis to axillary from the other malignancy.<sup>2</sup> We think that this result was affected by the fact that our hospital is a tertiary academic medical center to which patients are referred for further examination and treatment from surrounding provinces.

Male breast cancer is usually diagnosed at an advanced stage. Anatomical reasons play a role in this, namely small breast tissue, its close location to the nipple and, therefore, having early lymphatic and dermal spread.<sup>23</sup> Some authors have suggested that cases are diagnosed at a late stage when they are symptomatic due to the lack of a widely accepted screening program for men. Most of our cases (59.1%) had stage 2-3 disease at the time of the diagnosis, which is in accordance with the literature <sup>3</sup>. In our study, the rate of axillary metastases (40.9%) was higher than the rate reported in a study by Lawson (31%), but lower than the rate reported in the study by Gao (58.3%).<sup>2,3</sup>

As a result, mastectomy and axillary dissection are generally preferred for surgical approach in MBC, as in our study.<sup>24</sup> We did not have a primary breast cancer patient who underwent breast conserving surgery. A recent study found that male patients showed low compliance with radiotherapy after breast conserving surgery.<sup>25</sup> The limitation of our study is a retrospective evaluation of a relatively small group of patients in a single center.

In conclusion, there is no diagnostic value

difference between only MLO-view and routine two- view MM in men. Mediolateral oblique view is sufficient in terms of characterization, spread and localization of the lesion, as long as mastectomy is preferred and the findings are supported by US. Thus, it will prevent the potential adverse effects of extra radiation exposure and this is an important advantage. If there is no suspicious calcification and structural distortion on MLO view and if breast conserving surgery is not being planned, CC view may not be required. However, this diagnostic new approach must be supported by large series.

# **Conflict of Interest**

The authors have no conflict of interests to declare.

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# Criteria for Excision of Suspicious Calcifications Using the Breast Lesion Excision System (BLES)

Alexandra Christou\*<sup>a</sup>, Vassilis Koutoulidis<sup>b</sup>, Dimitra Koulocheri<sup>c</sup>, Afrodite Nonni<sup>d</sup>, Constantinos George Zografos<sup>e</sup>, Georgios Constantinos Zografos<sup>f</sup>

<sup>a</sup> Brighton and Sussex University NHS Hospitals, Brighton, UK

<sup>b</sup> Department of Radiology, National and Kapodistrian University of Athens, Athens, Greece

<sup>°</sup> Department of Radiology, Breast Unit Hippokration Hospital, Athens, Greece

<sup>d</sup> Department of Pathology, National and Kapodistrian University of Athens, Greece

<sup>®</sup> Department of Surgery, Nikaia General Hospital, Athens, Greece

Department of Surgery, School of Medicine, National and Kapodistrian University of Athens, Greece

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#### ABSTRACT

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Key words: Breast lesion excision system (BLES), breast cancer, calcifications, biopsy **Background:** The aim of the study was to retrospectively evaluate possible imaging and histopathology criteria that can be used in a clinical basis to assess the success of excision of suspicious calcifications using the breast lesion excision system (BLES).

**Methods:** We investigated 400 BLES stereotactic biopsies of suspicious calcifications with the mean size of 15.38 mm (st. dev.= 13.579 mm, range 3-78 mm) using a 20 mm probe performed in our department between January 2014 and 2016. The mean age of our population was 58.5 years old (range 39-78 years). The pathology results of BLES specimens were compared with the final surgical results to assess excision success rates. Possible imaging and histopathology criteria for removal were statistically analyzed (mammographic size, disease free margins, grade, comedo phenotype, molecular type).

**Results:** The results showed that 90/400 (22.5%) biopsies were cancers (80% DCIS) and 38/400 were lesions with cell atypia (9.5%) of which 29/38 had subsequent surgery and were included in the study. Excision was achieved in 31/90 cancers (34.4%) and in 23/29 lesions with cell atypia (76.3%). The imaging and histopathology criteria for BLES excision that could be potentially clinically assessed were the initial mammographic size (p<0.001), the distance of the lesion from the specimen margins (p<0.001), the presence of comedo necrosis (p=0.014) and the grade of the cancers (p=0.021). The underestimation rate was 15.5%.

**Conclusion:** the mammographic size, grade, comedo presence and disease-free margins, were the main criteria for BLES success rate of excision of suspicious calcifications.

# Introduction

Breast lesion excision system (BLES) biopsy technique has been used in recent years for percutaneous biopsy of suspicious breast lesions

\* Address for correspondence: Alexandra Christou, MD, PhD Address: 177 Preston Rd, Brighton BN1 6AG, United Kingdom Tel: +44 1273 664773 Email: <u>alexandrachristou@gmail.com</u> either under stereotactic or ultrasound guidance with high effectiveness and safety. It has been shown that it is a good alternative to core needle biopsy (CB) and vacuum assisted biopsy (VAB) techniques with similar accuracy and complication rates.<sup>1-5</sup> The underestimation rates of the technique are also comparable to those of VAB regarding high-risk lesions and ductal carcinoma in situ (DCIS).<sup>6,7</sup>

Moreover, due to its unique advantage of excising an intact piece of breast tissue with preserved tissue margins, the effectiveness of the technique in excision of the targeted breast lesions has been recently shown. The literature has reported that BLES performance in excision of suspicious breast lesions is promising and suggestive of potential use of the technique in excision of small breast benign and malignant lesions.<sup>7-10</sup>

A 2-mm disease free margin and no ink on tumor are considered the standards for an adequate surgical margin in DCIS and invasive cancer respectively.<sup>11,12</sup> As a result of these limited disease free margins that have been recently required for safe excision of breast cancer and the decrease in the volume of the breast tissue needed to be removed, the role of BLES technique as a potential therapeutic tool has emerged. The probes that are currently used for BLES biopsy are 12 mm, 15 mm or 20 mm. According to the BLES manufacturer guidelines, a spheroid piece of intact breast tissue can be removed weighing 1.1 gr and with dimensions 12 mm x 17 mm in case of a 12 mm probe; 2.1 gr weight and 15 mm x 21 mm dimensions in case of 15 mm probe; and 3 gr weight with 25 mm x 20 mm dimensions in case of 20 mm probe.<sup>13</sup>

The aim of this study was to evaluate the potential role of BLES using a 20 mm probe under stereotactic guidance to excise suspicious microcalcifications and identify potential imaging and histopathology criteria that could be used in a clinical basis to assess the possible success or failure of the excision.

#### Methods

Between January 2014 and 2016, 394 women (mean age 58.5 years old; range from 39 to 78 years old) undergone stereotactic breast biopsy due to suspicious calcifications found on mammogram at our breast unit. A total number of 400 cases of calcifications (6 women had 2 areas of suspicious calcifications) were biopsied under stereotactic guidance using the BLES device during that period.

The main criteria for avoiding a BLES biopsy have been previously reported in papers from our unit, with the lesion positioning and the thickness of the breast being the most frequent ones.<sup>14,15</sup>

The inclusion criteria for the study were only first BLES biopsies and histologically proven cases to be either malignant or high-risk lesions with cell atypia and subsequent surgery (90 malignant and 29/38 high-risk lesions); the surgical result was used to determine the final pathology result in all cases. The lesions with no surgical result were excluded from the study (n=9 high risk lesions with cell atypia). The high-risk lesions with no cell atypia, such as benign papillomas (n=33) were not included in the study either and categorized as benign.

Informed consent for the percutaneous biopsy was obtained from all patients and ethical approval for the conduction of the study was also obtained.

The biopsy equipment consisted of the Fischer prone digital stereotactic device (Mammotest;

Fischer Imaging) and the BLES device (Breast Lesion Excision System<sup>®</sup> -B.L.E.S.-; Intact Medical).

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All BLES biopsies were performed with an 8gauge probe and 20 mm baskets by two experienced radiologists working in the department with 8 and 3 years of experience, respectively. All patients were placed in a prone position.

The procedure has been previously described in reports from our department.<sup>14,15</sup> Briefly, after careful stereotactic localization of the targeted area in order to be centrally positioned, 20 ml of local anesthetic (lidocaine 2%) is applied around the area (12, 3, 6 and 9 o'clock positions). A post anesthesia stereotactic image is then taken to assess the accuracy of the positioning. In case of deviation of the targeted lesion from the initial position relocation is obtained. Then the BLES stereotactic biopsy is performed. Through a small skin cut, the end-sharp probe is navigated through the breast tissue to the targeted area and, using radiofrequency (RF), an intact piece of breast specimen is retrieved and harvested inside the BLES basket at the end of the probe, which separates and removes the tissue. Due to RF application, a patient return electrode is applied to the upper back on the contralateral side of the breast to be biopsied.

After the biopsy, a radiographic image of the removed specimen is performed to assess the presence of the target lesion and a clip marker is positioned in the cavity through the biopsy channel. The sample is then placed in formalin and sent to the pathology department for histopathology test.

The patient follows the post biopsy treatment with local compression, dressing the skin incision site and applying a compressive bandage. Post biopsy control mammogram of the breast is performed to show the correct placement of the clip marker at the cavity site and the presence or absence of residual calcifications and any immediate biopsy complications. A post procedure clinical follow up appointment 48 hours after the biopsy is then booked to check the healing of the skin incision and deal with any complications, such as hematoma or infection.

The histopathology analysis of the specimen consists of the macroscopic measurement of the size of the BLES biopsy specimen and inking of the margins of the specimen. Microscopically, the presence and the type of cancer or cell atypia, the size of the included lesion and its distance from the margins (mm) are mentioned in all pathology reports. The distance of the lesion from the margins mentioned in the pathology result is the shortest distance of the lesion from the ink of the margins. Thermal artefacts if present and significant for the pathology diagnosis are also noted in the final report. Regarding cancers, the grade (G1-G3, high, intermediate and low grades DCIS) and the molecular type (luminal cancers, HER2 positive cancers and triple negative cancers)

Tumour Characteristics	Number	Percentage
DCIS DCIS +Microinvasion DCIS + Invasion	72/90 10/72 13/72	80%
Invasion Intraductal Lobular Tubular Papillary	18/90 12 3 2 1	20%
Comedo	21/90	23.3%
Grade 1	23/90	25.5%
Grade 2	41/90	45,5%
Grade 3	26/90	28.8%
Luminal Her 2 Positive Triple Negative Mean Size (N=90)	69/90 18/90 3/90 15.38 mm (st. dev.= 13.579 mm, range 3-78 mm)	76.6% 20% 3.3%

are reported. In the surgical results, the presence or absence of the cavity from the previous BLES biopsy and any residual disease (malignancy or benign highrisk lesion) around the cavity are mentioned in the report. The size (in mm) of the residual disease is also mentioned.

The statistical analysis parameters were as follows: A) the mean values and the respective standard deviations were used to describe scale measurements such as the mammographic size and the margins in mm; B) frequencies and percentages were used for categorical variables such as BLES and surgical results and the lesion types; C) the mammographic size, the distance (mm) of the targeted lesion from the margins of the specimen, the grade and the molecular type of the cancers and the presence of comedo necrosis were the imaging and histopathology criteria that were statistically analyzed for the purposes of the study. For statistical reasons, the high, intermediate, and low grades DCIS were mentioned as grade 3, 2 and 1, respectively along with the grade of the invasive cancers. The statistical analysis of the cancers and the high risk lesions was performed separately (the results are reported for 90 cancers and 29 high risk lesions, separately). Mann Whitney test, Pearsons Chi-Square and Fisher's test were used to assess statistical differences and associations. ROC analysis was used for the estimation of effective cut off sizes of the variants and their associated sensitivity and specificity; D) SPSS v22.0 Software was used for the analysis and the statistical significance was set at 0.05.

#### Results

There were 90 confirmed malignant cases and 29 confirmed high-risk lesions with cell atypia with subsequent surgery. The main characteristics of the cancers are presented in Table 1.

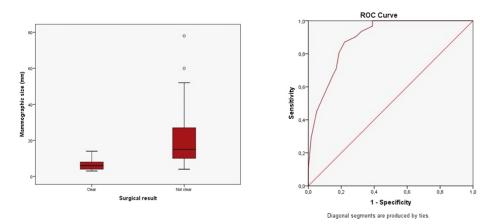
The high-risk lesions included in the study were mainly flat epithelial hyperplasia (13); lobular neoplasia type 1 and 2 (8); atypical ductal hyperplasia (4), papillomas with cell atypia (3) and mucocele like lesion with atypia (1).

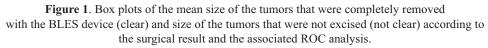
BLES excision was achieved in 31/90 (34.4%) cancers and in 23/29 (79.3%) high risk lesions with cell atypia. From these cancers, 25 were pure DCIS, 3 were pure invasive cancers (1 tubular, 1 IDC and 1 ILC) and 3 were invasive cancers with additional DCIS.

There was a statistically significant association between the initial mammographic size and the achievement of excision (Mann Whitney test, p < 0.001). The size of the lesions that were excised was significantly smaller (mean size = 6.32 mm) than the size of the lesions that were not excised (mean size 20.14 mm) (Table 2, Figure 1). ROC analysis showed a cut off size of 14 mm over which none of the tumors were excised (sensitivity 100%, specificity 39% and area under curve (AUC) 0.892) (Figure 1). The success rate of excision was increased with the decrease in the size of the tumor. Specifically, 57.4% cases were excised with a size smaller than 14 mm, 71.8% at a cut off size of 8 mm (sensitivity 80.6 %, specificity 81.4%) and 95.7% at a cut off size of 4.5 mm (sensitivity 29%, specificity 98.3%).

**Table 2.** The mean mammographic size of the cancers that were excised and the cancers that were not excised using the BLES device

Mean Mammographic size / std deviation	Number of cases n=90
6.32mm / 2.737mm	Excised cases n=31
20.14 mm/ 14.568mm	Not excised cases n=59





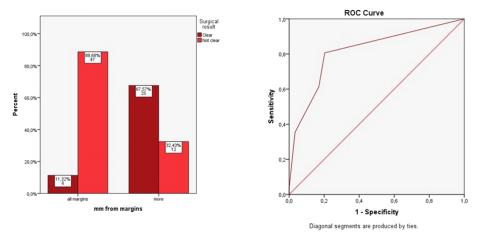


Figure 2. Bar charts of the distance in mm of the tumours from the specimen margins (mm from margins) of disease-free BLES specimen margins (more) and of involved BLES specimen margins (all margins) and the achievement of BLES removal of the cancers (clear-not clear on surgical result) and the associated ROC analysis.

 Table 3. Number of tumors with involved BLES specimen margins and with disease-free BLES

 specimen margins and the achievement or failure of BLES removal according to the final surgical result.

BLES specimen margins	Excision on surgical specimen	Residual disease on surgical specimen
Margins involved	6	47
Disease free margins	25	12

There was a statistically significant association between the distance of the tumors from the BLES specimen margins and the achievement of excision. In cases where the margins were disease-free the achievement of excision was 67,57% (25 cases) and in cases where the margins were involved was 11,32% (6 cases) (Pearson Chi-Square test, p<0.001). ROC analysis showed that at a cut off distance of 0.75 mm the specificity was 96,6%, the sensitivity was 35.5% and the AUC was 0,816 (Table 3, Figure 2).

There was a statistically significant association between the presence of comedo necrosis and the failure of excision (Pearson Chi-Square, p=0.006). In comedo cases, the failure of excision was higher (90,48%, 19 cases) whereas in cases where comedo necrosis was absent the failure of complete removal was lower (57,97%, 40 cases) (Table 4, Figure 3).

 Table 4. Comedo necrosis cases (present/not present) and the achievement or failure of BLES excision in the final surgical result (excision/residual disease)

Comedo necrosis cases	BLES excision	Residual disease
Present	2	19
Not present	29	40

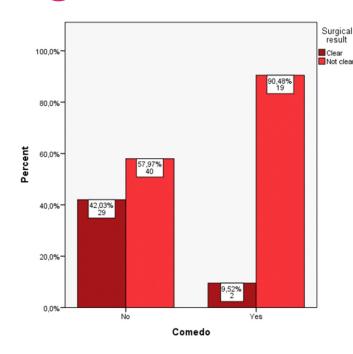


Figure 3. Bar charts of the cases with and with no comedo necrosis and the achievement or failure of BLES excision (clear-not clear on surgical result).

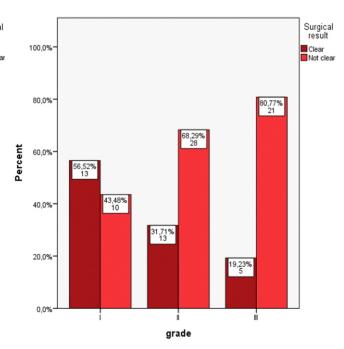


Figure 4. Bar charts of G1-G3 cancers and the achievement of BLES excision (clear-not clear on surgical result).

**Table 5.** Correlation of the grade of the tumor (I-III) and the achievement or failure of BLES excision according to the final surgical result (excision/residual disease)

Grade of tumors	BLES excision	Residual disease
Ι	13	10
II	13	28
III	5	21

There was a statistically significant association between the grade of the cancers and the achievement of complete removal (Pearson Chi-Square, p=0.021). In G3/high grade DCIS cases, the failure of excision was statistically higher (80,77%, 21 cases) whereas in G1/low grade DCIS cases, the failure was lower (43,48%, 10 cases) (Table 5, Figure 4).

No statistical association was seen between the molecular type and the BLES excision (Pearson Chi-Square, p=0.797) (Table 6).

The only statistically significant finding for the achievement of excision of the high-risk lesions was the distance of the lesion from the specimen margins (p=0,041 Mann Whitney test). Also, 94.7% of the cases (18/19 lesions) were removed when the

distance was over 1 mm, whereas 50% of the cases (5/10 lesions) were removed when the distance was 0.5 mm or 0 mm from the specimen margins.

The underestimation rate of cancers was 15.5% (14/90 cases); 7.7% (7/90 cases) was the underestimation rate of DCIS to invasive cancer; 3.3% (3/90) was the underestimation rate of DCIS to microinvasion; and 4.4% (4/90) was the underestimation rate of microinvasion to invasive cancer. No underestimation rate was found regarding the high-risk lesions. The complication rate was 8.75%.

Figures 5 and 6 illustrate cases of calcifications found to be DCIS and ILC respectively, which were completely removed using the BLES device (Figures 5, 6).

**Table 6.** Correlation of the molecular type of the cancers and the achievement or failure of BLES removal according to the final surgical result (excised/residual disease)

	8 8	/
Molecular type	BLES excision	Residual disease
Luminal	25	44
HER2 positive	5	13
Triple negative	1	2

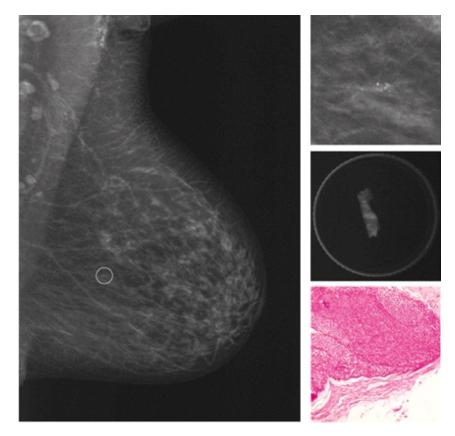


Figure 5. Mediolateral view (MLO) of the left breast shows a small group of suspicious calcifications (left image). Magnification view of the group of suspicious calcifications (right upper image). BLES specimen X-RAY shows that the group of the calcifications is totally included in the specimen (right middle image). Magnification view (x 100) of the histopathology image of the BLES specimen reveals pleiomorphic LCIS (DCIS) was completely removed (right lower image). Subsequent surgery confirmed the achievement of complete removal and no residual disease was found.

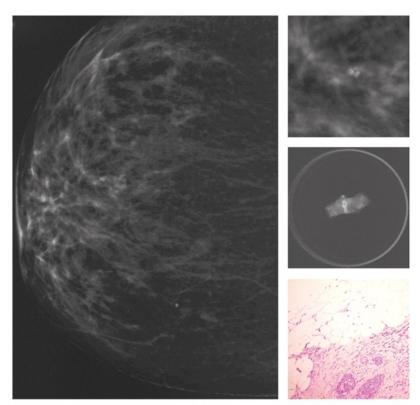


Figure 6. Right craniocaudal (CC) view shows a 5 mm cluster of suspicious calcifications (right image). Magnification view of the cluster of the calcifications (left upper image). BLES specimen X-RAY shows that the cluster of the microcalcifications is included in the specimen (right middle image). Histopathology image of the BLES specimen shows ILC with 1mm free disease margins (lower left image). No residual disease was found in the final surgical result.



## Discussion

We report a study of 90 histopathologically proven cancers and 29 high risk lesions with cell atypia that underwent subsequent surgical excision. According to our results, the main criteria that could potentially be used in a clinical basis to assess BLES effectiveness in suspicious calcifications removal are the mammographic size, the distance of the lesion from the BLES specimen margins, the grade and no comedo phenotype.

We achieved complete removal of cancers in 31/90 cases (34.4%) and of high risk lesions with cell atypia in 23/29 cases (79.3%) using the intact BLES biopsy device.

Regarding cancers, the BLES success rate of excision is in agreement with previously published results with rates between 30% and 66%.<sup>5, 7-10</sup> Our study included calcifications only and no size limitation of the target lesion and this can explain the relatively low effectiveness of the method as there were lesions larger than the size that potentially the BLES probe can excise. The rationale behind this was to identify effective cut off sizes and other possible criteria, such as the grade and molecular type of the tumor, the presence of comedo necrosis and the distance of the tumor from the margins which either alone or in combination could be useful to assess the potential successful excision using the BLES device.

We found the size of the target lesion with a cut off size at 14 mm to be a major factor in excision. In cases smaller than 14mm, the success of excision was 57.4% (31/54 cases) and over that size, none of the cases was excised. Sub-analysis of smaller sizes showed a high success rate of excision of 71.4% at a cut off size of 8mm with specificity of 81.4% and in even smaller cases -less than 4.5mm-the excision rate was 95.7% with specificity of 98.3%. Apart from the probe size limitation, another reason that could explain the higher success rate of excision of smaller lesions is that a centrally targeted position of smaller lesions in the specimen can be potentially more easily achieved and thus more frequently disease free margins can be observed. The mean size of the excised tumors was 6.32 mm. The safest cut off size we found was 4.5 mm with specificity of 98.3%. However, in order to clinically assess the success of excision, radiology-pathology correlation should be in place taking into consideration other factors as well, such as the disease-free margins, the aggressiveness and the nature and type of the tumor (DCIS, invasive, invasive with DCIS, calcified-non calcified part of DCIS).

The distance of the lesion from the specimen margins was the main criteria we found for adequate excision of the target lesions. In cases of disease free margins, the achievement of excision was 67.5%. We found a safe cut off point of 0.75 mm distance of disease free margins with specificity of 96.6%. It is

known that both the type of tumor excised and the distance of the margins are significant factors in residual disease found on re-excision; invasive cancers have a lower rate of residual disease than DCIS.<sup>16</sup> We had only 6 cases of invasive tumors (3 of them with additional DCIS) that were completely excised. Most of our excised cases were small cases of DCIS (25 cases with size <10mm). However, it has been suggested that histopathologically small areas of DCIS show lower incidence of residual disease in re-excision when found close to the margins.<sup>17,18</sup>

The lower grade of the tumors and the absence of comedo necrosis were found to be the criteria for complete removal. This is in agreement with results from surgical excisions that have previously shown that factors such as the high nuclear grade and the presence of comedo necrosis are associated with increased risk of residual disease in re-excisions and presence of microinvasion, respectively.<sup>19-21</sup> In fact, in our population, 3/4 cases with microinvasion upgraded to invasion in the final result had shown comedo necrosis in the initial BLES specimen. Also, the tendency of the lower grade tumors to show higher excision rates could be potentially related to the more accurate imaging estimation of the extent of these tumors and thus the more accurate targeting.<sup>22</sup>

Regarding the high risk lesions, we found that the distance of the lesion from the specimen margins was the only criteria for the adequate excision using the BLES device and as reported previously from our department, high risk lesions were mainly removed when the distance of the lesion from the margins was over 1mm.<sup>23</sup> High risk lesions belong to a heterogeneous group of unknown potential of malignancy and adequate sampling with potential complete removal is the main clinical practice in order to rule out upgrading. No underestimation was found for the high-risk lesions which is the lowest of previously reported underestimations (0-9.5%)<sup>5, 7-10</sup> supporting the adequate sampling. The safe removal of the high risk lesions supports the view of future potential use of the method to excise malignant lesions as well.

The underestimation rate of the cancers was 15.5% which is in agreement with previously reported underestimations, ranging from  $3.2-21.4\%^{2.3,7,9,10}$ ; however, the underestimation of DCIS to invasion was only 7.7%. The complication rate was also low, i.e., 8.75%. Both the underestimation and the complication rates have been previously reported in a study from our department.<sup>14</sup>

There are several limitations in the study. Firstly, only cases of calcifications were included, so solid masses were not investigated. Secondly, we included all sizes of calcifications and not only small clusters that can be potentially excised with the BLES probe; therefore, further research on the performance of BLES in small groups of calcifications is required. Thirdly, we did not analyses the subtypes of DCIS apart from the comedo necrosis as this type is the most aggressive one and, instead, we analyzed the grade of the cancers. However, both the grade of DCIS and invasive cancers were included in the same groups to simplify the statistical analysis. Fourthly, we included the data and the analysis of the high-risk lesions with cell atypia separately in this study to present an overview of the performance of BLES in excision of calcifications, thereby avoiding the mixing of the data analysis. Finally, our study is a retrospective one, so a possibility of bias cannot be excluded.

In conclusion, the small size and low grade of the cancers no comedo presence and disease free specimen margins were found to be the main criteria for suspicious calcifications excision using the BLES device, supporting the idea that potential consideration of these factors can play a role in the future clinical assessment of BLES as a possible removal tool in selected cases of suspicious calcifications.

# **Conflict of Interest**

None.

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# DOI: 10.32768/abc.202182109-114 The Added Value of Preoperative Magnetic Resonance Imaging of Breast in Surgical Decision

Marwa Makboul\*<sup>a</sup>, Shimaa Farghaly<sup>a</sup>, Murad A Jabir<sup>b</sup>, Ramy A. Hassan<sup>c</sup>, Mahmoud Refaat Shehata<sup>c</sup>

<sup>a</sup> Department of Radiodiagnosis, Faculty of Medicine, Assiut University, Assiut, Egypt

<sup>b</sup> Department of Surgical Oncology, South Egypt Cancer Institute, Assiut, Egypt

<sup>c</sup> Department of General surgery, Faculty of Medicine, Assiut University, Assiut, Egypt

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#### ABSTRACT

**Background:** Breast MRI has demonstrated excellent sensitivity in determining the extent of breast cancer and occult malignancy. This study aimed to evaluate the added value of breast MRI compared to mammography and ultrasonography in surgical decision.

**Methods:** A cross-sectional study was conducted on 84 female patients with newly diagnosed breast cancer by ultrasonography and mammography where conservative breast surgery was initially considered, and then bilateral breast MRI was performed and the multidisciplinary decision was revised. The data was analyzed to determine the additional value of breast MRI in treatment planning.

**Results:** Eighty-four patients were included of whom 44 (52.4%) patients had additional findings on MRI. The treatment plan was changed after the second ultrasonography and histopathology in 44 patients (52.4%) with a remarkable increase in the rate of modified radical mastectomy after MRI.

**Conclusion:** Preoperative breast MRI has great value in defining the surgical decision in patients with early breast cancer.

Introduction

magnetic resonance imaging,

oncoplastic breast surgery

Conservative and oncoplastic breast surgeries have become the standard treatment in early-stage breast cancer, as they have survival rates equivalent to mastectomy with better cosmetic and psychological outcomes.<sup>1</sup>

Conservative breast surgery is essentially the complete removal of the mass and some surrounding grossly normal tissue with negative histological margins to decrease the risk of local recurrence, as positive margins could result in further surgery either as re-excision or mastectomy with poor cosmetic outcome.<sup>1</sup>

\* Address for correspondence: Marwa Makboul, MD Lecturer of radiodiagnosis, Radiodiagnosis Department, Faculty of Medicine, Assiut University, Assiut, Egypt. Tel: +201006541595 Email: <u>makboul@aun.edu.eg</u> <u>drmarwa201198@ymail.com</u> Oncoplastic breast surgery is independent of adjuvant treatments; it is defined as surgical techniques which allows larger resections with acceptable cosmetic results with conservative forms of breast surgery in more advanced breast cancer with immediate breast reconstruction, where main procedures are volume displacement or volume replacement techniques.<sup>2</sup>

The primary aim of any diagnostic imaging procedure is accurate detection of the lesion and extent of the disease for optimization of the best treatment plan and surgical intervention.<sup>3</sup>

Breast ultrasonography (US) and mammography have been considered as primary imaging modalities for breast cancer diagnosis for a long time, but residual disease and the presence of extensive intraductal components after conservative breast surgery are considered the most important factors for local recurrence.<sup>4</sup>

Patients with newly diagnosed breast cancer are at risk of having another occult ipsilateral lesion or contralateral breast cancer which may be not detected accurately by ultrasonography and mammography; thus more sensitive methods are needed especially when breast conservation is considered.<sup>5</sup>

Contrast-enhanced breast magnetic resonance imaging (MRI) has been used as the most sensitive imaging modality for local staging of breast cancer especially in detection of multifocality, and multicentricity of breast cancer and the presence of contralateral breast disease. Numerous studies have confirmed the impact of MRI breast and its superiority in diagnostic performance compared to routine imaging procedures (US and mammography).<sup>6</sup>

This study aims to determine the extent to which the addition of breast MRI to the routine radiological assessment of breast cancer patients affects the overall surgical decision.

#### Methods

This cross-sectional study was done on 84 female patients with breast cancer diagnosis from January 2019 to February 2020. Patients were referred from the general surgery outpatients' clinic to the radiology department at Assiut University Hospitals for ultrasound (US) and mammography assessment, and then magnetic resonance imaging of breast (MRI) was done for each patient.

#### Ethics approval and consent to participate

This study was approved by the research ethics committee of the Faculty of Medicine at Assiut University, and all patients included in this study gave written informed consent to participate in this research. All patients also gave written informed consent to publish the data contained within this study.

#### Inclusion criteria

Female patients with breast cancer who were considered for conservative or oncoplastic breast surgery were recruited for this study.

#### Exclusion criteria

The exclusion criteria included patients with general contraindication for MRI examination (as metallic prosthesis or pacemaker) and patients with advanced breast cancer.

Breast lesions assessment by US and mammography Mammography examination was done using GE Alpha RT, and US examination was performed using Phillips IU22 ultrasound system (Philips Healthcare, Bothell, Wa, USA) according to breast imaging protocol, with a linear transducer 5-12 MHz. Scanning parameters were optimized for each case. Then breast MRI was performed for each patient.

#### MRI imaging protocol

MRI examination was performed in the prone

position using a 1.5 Tesla system (Avanto Siemens Healthcare), and a four-channel breast coil was used. The sequence was as follows: Axial 3D T1 weighted image TR/TE, 8.6/7.4, the field of view 400mm, slice thickness 1mm, acquisition time 1:34 minutes; Axial T2 fat suppression TR/TE, 5250/60, the field of view 380mm, slice thickness 4mm, acquisition time 2:44minutes; Axial diffusion-weighted imaging TR/TE, 5300/91, the field of view 460mm, slice thickness 4.5mm, acquisition time 2:39 minutes.

DWI was acquired before dynamic sequences with a spin-echo EPI (echo-planner imaging) in the axial plane, and sensitizing diffusion gradients were applied along the x, y, and z directions with b values of 50, 400, and 800 s/mm<sup>2</sup>. Intravenous injection of contrast agent (Gadolinium-dimeglumine) (Gd-DTPA) (Magnevist, Schering AG Berlin, Germany) using power injector at a dose of (0.1 mmol/kg) at a rate of 2ml/s, was followed by a 20 ml saline flush administered using an automatic injector.

#### Data analysis and image interpretation

All MRI images were transferred to the work station (Syngo Siemens Medical Solutions software) for image analysis by the expert radiology team. Tumor factors including size, number, and location of breast lesions, the density of breast, and presence or absence of calcification were detected by US and mammography, and accordingly, the initial surgical plan was firstly decided based on the findings of US and mammography.

Then MRI findings regarding tumor size, multifocality, multicentricity, and contralateral suspicious breast lesions were analyzed, followed by a second US and the final surgical decision was taken by the multidisciplinary team accordingly, based on MRI findings after histopathological correlation.

Breast cancer was treated with modified radical mastectomy, conservative breast surgery, and or oncoplastic breast surgery. The comprehensive treatment plan including Neo-adjuvant or adjuvant chemotherapy, radiotherapy, and hormone therapy was tailored according to histopathological results and tumor characteristics.

#### Statistical analysis

Data were collected and analyzed using the statistical package for social sciences, version 20 (IBM Corp., Armonk, New York, USA). Continuous data were expressed in the form of mean, SD, or median (range), frequency (percentage), FP: false positive; and FN: false negative.

#### Results

Eighty-four female patients with histologically confirmed breast cancer were employed in this study. The age range was between 27 and 63 years.

Variables		N= 84
Age (Range, years)		27-63
Mass laterality	Right breast mass	36 (43%)
Ultrasound evaluation	Left breast mass	48 (57%)
Mammography	Unifocal mass	84 (100%)
	Unifocal mass Calcification	84 (100%) 22 (26%)
	Density ACR-A ACR-B	16 (19%) 32 (38%)
	ACR-C ACR-D	33 (39.3%) 3 (3.6%)

**Table 1.** Characterictics data of the participants(n=84)

The majority (57%) of women had left breast mass. Based on mammography and breast ultrasound, all studied women had unifocal breast mass. The density of the breast was classified into ACR-A, ACR-B, ACR-C, and ACR-D (Table 1).

Regarding breast MRI findings, it was noticed that 44 (52.4%), 12 (14.3%), and 28 (33.3%) patients had unifocal, multifocal, and multicentric mass(es), respectively. In 44 (52.4%) women, MRI was able to detect additional findings while mammography and breast ultrasound failed to detect such findings as suspected enhancement in

the other contralateral breast, suspected enhancement in the same breast, suspicious small mass in the other breast, and retro-areolar segmental enhancement (Table 2).

Concerning the histopathological evaluation. 8 (9.5%), 6 (7.1%), and 70 (83.33%) women had ductal carcinoma in situ, invasive lobular carcinoma, and invasive ductal carcinoma respectively (Table 3).

Regarding the surgical decision among the participants, the initial decision was changed in 44 (52.4%) women based on MRI findings while the decision was not changed in 40 (47.6%) women.

**Table 2.** MRI findings of the participants (n=84)

Variables		N= 84
Pattern of the mass	Unifocal Multifocal Multicentric	44 (52.4%) 12 (14.3%) 28 (33.3%)
Presence of other findings	Suspected enhancement in another breast Suspected enhancement in the same breast Suspicious small mass in the other breast Retro-areolar segmental enhancement	44 (52.4%) 12 16 12 4

**Table 3.** Histopathological evaluation of breast masses among the participants

Histopathology	N= 84
Ductal carcinoma in situ	8 (9.5%)
Invasive lobular carcinoma	6 (7.1%)
Invasive ductal carcinoma	70 (83.33%)

On subset analysis of those with the changed decision, twelve patients (27.2%) with an initial surgical decision of unilateral oncoplastic breast surgery based on the sono-mammographic findings finally underwent bilateral oncoplastic surgery after additional MRI findings and a second US in the form of contralateral breast solitary masses.

Four patients (9%) initially were planned for oncoplastic surgery but when MRI revealed retroareolar segmental enhancements the decision was changed to modified radical mastectomy.

Sixteen (36.4%) women were initially planned for breast conservative surgery but based on additional MRI findings, the decision was changed to modified radical mastectomy due to multicentricity.

Regarding the postoperative histological

evaluation, this decision was appropriate in 12/16 (75%) women but in four women, the histopathology revealed more extensive lesions.

In twelve (27.2%) women, the initial decision was conservative breast surgery but this decision was changed to modified radical mastectomy with contralateral excisional biopsy based on MRI findings (Figure 1). Histopathological evaluation was in concordance with MRI findings in 10/12 but two women had benign lesions on the contralateral breast on histopathological evaluation.

According to our study, the utility of MRI in assessing the local extent of early breast cancer changed the surgical decision in 44 (52.4%) patients and these changes proved to be appropriate by the post-operative histopathological evaluation in 42

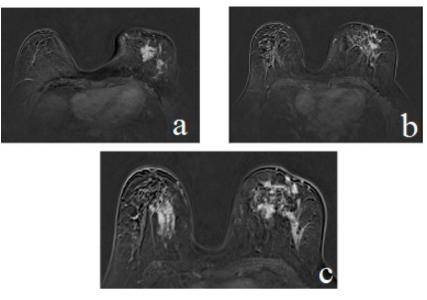


Figure 1. 37 year old female patient diagnosed with left breast cancer by US and mammography with extremely dense breast on mammography (ACR d), preoperative MRI was done and revealed a) Speculated outline malignant featuring left breast mass at about 6 o'clock.

b) Multiple enhancing foci seen surrounding the mass with ductal enhancement denoting multicentricity.c) Suspicious non-mass enhancement at the upper inner quadrant of right breast. Histopathological examination proved multicentric

invasive ductal carcinoma of the left breast and invasive ductal carcinoma of the right breast.

patients, and a final decision was still the same in 40 patients (Figure 2), (Table 4).

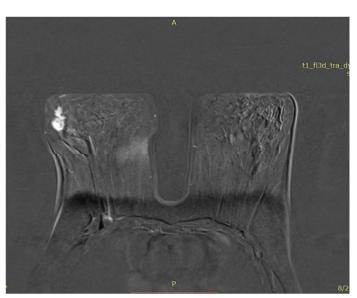
# Discussion

Breast conservation surgery and radiotherapy are considered as the standard therapy for early breast cancer as it provides the same overall survival as mastectomy. 7

Although triple assessment remains the standard practice, the advantageous impact of MRI on surgical management has been frequently investigated.<sup>2,3,10,14</sup>

In this study, we evaluated the added value of

Treatment change	Number	FP	FN
Modified radical mastectomy 1-Alone 2- Excisional biopsy	20/ 44(45.5%) 12/44 (27.2%)	0 2	0
Bilateral oncoplastic surgery Total	12/44 (27.2%) 12/44 (27.2%) 44 (100%)	0 2 (4.5%)	0 0



**Figure 2.** Female patient, aged 57 years old, diagnosed as right breast cancer by US and mammography, and preoperative breast MRI revealed malignant featuring unifocal mass at the lower outer quadrant of right breast, no other enhancing lesions of right breast, normal left breast. Histopathological evaluation proved invasive ductal carcinoma of the right breast.

preoperative breast MRI in the surgical decision, and whether MRI could potentially lower the rate of incompletely excised malignant lesions by identifying another occult malignant lesion or contralateral lesions. The treatment plan based on US and mammography was changed based on MRI examination including conversion from oncoplastic breast surgery to mastectomy.

In the current study, 84 patients underwent preoperative breast MRI which detected findings that could not be detected by US and mammography in 44 (52.4%) patients. This agrees with the previous study showing that MRI detected 30% additional lesions not detected in conventional imaging.<sup>8</sup>

Our study has considered the correlation of histopathological assessment with the proposed surgical procedure.<sup>14</sup> The reported correlation of the final pathology with MRI findings in our study confirms its advantageous impact in 47.6% of our patients with minimal chance for over-treatment. Our results are consistent with earlier studies and reported a significant change in surgical management.<sup>2,3,10-14</sup>

According to our study, the utility of MRI in assessing the local extent of early breast cancer changed the surgical decision remarkably in 47.6% of cases, and this is slightly higher than previously published reports which demonstrated changes in 14-35% of patients.<sup>9,10,11,12</sup>

In twelve patients (27.2%) from those with a changed decision in our study, the surgical decision was changed from oncoplastic surgery to bilateral oncoplastic surgeries due to detection of the contralateral lesion. This finding was higher than the results of a previous study that revealed contralateral breast lesions after MRI in 7%.<sup>13</sup>

In our study 16 patients (36.4%) were initially planned for breast conservative surgery but based on MRI finding the decision was changed to modified radical mastectomy due to multicentricity, and this was in agreement with results of an earlier study which revealed a potential increase in mastectomy rate after MRI to about (44%).<sup>14</sup>

In our study, a negative correlation (falsepositive results) between MRI findings and final histopathological assessment was found only in less than 5% of cases (2/44) in the form of benign breast lesions, which is lower than earlier studies and did not result in undue mastectomy.<sup>3</sup>

The limitations of this current study were a small sample size due to the high cost of preoperative MRI which precludes its widespread use in the general population despite its great role in accurate evaluation of breast cancer.

Also, accurate determination of invasive versus in situ disease by MRI and estimating the extent of ductal carcinoma in situ (DCIS) was limited.

In conclusion, despite these limitations, our study confirmed the advantageous impact of MRI for

assessing early breast cancer disease and defining the surgical plan.

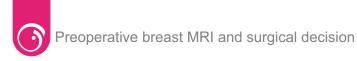
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# **Conflict of Interest**

The authors declare that they have no conflict of interest.

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# Treatment of Post-Breast Surgery Pain Syndrome with Botulinum Toxin: Analysis of The Response to the Addition of Levobupivacaine and to the Type of Surgery

Ana-Belén Puentes-Gutiérrez\*<sup>a</sup>, María García-Bascones<sup>a</sup>, Marcelino Sánchez-Casado<sup>b</sup>, Laura Fernández-García<sup>a</sup>, Rebeca Puentes-Gutiérrez<sup>c</sup>, María-Angustias Marquina-Valero<sup>a</sup>

<sup>a</sup> Department of Physical Medicine and Rehabilitation, Complejo Hospitalario de Toledo, Toledo, Spain

<sup>b</sup> Department of Intensive Care Unit, Complejo Hospitalario de Toledo, Toledo, Spain

<sup>°</sup> University College of Nursing, Valladolid, Spain

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## ABSTRACT

**Background:** The spasm and/or contracture of the pectoralis major contribute to the post-breast surgery pain. The purpose of our study was to evaluate changes in the post-breast surgical pain syndrome after the infiltration of botulinum-toxin type-A (BTX-A), according to the type of surgery and the reconstitution of the botulinum-toxin.

**Methods:** This retrospective study was conducted at the Rehabilitation Department with two cohorts: BTX-A reconstituted with saline solution (SS group) or with levobupivacaine (LV group). Data about pectoralis major contracture and pain (global, at night, at rest and during activity) before the infiltration and six weeks after that were collected from the medical records and compared between SS and LV groups, and between conservative breast surgery and mastectomy cases.

**Results:** in the study, 48 women aged 53.3 ( $\pm$ 11.10) years were included, with 26 (54.2%) in SS group and 22 (45.8%) in LV group. There were no differences between both groups except transitory paresis (3.8% vs 22.7%; P=0.022). In all patients, baseline circumstances vs after 6 weeks were compared, and we found significant differences in contracture (1,77 ( $\pm$ 0.57) vs 0.97 ( $\pm$ 0.79)), VAS global (5.45 ( $\pm$ 1.92) vs 3.46 ( $\pm$ 2.48)), VAS night (3.17 ( $\pm$ 3.13) vs 1.61 ( $\pm$ 2.29)), VAS rest (2.14 ( $\pm$ 2.56) vs 1.21 ( $\pm$ 1.98)) and VAS activity (4.31 ( $\pm$ 2.55) vs 2.78 ( $\pm$ 2.58)). We found higher improvements in the breast conservative surgery.

**Conclusion:** A significant lower pain and contracture after BTX-A injection in the pectoralis major was observed, but its reconstitution in levobupivacaine may not be an effective method to increase the analgesic effect. There were higher improvements in the breast conservative surgery than in the mastectomy.

# Introduction

Key words:

syndrome,

syndrome,

breast cancer,

botulinum toxin.

Post-mastectomy pain

post-breast-surgery pain

The post-mastectomy pain syndrome or postbreast surgery pain (PBSPS) is defined as a chronic pain with neuropathic qualities, located in ipsilateral

\* Address for correspondence: Ana Belén Puentes Gutiérrez, MD, PhD Physical Medicine and Rehabilitation Department, Hospital Universitario de Toledo. Toledo Address: Avenida del Rio Guadiana, 45007. Toledo. Spain. Tel: +43 639164316 Email: <u>anapuentes@gmail.com</u> breast/chest wall, axilla and/or medial arm which lasts at least 6 months following any breast surgery.<sup>1-4</sup> This is an underdiagnosed entity which impacts on the patients' quality of life.<sup>1-4</sup>

Considering that the spasm and/or contracture of the pectoralis major contribute to the PBSPS, there are some pre-operative analgesic therapies (nerve blocks, infiltration of botulinum toxin type A–BTX-A, etc.) which act on this muscle.<sup>14</sup>

The purposes of this study were: (i) to quantify changes in the pain intensity and in the severity of the



pectoralis contracture in the PBSPS six weeks after the intramuscular infiltration of the BTX-A; (ii) to assess if there were differences in the response according to the type of surgery; and (iii) to verify if the reconstitution of the BTX-A with levobupivacaine increased the analgesic and relaxing effect versus the reconstitution with saline solution.

#### Methods

Infiltration of BTX-A in the pectoralis major, due to a painful contracture of this muscle after breast cancer treatment is an usual clinical practice in Rehabilitation. All patients were duly informed and signed the consent before the infiltration. BTX-A is often infiltrated intramuscularly with 100 units (U) of onabotulinumtoxin-A or 250 U of abobotulinum-A in just one point. Some physicians reconstitute the BTX-A with 1.1 ml of saline solution and others with 1.1 ml of levobupivacaine. In order to study which is the best cohort (saline solution or levobupivacaine), a retrospective cohort study was conducted. Data of all women treated from January 2018 to January 2020, with infiltration of BTX-A in the pectoralis major reconstituted with saline solution or with levobupivacaine were obtained from the Physical Medicine and Rehabilitation Department database.

Demographic data and information on risk factors related to the development of chronic pain were collected on type of breast surgery, mastectomy versus conservative surgery (excision of the tumor and/or adjacent tissues), number of axillary lymph nodes removed, adjuvant therapy and number of months from breast surgery until infiltration. Before the BTX-A infiltration and six weeks later, the

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severity of the pectoralis contracture was evaluated from 0 (none) to 3 (severe) and the pain (global during the entire day, at night, at rest and during activity) with the visual analog scale (VAS) from 0 to 10, the satisfaction of patients from 0 (none) to 10 (maximum) and the adverse effects were assessed.

This work was approved ethically by the Research Committee.

#### Statistical Methods

To determine the sample size, a pilot study was conducted. Previous data from ten patients were obtained where the VAS average was  $5.45 \pm 1.5$ , and with a confidence level of 95%, 80% statistical power and an effect size of 1 in the VA between groups, a sample size of 48 patients was decided. The descriptive analysis was carried out using an arithmetic mean (standard deviation) for quantitative variables and the counting (percentage) for qualitative variables. To compare differences between infiltration with saline and levobupivacaine, we used the Mann-Whitney U-Test for the quantitative variables and Chi-Square test for the categorical variables, with the Fisher's approximation if the application conditions were not met. To compare the values before and 6 weeks after the infiltration, we used the Wilcoxon Signed-Rank Test. A test was considered statistically significant when the corresponding p value was less than 0.05.

## **Results**

In the study, 48 patients aged 53.3 (11.10) years were included. In 21 (44.7%) of them a mastectomy was performed, and 6.13 (4.5) axillary lymph nodes

Table 1. Comparison of the different studied variables according to the administration of
BTX-A reconstituted in levobupivacaine or in saline groups in all the patients.

	Levobupivacaine (N=22) (45.8%)	Saline (N=26) (54.2%)	Р
Age (years)	54 (10.91)	52.76 (11.45)	0.706
Body Mass Index	27.5 (4.97)	25.8 (4.23)	0.467
Breast-surgery			
conservative	12 (54.5%)	15 (57,7%)	
mastectomy	10 (45.5%)	11 (42.3%)	0.827
Expander	2 (9.5%)	4 (15.4%)	0.678
Number of lymph node removed	5.80 (4.58)	6.13 (4.70)	0.862
Radiotherapy	19 (86.4%)	21 (80.8%)	0.710
Months from breast surgery until infiltration	54.05 (36.96)	50.26 (52.42)	0.321
Number of infiltrations	1.45 (1.14)	1.6 (1.00)	0.508
Contracture	1.81 (0.64)	1.70 (0.50)	0.484
VAS global basal	5.81 (1.93)	5.29 (2.03)	0.375
VAS night basal	2.83 (3.20)	3.76 (2.84)	0.305
VAS rest basal	2.29 (2.51)	2.11 (2.58)	0.826
VAS basal activity	4.47 (2.72)	4.06 (2.41)	0.594
Contracture 6 weeks	0.82 (0.89)	1.11 (0.68)	0.245
VAS global 6 weeks	3.47 (2.45)	3.28 (2.56)	0.808
VAS night 6 weeks	1.33 (2.16)	1.70 (2.37)	0.612
VAS rest 6 weeks	1.33 (2.19)	1.33 (2.24)	1.000
VAS activity 6 weeks	3.04 (2.64)	2.45 (2.52)	0.476
Patient's satisfaction	7.70 (1.79)	7.11 (2.69)	0.440
Secondary effects: excessive transitory paresis	5 (22.7%)	1 (3.8%)	0.022

The categorical data are expressed as counting (percentage) and the quantitative ones as average (standard deviation). VAS: visual analog scale

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(111) in patients	with mastectomy	•				
Variables All patients		atients	Conservative Breast Surgery		Mastectomy	
	Baseline	6 weeks	Baseline	6 weeks	Baseline	6 weeks
Contracture	1.77 (0.57)	0.97 (0.79)*	1.65 (0.48)	0.78 (0.67)*	1.92 (0.65)	1.21 (0.88)*
VAS global	5.45 (1.92)	3.46 (2.48)*	5.38 (2.17)	3.25 (2.48)*	5.52 (1.62)	3.72 (2.53)#
VAS night	3.17 (3.13)	1.61 (2.29)*	3.54 (3.07)	1.47 (2.21)*	2.65 (3.25)	1.81 (2.45)
VAS rest	2.14 (2.56)	1.21 (1.98)#	2.47 (2.69)	1.24 (2.13)#	1.64 (2.37)	1.18 (1.83)
VAS activity	4.31 (2.55)	2.78 (2.58)*	4.27 (2.46)	2.24 (2.40)*	4.37 (2.75)	3.53 (2.71)

**Table 2.** Comparison between the baseline circumstances and after 6 weeks with the contracture and the pain (VAS) at different time points, analyzed (i) in all the patients, (ii) in patients with conservative surgery and (iii) in patients with mastectomy.

The quantitative variables are expressed as an average (standard deviation). Statistical significance comparing every basal variable with its value after 6 weeks:  $p<0,001, p<0,01, \mu = 0,05$ .

were removed on average and 40 patients (83.3%) received radiotherapy. Out of 48 patients, 26 (54.2%) patients received BTX-A injections reconstituted in saline solution and 22 (45.8%) reconstituted in levobupivacaine. Also, 19 patients (34.1%) needed further injections when the effects of BTX-A decreased.

Table 1 shows the differences regarding the variables between the saline and levobupivacaine groups. Table 2 shows the differences in pain variables and the contracture before and 6 weeks after the infiltration, both in the mastectomy group and in the conservative surgery group, as well as the results in all patients.

In the analysis of the adverse effects, 6 (12.5%) presented a slight temporary paresis at the proximal upper limb. The average satisfaction 6 weeks after the post-infiltration of the whole sample was 7.37 (2.33).

## Discussion

Most of studies on the infiltration of the pectoralis major with BTX-A to treat PBSPS are optimistic due to its dual action, both relaxing and analgesic.<sup>2,3,5-7</sup> Some studies practice intra-operative BTX-A infiltration as a preventive treatment for post-surgery pain.<sup>6, 8</sup> The analgesic effect and obtaining a more natural breast appearance are two reasons for applying BTX-A in aesthetic surgeries of breast augmentation with implants.<sup>6,9,10</sup> The study of Lo et al. is one of the few studies that has not obtained any analgesic improvement infiltrating BTX-A as opposed to placebo after the breast reconstitution with expanders.<sup>8</sup>

The PBSPS is found most frequently after more invasive treatments such as mastectomy, axillar lymphadenectomy, reconstruction with expanders or after radiotherapy, but it can also be found also after any other surgical procedures involving breast parenchyma or underlying muscle.<sup>1-4, 6</sup> The fact that we have observed a more complete analgesic response to the BTX-A in conservative surgery over the mastectomy leads us to consider the possibility of additional factors in the mastectomy related to a greater tissue lesion.

The technique performed in our study can be extrapolated to other studies. Most of them use onabotulinumtoxin A, with the most frequent dose of 100 U (75-140U), reconstituted with 1 to 5 ml of saline solution.<sup>2,3,5,6</sup> The analgesic effect begins 6-36 hours later, with a maximum peak after 7-14 days, being reversible after 3-6 months; thus, further injections may be required.<sup>2</sup> The VAS is the most common method for assessing pain, although in many cases it is just the patient's report.<sup>2,6,8</sup>

Local anesthetics infiltration before the BTX-A have been used to decrease the pain during the technique. This has led some researchers to reconstitute the BTX-A with an anesthetic to reinforce the analgesic effect of both drugs, both in the short and the long term.<sup>11,12</sup> On the other hand, the anesthetics have also a myotoxic effect with the possibility of obtaining a faster onset of paresis.<sup>12</sup> Although there are few and heterogeneous studies, many of them are clinical cases, it seems that reconstituting with anesthetics does not limit the efficacy of the BTX-A.<sup>11</sup> A clinical trial to treat the hyperhidrosis, which compared the BTX-A reconstituted in a local anesthetic or in a saline solution, has shown lower pain during the injections, and no difference regarding the sweat production.<sup>11</sup>

The excessive level of transitory paresis was one of the most frequent adverse effects of the BTX-A.<sup>2,6,7</sup> In our study, this effect was more frequent when reconstituting it with an anesthetic and it was reversible in all the cases.

Reconstitution with local anesthetic has been applied to treat other painful pathologies such as the complex regional pain syndrome.<sup>13,14</sup>

The main limitation in the study is that it was retrospective with short follow-up period. Further properly conducted trials providing high-level evidence assessing benefits and risks of reconstitution in local anesthetic and maximizing the effect and duration of the BTX-A are required, since our study does not show any advantage with regard to the saline solution.

In conclusion, a single intramuscular BTX-A injection significantly decreased pain intensity and pectoralis major contracture after six weeks; this improvement was complete in the conservative breast surgery and incomplete in the mastectomy. The BTX-A reconstitution in levobupivacaine was not clinically relevant whereas it produced a higher level of reversible paresis. These findings need to be

Management of post-surgical breast pain

confirmed by prospective studies.

#### **Conflict of Interest**

The authors declare no conflict of interest.

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# Quality of Life for Women with Breast Cancer-Related Lymphedema: The Importance of Collaboration between Physical Medicine and Rehabilitation and General Surgery Clinics

Türkan Turgay\*<sup>a,b</sup>, Pınar Günel Karadeniz<sup>c</sup>, Göktürk Maralcan<sup>d</sup>

<sup>a</sup> Department of Physical Medicine and Rehabilitation, SANKO University, Gaziantep, Turkey

<sup>b</sup> Sani Konukoğlu Research and Practice Hospital, SANKO University Gaziantep, Turkey

<sup>°</sup> Department of Biostatistics, SANKO University, Gaziantep, Turkey

<sup>d</sup> Department of General Surgery, SANKO University, Gaziantep, Turkey

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#### ABSTRACT

**Background:** The aim of this study was to examine the clinical characteristics and quality of life (QOL) of patients with BCRL (breast cancer-related lymphedema).

**Methods:** In this cross-sectional descriptive study, patients' characteristics such as age, body mass index (BMI: kg/m<sup>2</sup>), history of chemotherapy (CT), radiotherapy (RT), hormone replacement therapy (HRT), neoadjuvant therapy (NT), cancer stages, and types of surgery were recorded. Patients were evaluated using the 'Disabilities of the Arm, Shoulder and Hand questionnaire' (DASH), the 'Lymphedema Quality of Life Questionnaire' (LYMQOL-ARM), and a visual analogue scale (VAS).

**Results:** A total of 68 women with the mean age of  $52.50\pm9.33$  and BMI  $29.240\pm5.05$  kg/m<sup>2</sup> were recruited after breast cancer surgery in this study: thirty-three patients (48.5%) in Stage 0; 24 (35.3%) in Stage 1; 10 (14.7%) in Stage 2; and 1 (1.5%) in Stage 3. No statistically significant difference was found in the QOL according to treatments received after the diagnosis of breast cancer surgery, RT (except the appearance domain of QOL), CT, HRT, or NT. In patients who had received axillary dissection in combination with RT, a statistically significant association was observed between QOL related to body image and symptoms (p=0.009 and p=0.017, respectively). A statistically significant difference was found only in body image and clinical symptom domains according to the lymphedema stage (p=0.027 and p=0.002, respectively). It was observed that as shoulder pain (VAS) and disability (DASH) scores increased, scores of all domains of QOL increased except the overall domain in QOL (p<0.05).

**Conclusion:** It was observed that clinical symptoms and body image parameters in QOL were associated with the lymphedema stage and the number of lymph nodes dissected. It was concluded that axillary dissection with axillary RT and RT alone after breast cancer surgery is associated with body image. Our study revealed that body image perception is related to the quality of life in patients with BCRL. Optimal management of the negative effects of self-reported lymphedema evaluated in the latency phase on quality of life requires coordination between Physical Medicine and Rehabilitation and General Surgery Clinics.

Key words: Breast cancer-related lymphedema, quality of life

\* Address for correspondence: *Türkan Turgay MD,* 

Faculty of Medicine, Department of Physiotherapy and Rehabilitation, SANKO University Sani Konukoğlu Research and Practice Hospital, Physical Medicine and Rehabilitation Clinic, Address: İncilipinar mah. Ali Fuat Cebesoy Blv. No: 45. Şehitkamil/Gaziantep, Turkey. 27090. Email: <u>turkanharunlar@hotmail.com</u>

# Introduction

Breast cancer is the most common malignancy in women worldwide. The GLOBOCAN project of the International Agency for Research on Cancer (IARC) shows the high incidence and mortality rates of breast cancer compared to other types of cancer worldwide. In 2018, 2.1 million new cases of breast cancer were detected worldwide, and this number is expected to reach approximately 3.2 million by 2050.<sup>1</sup> Similar to developed countries, 5-year survival rate in Turkey was found to be 86%.<sup>2</sup> Lymphedema is a chronic progressive condition characterized by impaired lymph drainage due to various reasons, including the accumulation of protein-rich lymph fluid in interstitial cell spaces, and progressive swelling in one or more body regions.<sup>3</sup> Breast cancer-related lymphedema (BCRL) occurs due to obstruction of lymphatic ducts or lymph nodes or their infiltration with tumor cells (lymphangitis carcinomatosis). Lymphedema after breast cancer treatment is one of the most frightening and disturbing complications of patients. In BCRL, the greatest risk for the development of upper extremity lymphedema is in the first two years after diagnosis and treatment.<sup>4</sup> In a recent meta-analysis, the incidence of BCRL was found to be approximately 21%.<sup>5</sup> Risk factors for the disease include invasive cancer diagnosis, axillary lymph dissection, radiotherapy (RT), local infection, advanced age, and obesity, but other factors may also contribute.<sup>6,7</sup> It is known that patients with BCRL are affected more in different aspects (physical, functional, psychosocial, and emotional states) of their quality of life (QOL) compared to patients without lymphedema.<sup>8</sup> In the literature, different results have been obtained in studies examining the relationship between different lymphedema levels and functional status of the upper extremity and QOL, considering the risk factors for lymphedema, and this relationship has not been fully understood.<sup>9</sup> Collaboration among institutions that manage breast cancer survivors is necessary to establish standard treatment guidelines and to prevent lymphedema occurrence. The aim of this study is to evaluate the clinical characteristics and OOL of patients with BCRL in cooperation and follow-up of Physical Medicine and Rehabilitation and General Surgery Clinics.

# Methods

#### Patients

A total of 68 patients diagnosed with BCRL, who applied to SANKO University, Faculty of Medicine, Physical Medicine and Rehabilitation and General Surgery Clinics, for the first time or for follow-up purposes, between December 2019 and February 2020, were included in this cross-sectional descriptive study. The study protocol was approved by the SANKO University Clinical Research Ethics Committee, dated 09.01.2020 and under approval number 2020/01-03. Written informed consent was obtained from all participants and the study was conducted in accordance with the Declaration of Helsinki Ethical Principles.

The inclusion criteria for participating in the study were: 1) an affected arm circumference of 2

cm greater than that of the unaffected arm in at least one of the two sites, 2) the presence of BCRL for at least 1 month or longer, 3) being a female aged 18 years or older, 4) volunteering for this study, and 5) not having received any lymphedema treatment. All patients who met these inclusion criteria were recruited in this study and no sample size calculation was made. Patients with advanced or metastatic cancer, those with bilateral breast cancer, and those with a previous history of orthopedic and/or neurological disease in the affected arm were excluded from the study. All survivors had finished their treatment including surgery, chemotherapy (CT) and RT at least 3 months before.

An Information Form was created to record the sociodemographic characteristics and the findings of examination of the patients who participated in this study. The age, body mass index (BMI; calculated as weight (kg)/height (m<sup>2</sup>)), (normal<25 kg/m<sup>2</sup>; overweight 25-29 kg/m<sup>2</sup>; obese≥30 kg/m<sup>2</sup>), disease and lymphedema durations, history of CT, RT, hormone replacement therapy (HRT), and neoadjuvant therapy (NT) were recorded. Cancer stages, types of breast and axillary surgery, sentinel lymph node biopsy (SLNB) and the number of dissected lymph nodes were also recorded from patient files.

# Evaluation of Patients

The intensity of arm pain was measured using a 10-cm Visual Analogue Scale (VAS). The Disabilities of the Arm, Shoulder, and Hand questionnaire (DASH), which evaluates disability and symptoms, daily activity limitations, and leisure time activity limitations, was used in this study. The DASH, developed by the American Academy of Orthopedic Surgeons, and validated in Turkish, is a self-rated questionnaire that measures upper extremity disability and symptoms. The DASH score takes values between 0 (no disability) and 100 (most severe disability). Higher scores point to greater disability.<sup>10</sup>

The severity of lymphedema was determined according to the difference between the extremities that was adopted by the American Physiotherapy Association (less than 3 cm: mild, between 3 and 5 cm: moderate, and above 5 cm: severe lymphedema).<sup>11</sup>

In this study, no imaging device (bioimpedance spectroscopy, dual-energy x-ray absorptiometry, magnetic resonance imaging, computed tomography, color doppler imaging, lymphoscintigraphy, or indocyanine green lymphography) was used. Bioimpedance spectroscopy is often used to monitor individuals at risk for arm lymphedema after breast cancer surgery to determine stage 0, and if this method is not available, self-reported symptoms may also be a valid assessment of this stage.<sup>12,13</sup>

Clinical lymphedema staging of the patients was evaluated according to the International Society of Lymphology with a degree between 0 and 3. In this respect, patients were classified as those who were Stage 0 (or 1a)- subclinical lymphedema (swelling is not yet evident despite impaired lymph transport, subtle changes in tissue fluid/composition, and changes in subjective symptoms); Stage 1 - spontaneous reversible (increase in upper extremity circumference, heaviness feeling and pitting edema); Stage 2 -spontaneous irreversible (non-pitting edema, tightness in soft tissue, fibrosis); and Stage 3-lymphostatic elephantiasis (severe lymphedema, trophic skin changes).<sup>14</sup>

The Lymphedema Quality of Life Questionnaire (LYMQOL-ARM) the validity and reliability of which has been assured was applied to the BCRL patients to assess their QOL.15 This questionnaire, which consists of 21 questions has four domains, namely function (effect on daily activities and leisure activities, dependence on other people), appearance/body image (effect on appearance, difficulty finding clothes to fit and wear, effect on one's feeling about oneself and effect on relationships with other people), clinical symptoms (causing pain, numbness in swollen arm, feeling of pins and needles, feeling of weakness, feeling heavy and feeling tired) and mood/emotions (trouble sleeping and difficulty concentrating on things, feeling tense, feeling worried, feeling irritated and feeling depressed). Each domain score has a range between 1 and 4. Item scoring in each domain is as follows: not at all=1, a little=2, quite a bit=3, and a lot=4. The total score for each domain is calculated by adding up all the scores together and dividing the total by the total number of questions answered. High scores show poor QOL. The last domain evaluates overall QOL on a scale from 0 (poor overall QOL) to 10 (excellent QOL).

#### Statistical Analysis

Descriptive statistics were given as mean± standard deviation and median (min-max values) for continuous variables, frequency, and percentages for categorical variables. Normality of data was evaluated with Kolmogorov-Smirnov test. Mann-Whitney U test was used for comparison of two groups, Kruskal-Wallis test was used for comparison of more than two groups. For assessing the relationship between continuous variables Spearman's rho correlation coefficient was used. P-values<0.05 were considered as statistically significant.

#### Results

A total of 68 women were recruited for evaluation after breast cancer surgery in this study. The mean age of the patients was  $52.50 \pm 9.33$ . Their average BMI was  $29.240 \pm 5.05$  kg/m<sup>2</sup>. Most of the patients were obese (Table 1). In patients with lymphedema, disabilities of the arm, shoulder, and hand (DASH) score was  $32.2 \pm 18.24$ .

Table	1.	Characteristics	of	patients
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Characteristics	
Age <sup>a</sup>	52.50±9.34
$BMI^{a}$	29.22±5.05
Cancer type <sup>b</sup> IDC ILC SAR MIXT	58 (85.3%) 7 (10.3%) 1 (1.5 %) 2 (2.9%)
Disease Duration (months) <sup>e</sup>	12 (1-108)
Disease Stage <sup>b</sup> (pathology report) 1 2A 2B 3A 3C	5 (7.4%) 20 (29.4%) 14 (20.6%) 22 (32.4%) 7 (10.3%)
The onset of BCRL (months) $^{\circ}$	3.5 (1-84)
Severity of Lymphedemab Mild Moderate Severe	33 (48.5%) 20 (29.4%) 15 (22.1%)
Lymphedema Stage <sup>b</sup> Stage 0 Stage 1 Stage 2 Stage 3	33 (48.5%) 24 (35.3%) 10 (14.7%) 1 (1.5%)
Type of Surgery <sup>b</sup> Modified Radical Mastectomy Simple Mastectomy Segmentectomy Standard Radical Mastectomy	35 (51.4%) 14 (20.6%) 18 (26.5%) 1 (1.5%)
Number of Dissected Lymph Node <sup><math>\circ</math></sup>	13 (0-59)
RT <sup>b</sup> Yes No	43 (63.2%) 25 (36.8%)
CT <sup>b</sup> Yes No	50 (73.5%) 18 (26.5%)
HRT <sup>b</sup> Yes No	26 (38.2%) 42 (61.8%)
NT Yes No	16 (23.5%) 52 (76.5%)
VAS <sup>c</sup>	5 (0-9)
DASH <sup>c</sup>	32.29±18.24
Functions- LQOL <sup>°</sup>	1.2 (1.0-2.7)
Appearance/Body image-LQOL <sup>°</sup>	1.05 (1.0-3.0)
Clinical symptoms-LQOL <sup>c</sup>	1.8 (1.0-3.6)
Mood/Emotions-LQOL <sup>c</sup>	2.0 (1.0-3.8)
Overall-LQOL <sup>°</sup>	5 (2-10)

BMI: Body Mass Index, IDC: Invasive ductal carcinoma, ILC: Invasive lobular carcinoma sarcoma, SAR: Sarcoma, MIXT: Mixed type (Invasive ductal carcinoma and Invasive lobular carcinoma), VAS: Visual Analogue Scale, DASH: Disabilities of the Arm, Shoulder and Hand, BCRL: Breast cancer related lymphedema, LQOL: Lymphedema Quality of Life. ALND: Axillary lymph node dissection, CT: Chemotherapy, RT: Radiotherapy, NT: Neoadjuvant therapy, HRT: Hormone replacement therapy, "(Mean±SD), <sup>b</sup>n (%), <sup>c</sup>(Median (Min-Max)).

Thirty-five (51.4%) patients had undergone modified radical mastectomy, 14 (20.6%) simple mastectomy, 18 (26.5%) segmentectomy, and 1 (1.5%) standard radical mastectomy (traditional radical mastectomy). Also, 34 patients with negative SLNB had not received ALND. The median number of dissected nodes was 13 (min-max=0-59).

The median disease duration was 12 (1-108) months, and median time from surgery to lymphedema onset was 3.5 (1-84) months for patients.

The majority (68.5%) of the lymphedemapositive patients had early-onset lymphedema of less than 12 months. It was determined that disease duration, lymphedema onset time, and the number of lymph nodes dissected influenced QOL related to the appearance and symptoms domains.

According to clinical lymphedema staging, thirty-three patients (48.5%) who had symptoms such as postoperative arm pain, feeling of heaviness in the arm, numbness, tingling (pins and needles), loss of strength, skin tightness and loss of flexibility were accepted as Stage 0. The stage distribution of other patients was as follows; 24 (35.3%) patients in Stage 1, 10 (14.7%) patients in Stage 2, and 1 (1.5%) patient in Stage 3.

Cancer types among patients were invasive ductal carcinoma (85,3%); invasive lobular carcinoma (10.3%); mixed type (invasive ductal carcinoma and invasive lobular carcinoma) (2.9%); and sarcoma (1.5%) (Table 1).

The pathological stages of the patients in the study were mostly stage 3A (32.4%) and stage 2A (29.4%). The socio-demographic and clinical features of the patients are shown in Table 1.

The QOL of the patients did not differ statistically according to cancer stage (p>0.05, Table 2).

Most of the patients who developed lymphedema after surgery had received CT, (73.5%) and RT (63.2%). In our study, no statistically significant difference was found in the QOL according to treatments received after the diagnosis of breast

	Functions- LQOL	Appearance/Body image-LQOL	Clinical symptoms -LQOL	Overall- LQOL	Mood/Emotions -LQOL
Disease Stage 2A-2B (n=34) 3A-3C (n=29) p	1.2 (1.0-2.6) 1.3 (1.0-2.7) 0.183	1.0 (1.0-2.4) 1.2 (1.0-2.6) 0.744	1.55 (1.0-2.8) 2.0 (1.0-3.6) 0.091	2.0 (1.0-3.3) 2.0 (1.0-3.8) 0.533	5 (3-10) 5 (2-10) 0.839
Severity of Lymphedema Mild (n=33) Moderate (n=20) Severe (n=15) p	1.2 (1.0-2.6) 1.2 (1.0-2.0) 1.4 (1.0-2.7) 0.721	1.0 (1.0-2.4) 1.1 (1.0-3.0) 1.5 (1.0-2.6) 0.249	1.6 (1.0-2.8) 1.6 (1.0-3.6) 2.0 (1.0-3.0) 0.327	2.0 (1.0-3.6) 1.9 (1.3-3.3) 2.6 (1.0-3.8) 0.554	5 (2-10) 5 (2-10) 5 (2-10) 0.437
Lymphedema Stage Stage 0 (n=32) Stage 1 (n=24) Stage 2 + Stage 3 (n=10) p	1.15 (1.0-2.4) 1.35 (1.0-2.6) 1.25 (1.0-2.7) 0.299	1.0 (1.0-2.0) 1.3 (1.0-2.6) 1.7 (1.0-3.0) 0.027	1.5 (1.0-2.6) 1.9 (1.0-3.0) 2.1 (1.6-3.5) 0.002	1.8 (1.0-3.3) 2.3 (1.1-3.8) 2.2 (1.0-3.3) 0.244	5 (2-10) 5 (3-6) 4.5 (2-6) 0.425
Type of Surgery ALND (+) (n=46) ALND (-) (n=22) p	1.25 (1.0-2.7) 1.15 (1.0-2.1) 0.435	1.2 (1.0-3.0) 1.0 (1.0-2.6) 0.897	2.0 (1.0-3.6) 1.5 (1.0-3.0) 0.482	2.0 (1.0-3.6) 1.95 (1.0-3.8) 1.000	5 (2-10) 5 (3-10) 0.272
RT yes (n=43) no (n=25) p	1.2 (1.0-2.7) 1.3 (1.0-2.6) 0.247	1.2 (1.0-3.0) 1.0 (1.0-2.0) 0.026	2.0 (1.0-3.6) 1.5 (1.0-2.6) 0.156	2.0 (1.0-3.8) 2.0 (1.3-3.6) 0.601	5 (2-10) 5 (2-10) 0.907
CT yes (n=50) no (n=18) p	1.2 (1.0-2.6) 1.3 (1.0-2.7) 0.469	1.15 (1.0-3.0) 1.0 (1.0-2.0) 0.188	1.9 (1.0-3.6) 1.55 (1.0-2.3) 0.095	2.0 (1.0-3.8) 2.5 (1.5-3.6) 0.183	5 (2-10) 5 (2-10) 0.575
HRT yes (n=26) no (n=42) p	1.2 (1.0-2.7) 1.25 (1.0-2.4) 0.409	1.15 (1.0-2.4) 1.0 (1.0-3.0) 0.334	1.7 (1.0-2.8) 1.8 (1.0-3.6) 0.756	2.1 (1.0-3.3) 1.8 (1.3-3.8) 0.771	4.5 (2-10) 5 (2-10) 0.588
NT yes (n=16) no (n=52) p	1.25 (1.0-2.7) 1.2 (1.0-2.6) 0.853	1.05 (1.0-2.0) 1.05 (1.0-3.0) 0.434	1.6 (1.0-3.6) 1.8 (1.0-3.5) 0.913	1.8 (1.0-3.0) 2.05 (1.0-3.8) 0.173	5 (2-9) 5 (2-10) 0.240
RT with ALND (n=34) RT without ALND (n=34) p	1.25 (1.0-2.7) 1.2 (1.0-2.6) 0.656	1.4 (1.0-3.0) 1.0 (1.0-2.6) 0.009	2.0 (1.0-3.6) 1.5 (1.0-3.0) 0.017	1.9 (1.0-3.3) 2.2 (1.0-3.8) 0.155	5 (2-10) 5 (2-10) 0.448

LQOL: Lymphedema Quality of Life. ALND: Axillary lymph node dissection, CT: Chemotherapy, RT: Radiotherapy, NT: Neoadjuvant therapy, HRT: Hormone replacement therapy. (Median (Min-Max)).

	Functions- LQOL	Appearance/Body image-LQOL	Clinical symptoms -LQOL	Mood/Emotions -LQOL	Overall- LQOL
BMI r p	-0.035 0.777	0.053 0.670	0.009 0.944	0.005 0.970	-0.202 0.098
Disease Duration (months) r p	-0.117 0.342	0.258 0.033	0.289 0.017	0119 0.332	-0.094 0.445
The onset of BCRL (months) r p	0.019 0.879	0.223 0.068	0.379 0.001	-0.021 0.863	-0.066 0.591
Number of Dissected Lymph Noo r p	de 0.224 0.068	0.347 0.004	0.402 0.001	0.103 0.408	-0.259 0.035
VAS r p	0.441 <0.001	0.252 0.038	0.484 <0.001	0.400 0.001	-0.242 0.047
DASH r p	0.633 <0.001	0.327 0.007	0.420 <0.001	0.367 0.002	-0.286 0.018

 Table 3. Relationship between patient characteristics and quality of life

r: Correlation Coefficient, BMI: Body Mass Index, VAS: Visual Analogue Scale, DASH: Disabilities of the Arm, Shoulder and Hand, BCRL: Breast cancer related lymphedema, LQOL: Lymphedema Quality of Life.

cancer surgery, RT (except the appearance domain of QOL), CT, HRT, or NT (p>0.05, Table 2). There was no significant difference between patients who underwent surgery with or without axillary lymph node dissection (ALND) (p>0.05, Table 2). In addition, patients who had received axillary dissection in combination with RT were associated with poorer QOL related to body image scores included (p<0.05, Table 2).

It was also observed that the increase in the stage of lymphedema worsened symptoms such as arm weakness, pricking, and feeling of heaviness and body image (p<0.05, Table 2). It was observed that as shoulder pain and disability scores increased, scores of all domains of QOL increased except the overall domain in QOL (Table 3). On these questionnaires, low scores indicate less pain (VAS) and disability (DASH).

#### Discussion

Lymphedema is one of the most feared complications after breast cancer treatment. It affects approximately one-third of all breast cancer survivors and may compromise patients' overall QOL due to symptoms such as limitation of upper limb movements, pain, feeling of limb heaviness, skin changes and increased risk of infection (e.g., cellulite). In addition, psychologically, women may experience negative emotions such as loss of selfesteem, anxiety, disappointment, sadness, and anger due to a disturbance in body image. In this study, we evaluated the clinical characteristics and QOL of patients who underwent breast cancer surgery.

In our study, the mean BMI was  $29.240 \pm 5.05$  kg/m<sup>2</sup> (range, 19.8 to 43.4 kg/m<sup>2</sup>). There are studies in the literature that show significant differences in

BMI and QOL score variables<sup>16,17</sup>, but in our study, no significant correlation was found between BMI and QOL.

Although age is assumed to be a factor associated with the risk of BCRL, few studies to date have documented the age-related incidence and prevalence of BCRL. The mean age of the patients was  $52.50\pm9.33$  (range, 36 to 74 years) in this study. However, we found no differences between age and QOL in BCRL patients. In cancer survivors, the normal aging process can affect cancer treatment and QOL over time. Studies show that breast cancer has a greater effect on QOL in younger patients than in the elderly.<sup>18,19</sup>

BCRL can occur even years after breast cancer treatment is completed.<sup>20</sup> In our study, the average time from surgery to the onset of lymphedema was 3.5 (1-84) months. One prospective study reported that 75% of the BCRL cases were evident in the first year after surgery.<sup>21</sup> Likewise, the study with the longest follow-up (11 years) reported the highest incidence.<sup>22</sup>

While more imaging modalities are needed to investigate the etiology of lymphedema symptoms, the accumulation of lymph fluid in the physiologically affected arm can create a feeling of heaviness, tension, and stiffness, and may also cause neuropathic complaints. In this study, thirty-three (48.5%) of 68 patients were evaluated as stage 0 according to clinical lymphedema staging according to these symptoms. The diagnosis of lymphedema in most patients can be easily determined with anamnesis and detailed physical examination. Objective assessment of lymphedema, such as lymphoscintigraphy and circumferential band measurement, may not detect the early stage, and selfreported symptoms can potentially be a useful and low-cost tool for early screening of lymphedema.<sup>23</sup> In addition to the fear of cancer, these symptoms related to lymphedema are among the most important factors that cause stress in breast cancer patients and negatively affect their QOL.<sup>24</sup> Therefore, it is emphasized that referring patients to physical therapy and rehabilitation clinics immediately after surgery helps to detect early lymphedema in this study. We believe that the negative effects of self-reported lymphedema evaluated in the latency phase with stage 0 on quality of life should not be ignored.

In the earliest stages of BCRL, mild changes occur, along with a feeling of heaviness in the arms or hands, discomfort, or both. In the mid-advanced stage, limb edema does not subside with elevation or external pressure, and the affected area may become enlarged and show severely dry, scaly thickened skin. In this study, women with advanced lymphedema stage had statistically significantly lower median QOL scores for body image and clinical symptoms. Clinical symptoms may vary depending on the severity and course of BCRL.<sup>25,26</sup>

There are studies showing that BCRL is related to specific treatment modalities, particularly ALND, a greater number of lymph nodes dissected, RT and CT, alone or in combination. Surgical technique, CT, and RT after surgery extend the life of patients but negatively affect their QOL.<sup>5,27-29</sup> In our study, 34 patients with negative SLNB had not received ALND and there was no significant difference between the patients who underwent ALND and those who did not undergo ALND in terms of QOL. This finding was concordant with a study by Velanovich et al., showing that ALND alone does not impair QOL.<sup>30</sup> There are some conflicting results in the literature. In 3 studies with 1755 participants, it was reported that the quality of life was better after SLNB than ALND in two studies and no difference was observed in the other study.<sup>31</sup>

In our study, the median number of dissected nodes was 13 (min-max=0-59). These results suggest that QOL in BCRL associated with axillary surgery may depend on the number of nodes removed in the lymphatic system. In addition, in this study, a significant relationship was found between the number of lymph nodes dissected and scores for QOL in the domains of clinical symptoms and body image.

In addition to possible risk factors for BCRL, there are conflicting results on adjuvant and NT. In this study, no statistically significant difference was determined between CT, HRT, NT implemented following the diagnosis of breast cancer, and all domains in QOL. Some studies show that adjuvant CT is a potential risk factor for BCRL and reduces the QOL.<sup>32,33</sup> HRT has been associated with various side effects (arthralgia, osteoporosis) that reduce the QOL and treatment compliance of breast cancer patients.<sup>34-36</sup>

Radiation therapy is an important and approved treatment method in all clinical stages of breast cancer patients. Skin injury is a common side effect of breast cancer radiation therapy. Changes such as sunburn-like rash, skin peeling, and darkening of the skin can be seen in the treated area, which highly affect the body image of the patients. In this paper, we observed that women who received RT had negative QOL body image scores, negative feelings towards themselves and relationships with other people. Negative body image inevitably affects a woman's mood and interpersonal relationships, leading to social stigma and hence social isolation. Conditions including scarring, pigmentary changes, chronic radiation dermatitis, and radiation fibrosis have been associated with decreased QOL and impaired function.<sup>37</sup>

In our study, QOL of patients who received the combination of ALND and RT, was statistically lower regarding the body image domain. Some studies conducted on breast cancer survivors report that RT is associated with a 2-4.5 times higher risk of lymphedema, while RT received in combination with ALND poses an 8-10 times higher risk of lymphedema.<sup>38</sup>

Lymphedema can cause limitations in range of movements, pain, weakness, paresthesia, dysesthesia, stiffness, and upper limb function limitation in the affected arm. In this study, a statistically significant correlation was found with shoulder pain and disability scores in all domains of the QOL of patients with lymphedema. A recent study showed that shoulder abnormalities on ultrasonography (e.g., supraspinatus tear, biceps tenosynovitis, acromioclavicular arthritis, subdeltoid bursitis, and adhesive capsulitis) and pain factors are associated with upper extremity dysfunction and poor quality of life in patients with BCRL.<sup>39</sup>

The current study has some limitations. First, the sample size was small. Second, this study is not a longitudinal study; it is cross-sectional. Therefore, we could not observe a longitudinal change in quality of life in patients, as we included patients in different postoperative periods. Therefore, future longitudinal and large-scale prospective studies are needed.

In conclusion, although QOL did not differ significantly in terms of clinical and demographic characteristics, it was observed that clinical symptoms and body image in QOL worsened as the lymphedema stage and the number of lymph nodes dissected increased. Based on the results of this study, ALND plus RT and RT alone after breast cancer surgery were associated with a lower score in body image domain in QOL. In addition, this study revealed how much body image perception can be related to the QOL in patients with mostly subclinical lymphedema. These negative effects of lymphedema on quality of life can be minimized with collaboration between Physical Medicine and Rehabilitation and General Surgery Clinics.

## **Conflicts of Interest**

The authors declare that they have no conflict of interest.

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# Breast Cancer Prevalence and Management in Hispanic Women: Comparison to Black and White Women at a Regional Medical Center

Sara Perregaux<sup>a</sup>, Stella Self<sup>b</sup>, Justin Collins<sup>c</sup>, Sarah Renfro<sup>d</sup>, Charlotte VanHale<sup>a</sup>, Ahmer Ansari<sup>a</sup>, Brian McKinley<sup>e</sup>, Mary Rippon<sup>e</sup>, Christine Schammel<sup>\*f</sup>, David Schammel<sup>f</sup>

<sup>a</sup> University of South Carolina School of Medicine Greenville, Greenville SC, USA

<sup>b</sup> Department of Mathematics and Statistics, Clemson University, Clemson SC, USA

<sup>°</sup> Medical University of South Carolina, Charleston SC, USA

<sup>d</sup> University of North Carolina Chapel Hill, Chapel Hill NC, USA

<sup>e</sup> Department of Surgery, Prisma Health, Greenville SC, USA

<sup>f</sup> Department of Pathology, Greenville SC, USA

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# ABSTRACT

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Key words: Hispanic ethnicity, breast cancer, disparities, epidemiologic strategies, state versus SEER **Background:** While stage and grade of breast cancer determines prognosis and outcome, race also impacts survival. While Black and White women have been studied, data for Hispanic women is sparse.

**Methods:** Age-matched Hispanic, Black and White women diagnosed/treated with breast cancer at a single institution were retrospectively evaluated regarding prevalence, treatments and outcomes.

**Results:** Overall, 120 women were included in the study (40 per race). No demographic/histologic variables were significantly different among races. ER+/PR+ tumors were less frequent in Hispanics than Whites, but higher than Blacks. Prevalence of triple negative breast cancers in Hispanic women was between the Black and White cohorts (p=0.025 and p=0.011, respectively). Stage II and III diagnoses (p=0.025) were more frequent in Hispanics and they opted for chemotherapy more often (p=0.034); however, there were no significant differences in outcomes and mortality among groups. When compared to the State tumor registry, our population had more LCIS diagnoses (p=0.01), earlier stages (I p=0.02; II p=0.006), received more treatment overall (radiation p=0.02, chemotherapy p=0.0001) and experienced better survival (p=0.004). In comparing the study population to the SEER database, higher rates of LCIS and IDC and lower rates of ILC and mixed histology in the study population were noted. LCIS and IDC were more prevalent in our cohort than SEER data (p=0.005, p=0.05, respectively), although we noted less ILC and mixed histology (p=0.03 and p=0.04).

**Conclusion:** These data are the first reported for Hispanics in our state and highlight the need for larger studies to better serve this growing demographic.

Introduction

In the United States, women have a one in eight lifetime risk of developing breast cancer, <sup>1</sup> the second

\* Address for correspondence: Christine Schammel, PhD Department of Pathology, Pathology Associates, Associate Director of Medical Student Research Address: University of South Carolina School of Medicine Greenville Greenville SC 29605 Tel: +1 864-363-6225 Email: <u>Christine.schammel@prismahealth.org</u> leading cause of cancer-related mortality in women (21.5 deaths per 100,000 women).<sup>2</sup> While stage at diagnosis and hormone receptor status affect prognosis, factors such as age, race and socioeconomic status also have an impact. <sup>3,4</sup> While the impact of these factors between Black and White women has been extensively studied, <sup>1, 2, 5-14</sup> data concerning Hispanic women is sparse. <sup>15,16</sup>

An evaluation of disparities in stage at diagnosis, prognosis and outcomes among Hispanic, Black and

White women with breast cancer <sup>5,17</sup> revealed higher stage/grade cancers at diagnosis for Hispanic and Black women (stage III and IV; >50%), portending a poorer prognosis. Other studies noted that Hispanic women, like Black women, were more likely to have ER-, PR-, and aggressive ER-/PR-/HER-2- tumors,<sup>18</sup> also resulting in limited therapy options.<sup>19-21</sup>

While pathologic differences in breast cancer have been noted, the etiology has not been definitively elucidated. Estrogen exposure variation, <sup>22-25</sup> influenced by cultural norms, may play a role. For example, estrogen exposure fluctuates with parity and duration of breastfeeding, impacting the prevalence of more aggressive phenotypes of breast cancers.<sup>22-25</sup> Increased parity is associated with a greater incidence of Her-2 positive breast cancer, for which there is a targeted therapy; breastfeeding for >36 months may be protective from development of ER-/PR-/Her-2 lesions<sup>23,24</sup> while an earlier age at first pregnancy/late menopause is associated with an increased incidence.<sup>22</sup> Interestingly, estrogen activity in Hispanic women was found to increase commensurate to length of US residency, correlating with breast cancer incidence.<sup>25</sup> These data suggest that different environmental/social/cultural factors may impact the underlying pathophysiology of breast cancer development and, thus, treatment options and prognosis.

In the United States, the Hispanic population continues to grow, particularly in the Southeast.<sup>21</sup> The Hispanic population in South Carolina grew 154% during 2000-2011, the second fastest in the US. While recently this rate has slowed, comparatively, this region still has the largest growth in the US (33%; 2008-2018).<sup>27</sup> These data necessitate an assessment of breast cancer management, diagnosis, treatment, and outcomes, for Hispanic women in our area. We performed a retrospective review to compare pathologic factors at diagnosis, treatments and outcomes in an age-matched cohort of Hispanic, Black, and White women at a single regional medical center in order to better elucidate patterns that could directly impact patient care of this demographic.

## Methods

Following IRB approval, all female breast cancers diagnosed and/or treated at a single institution between 1/1/2000 and 12/31/2010 were retrospectively evaluated. Those for whom race information and complete records were unavailable were excluded. Race comparison was made using three categories: Hispanic, Black and White. While Hispanic is an ethnicity, individuals that self-reported as Hispanic or Latino were included in this cohort; sub-classifications of black-Hispanics and white-Hispanics were not considered. Patients that met inclusion criteria were initially age-matched across race classification. Specifically, 40 Hispanic patients were age-matched with 40 Black and 40 White patients. Demographic and clinicopathologic data were collected for each patient to include age at diagnosis, diagnostic modality, histologic type, stage and grade at diagnosis, hormone receptor status, treatments, and outcomes. Histology included lobular carcinoma insitu (LCIS), ductal carcinoma insitu (DCIS), invasive lobular carcinoma (ILC) and invasive ductal carcinoma (IDC). Histology, stage, grade and receptor status for each patient was verified by a board-certified, fellowship trained breast pathologist. Hormone receptor status was not included for any patients with LCIS, as testing hormone receptors is not protocol for this histological type. Her-2 status was not evaluated for patients with DCIS per College of American Pathology (CAP) protocol.<sup>28</sup> As our institution is the referral center for community, need-based medical providers, referral origin and insurance status were also collected. Insurance status was defined as no insurance, private insurance, public/government insurance, and insurance not otherwise specified. As our institution is a small academic medical center, care is provided to patients regardless of their ability to pay. To further estimate socioeconomic status, zip code was collected; the US Census Bureau's website (factfinder.census.gov)<sup>29</sup> was used to collect median income, educational status, and poverty statistics for each zip code as an estimate of socioeconomic impact. The average of the median income data collected for the entire cohort was calculated and patients were stratified as 'above' or 'below' the average. Follow up/survival was considered from the date of diagnosis. While diagnosis dates varied, all diagnoses took place in 2000-2010, with follow-up until 8/2019. Kaplan Meier survival curves were used to represent these data with varied stratifications.

Our institutional tumor registry provided breast cancer diagnosis data from the hospital system for comparison to the age-matched cohort; data provided included demographic and clinicopathologic data such as race, age at diagnosis, histologic type, stage and grade at diagnosis, hormone receptor status, and outcomes. In addition, our institutional tumor registry was able to procure data from the state cancer registry<sup>29</sup> (SCCR) for comparison to the age-matched cohort to include race, age, histology and overall outcomes for new breast cancer diagnoses in the state. Per reporting guidelines, state cancer registry data includes data from our institution.

National data was procured from the Surveillance Epidemiology and End Results (SEER) program database (SEER)<sup>30</sup> using SEER 18 as a comparison. Hispanic, Black and White data was extracted excluding other races.

Analysis of variance (ANOVA) was performed if the data was non-binary and Pearson's Chi-square statistical test was performed if the data was binary in order to compare outcomes between the race cohorts. If the results of the ANOVA showed significance, Levene's test for equal variances was performed to determine if the variances between the groups were equal. If they were equal, then a Fisher's Least Significant Difference test was performed. If the variances were not equal, then Tamhame and Dunnett's post-hoc tests were performed. A multivariate linear regression was used to measure collective influence of variables on mortality. The  $\alpha$ level was set at 0.05. All results were analyzed through the statistical program SPSS (Version 23, Copyright 2015). Data collected was compared to state breast cancer data from 1/1/1997-12/31/2007 stratified by race. The National SEER database (SEER 18) was queried to compare local and state data to national breast cancer trends. All data was normalized for appropriate local, state and national comparison. The size of the cohort was considered for all analyses.

#### Results

The average age for the cohort was 55.88 with the average age of Hispanic women 56.13, Black women 55.75 and 55.93 years for White women (p=0.947; data not shown); there was no significant difference across the three races for histological type (p=0.745; data not shown). While 16.7% of the patients were diagnosed with DCIS, the prevalence was greater in the Black cohort (22.5%) when compared to the White/Hispanic cohorts (15% and 12.5% respectively). LCIS was diagnosed in 5.8% of the patients, with a prevalence of 7.5% in both Black/White cohorts and 5% in the Hispanic group. IDC was the most common histological presentation

Table 1.	Demograp	hics of	the	cohort
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of breast cancer in our group (70.8%), affecting 82.5% of Hispanic, 70% of White, and 60% of Black women. ILC was found in 4.2% of patients, most frequently in Black women (7.5%) compared to White (2.5%) and Hispanic (2.5%) groups.

Individual hormone receptor status was evaluated for each cohort. ER status was not significantly different (p=0.253; data not shown) nor was Her-2 status (p=0.503; data not shown). PR status (PR+ or PR-) was significantly different, with White women having more PR+ lesions (p=0.011; data not shown). Combination ER+/PR+ (p=0.025) and triple negative cancers (p=0.009) were found to be significant across the three races; however, ER+/PR+/Her-2+ status was not significant (p=0.780; Table 1).

Stage at diagnosis was also found to be significant between the races (p=0.025; Table 1). While insurance status was not significantly different across the three races (p=0.2422; Table 1), it was significantly different between Hispanic and White women (p=0.005; data not shown). Treatments included surgery, radiation, chemotherapy, hormone therapy, or a combination of multiple therapies (Table 1). The majority of patients opted for surgery (88.33%; n=106), while 56 (46.7%) opted for radiation. Hispanic women received chemotherapy significantly more often than Black/White patients (63% versus 43% and 48%, respectively; p=0.0304; Table 1). No significant difference was noted between the cohorts for use of hormone therapy (p=0.740). As expected, the majority of our patients (95%) had multiple modality treatment.

In addition to stratification by race, the data was stratified by stage at diagnosis. As mentioned

	Total	Hispanic	Black	White	P*
Receptor Status†	n=95	n=35	n=29	n=31	
ER+/PR+	63 (82.3%)	22 (57.5%)	14 (32.5%)	27 (72.5%)	0.025
ER+/PR+/Her-2+	13 (11.7%)	6 (15%)	3 (7.5%)	4 (12.5%)	0.780
ER-/PR-/Her-2-	19 (15.8%)	9 (20%)	9 (25%)	1 (2.5%)	0.011
Stage	n=120	n=40	n=40	n=40	0.025
0	25 (20.8%)	5 (12.5%)	11 (27.5%)	9 (22.5%)	
1	33 (27.5%)	9 (22.5%)	9 (22.5%)	15 (37.5%)	
2	51 (42.5%)	21 (52.5%)	15 (37.5%)	15 (37.5%)	
3	6 (5%)	5 (12.5%)	1 (2.5%)	1 (2.5%)	
4	5 (4.2%)	1 (2.5%)	4 (10%)	0 (0%)	
Insurance Status					0
Not insured	19 (16%)	11 (27.5%)	4 (10%)	4 (10%)	
Private	61 (51%)	15 (37.5%)	18 (45%)	28 (70%)	
Public	24 (20%)	7 (17.5%)	13 (32.5%)	4 (10%)	
NOS	16 (13%)	7 (17.5%)	5 (12.5%)	4 (10%)	
Mortality Status‡					
Alive	106 (72.5%)	37 (92.5%)	31 (77.5%)	38 (95%)	
Dead	14 (11.7%)	3 (7.5)	9 (22.5%)	2 (5%)	
Treatment§					
Surgery	107 (88.33%)	35 (90%)	33 (80%)	38 (95%)	0.104
Radiation	56 (46.7%)	18 (45%)	18 (45%)	20 (50%)	0.967
Chemotherapy	59 (48.3%)	25 (62.5%)	15 (35%)	19 (47.5%)	0.034
Hormone Therapy	63 (50.8%)	22 (55%)	19 (42.5%)	22 (55%)	0.740
Combination	114 (80.8%)	39 (92.5%)	38 (95%)	37 (55%)	0.137

\*p-values are based on a comparison of all three race cohorts; †Hormone receptor status completed per AJCC/CAP guidelines; ‡As of 2016 per the study completion date; §Patients may have received multiple treatments.



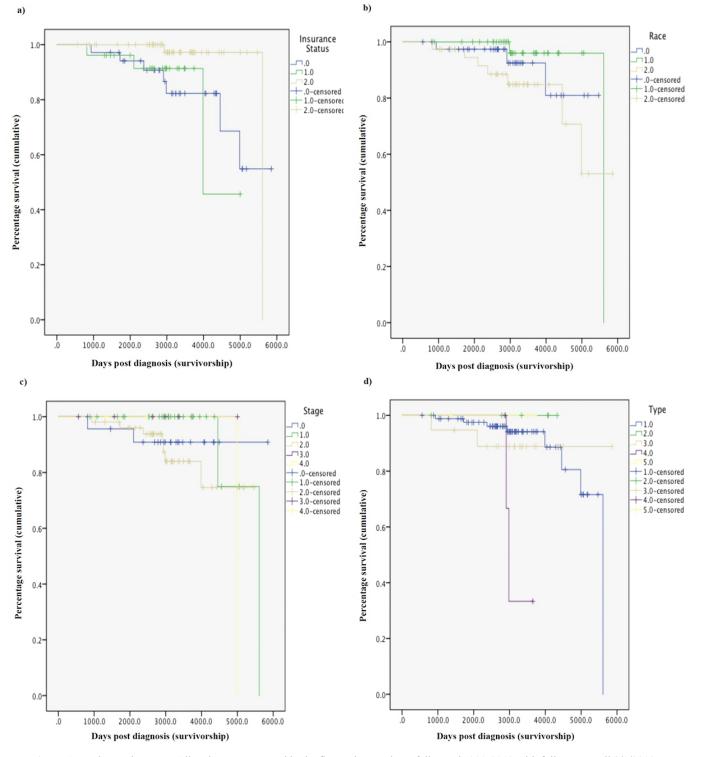


Figure 1. Kaplan Meir curves. All patients represented in the figures have a date of diagnosis 200-2010 with follow-up until 8/1/2019. a. survival based on patient insurance. 0 signifies no insurance, 1 government insurance (medicare or medicaid only) and 2 indicates private insurance (includes medicare with supplements). Survival is calculated in days based on a patient's date of last contact. The highest survival probability overall and at all time points where data exists is for private insurance patients. It is interesting to note that the difference between no insurance and government insurance is small at best with some time periods actually slightly favoring no insurance. b. survival based on race. 0 signifies Hispanic, 1 signifies Black and 2 signifies White patients. Black patients appear to outlive both Hispanic and White women; although all races have similar survival near the end of the study. This indicated that there may be little difference between the groups. c. survival by stage at diagnosis. Stage classification is noted. Patients diagnosed at stage 0 have an immediate worse survival than all other stages but have better survival overall. As expected, stage 1 patients have high survival until approximately 4500 days where survival matches that of stage 2 patients. Though stage 3 and 4 are included on the diagram, the limited number of patients renders that information inconclusive. d. survival by histologic type. 1 is IDC, 2 is LCIS, 3 is DCIS, 4 is mixed IDC and ILC, and 5 is ILC. IDC was the most common type in our cohort. IDC's curve is most defined and has the second best survival. DCIS has fewer patients and a less robustly defined curve but the data does show better overall survival at the later point of the time period. Specifically, the patients with DCIS that do make it past a certain threshold tend to do better than all other patients even though they have a steeper decline in survival at the early time points. It is important to note that LCIS, mixed IDC/ILC and ILC all have such small patient cohorts that the survival curve here is not meaningful. As with all graphs, a chi-square statistic from a log-rank test would be needed to compare the curves for statistical significance.

previously, Stage IV disease was found to be significantly less represented compared to Stages 1/2 (p<0.001; data not shown). Surgery and radiation were found to be significant across stages (p=0.001, p=0.028, respectively; data not shown), as was chemotherapy (p<0.001); however, hormone therapy was not found to be significant (p=0.612). There was no significant difference between the races at each stage (data not shown).

Kaplan-Meir curves were performed to demonstrate survivorship outcomes based on patient insurance, race, stage at diagnosis, and histologic type (Figure 1). Survivorship based on race (Figure 1b) revealed all races have similar survival near the end of the study. Survivorship by stage at diagnosis (Figure 1c) and by histology (Figure 1d) are also represented.

In addition to our single institution's data, we compared data on staging between races from the state tumor registry. There were no significant differences between our institution and state data in terms of age, ER or PR status (data not shown). Interestingly, while Her-2 status was similar for institution and state Hispanic and White women, Black women at our institution had a significantly higher prevalence of Her-2 negative lesions than reported by the state (p=0.05; data not shown). Receptor combinations were not determined by the state eliminating comparison. No significant differences were noted for DCIS, IDC, ILC and mixed histology tumors between our institution and the state registry (Table 2); however, the prevalence of total LCIS and LCIS for Black and White women was significantly higher at our institution (total p=0.01; Black p=0.003; White p=0.04; Table 2). While the prevalence of LCIS for Hispanic women at our institution was also higher than in the state (5%)versus 3.9%, respectively), this was not significantly different (p=0.74). Stage at diagnosis was also compared. There were no significant differences in Stage 0 (total and across all races; Table 2); however, our institution had significantly less total Stage 1 diagnoses than the state (27.5% and 37.7%, respectively; p=0.02). Interestingly, the difference for each race cohort for Stage 1 at diagnosis was not significantly different (Table 2). Total Stage 2 diagnoses were significantly higher at our institution (42.5% versus 28.4%; p=0.006) manifesting across all race cohorts; only the prevalence in Hispanic women was significantly different (p=0.02; Table 2). Stage 3 diagnoses were lower at our institution across every group (total, Hispanic, Black and White) with only the Black prevalence significantly different (2.5% and 12.9%, respectively; p=0.05). Stage 4 diagnoses were not significantly different for any comparison (Table 2).

While surgical interventions were lower overall at our institution compared to the state (88.3% versus 91.1%; p=0.3), the incidence for each race cohort was not significantly different (Table 2).

Total radiation and chemotherapy treatments were significantly higher at our institution (p=0.02, p=0.001, respectively) than the state registry records. While hormonal therapy was given more often at our institution when compared to the state, this was not significant (50.8% and 44.7; p=0.18). The incidence of these treatment modalities was also significantly greater in Hispanic and White cohorts; however, while radiation in the Black cohort was greater than the state registry, chemotherapy and hormone therapy was less frequently used in this cohort at our institution. These differences were not significantly different (Table 2).

Outcomes were determined by mortality at the conclusion of the study period. Survival at our institution was higher than reported in the state registry across all comparisons. Total survival was 88% for our institution compared to the state registry at 77% (p=0.004; Table 2). Hispanic women had a significantly better survival at our institution (92.5% versus 71.4%; p=0.004; Table 2) as did White women (95% versus 79.3%; p=0.0001; Table 2). Black women also had a better survival when compared to the state registry (77.5% versus 72.7%); however, this was not significantly different (p=0.5).

When comparing state and SEER data, DCIS, LCIS, IDC and mixed (IDC/ILC) histologies were significantly different (p=0.0001, p=0.0013, p=0.0001, p=0.0001, respectively; Tables 2 and 3); ILC prevalence was not significantly different (p=0.056; data not shown). Only IDC was greater in the state data compared to SEER (69.4% and 63.1%, respectively). When comparing the data across individual races, LCIS was less for South Carolina Hispanic women (3.9% versus 10.8%; p=0.0001) as was mixed histology (4.2% versus 7.1%, respectively; p=0.05). DCIS was also less within the Hispanic cohort (12% state versus 15.1% SEER; p=0.14; Table 2 and 3), but IDC was more prevalent (74.9% state versus 73% SEER; p=0.46). Black and White women were significantly different across all histologic classifications with DCIS, LCIS, ILC and mixed histology less represented in the state registry. For both races, DCIS was significantly less frequent (Black 17% state versus 19.2% SEER; White 15.8% state and 18.1% SEER; p<0.001), with LCIS also being less frequent in the state data compared to SEER (1.6% Black and 2.9% White, respectively). IDC was also less frequent in the state registry for both races (73% and 68.3%) compared to SEER (75.1% and 71.5%, respectively; Tables 2 and 3). Likewise, ILC was identified in 5.6% of Black and 8% of White women while mixed histology was noted in 2.8% of Black and 5.5% of the White cohort; both were significantly less than SEER reports (p=0.0001).

The comparison of our histology data to SEER 18 histology is outlined in Table 3. While overall DCIS was more frequently diagnosed nationally then at



our institution, this was not significantly different (p=0.5). Likewise, our Hispanic and White cohorts had a lower prevalence of DCIS compared to national trends, but this was not significantly different (Table 3). Black women were more likely to be diagnosed with DCIS at our institution, but this was not significant (p=0.59). LCIS, IDC, ILC, and mixed histology were all significantly different at our institution when compared to SEER 18 (p=0.005, p=0.05, p=0.03, p=0.04, respectively; Table 3), with LCIS diagnosed significantly more often than SEER 18 (6.7% versus 2.6%, p=0.005). When comparing the prevalence of IDC between our institution and the SEER 18 data overall, our institution had a significantly higher prevalence (71.8% versus 63.1%, p=0.05) but we had significantly less ILC compared to the SEER 18 data (2.5% versus 7.7%, p=0.03). Our institution also had significantly less mixed histology when compared to the SEER 18 data (3.3% versus 8.4%, p=0.04).

#### Discussion

The Hispanic population comprises 9.3% of the population of which our healthcare system serves, and this percentage is predicted to continue to grow. <sup>26, 27, 31</sup> In terms of women's health in our state, race differences in breast cancer prevalence, treatments and outcomes have been evaluated for black and white cohorts; however, to date, no evaluation for Hispanic women has been completed. Our study addresses this deficiency.

Nationally, the average age of breast cancer patients at diagnosis is 62 years; (cancer.gov)<sup>32</sup> however, the average of our cohort was younger, 55.8 years. In particular, the Hispanic population we serve is primarily under 50 years (suburbanstats.org).<sup>33</sup> While histology and receptor status portend prognosis and outcome<sup>30</sup>, our data did not demonstrate any significance in histological subtypes between races, suggesting that differences in treatments and outcomes were not due to variables in biology. When evaluating histology between our institution and the state data, our population had

Table 2. Comparison of institutional data to state data.

	Total			Hispanic			Black			White		
	Our Study n=120	SC n=35646	p-value	Our Study n=40	SC n=297	p-value	Our Study n=40	SC n=8688	p-value	Our Study n=40	SC n=26661	p-value
Histology	n=120	n=30411		n=40	n=259		n=40	n=7192		n=40	n=22960	
DCIS	19(15.8%)	4873(16%)	0.86	4(10%)	31(12.0%)	0.71	9(22.5%)	1221(17.0%)	0.36	6(15%)	3621(15.8%)	0.88
LCIS	8(5.8%)	695(2.3%)	0.01	2(5%)	10*(3.9%)	0.74	3(7.5%)	116(1.6%)	0.003	3(7.5%)	569(2.5%)	0.04
IDC	86(70.8%)	21116(69.4%)	0.74	33(82.5%)	194(74.9%)	0.29	25(62.5%)	5251(73.0%)	0.06	28(70%)	15671(68.3%)	0.82
ILC	3(4.2%)	2256(7.4%)	0.18	0(0%)	13(5.0%)	0.15	2(5%)	402(5.6%)	0.6	1(2.5%)	1841(8.0%)	0.19
Mixed <sup>†</sup>	4(2.5%)	1471(4.8%)	0.23	1(2.5%)	11(4.2%)	0.19	1(2.5%)	202(2.8%)	0.91	2(5%)	1258(5.5%)	0.89
Stage	n=120	n=21437		n=40	n=198		n=40	n=5098		n=40	n=15615	
0	25(21.7%)	4154(19.3%)	0.64	6(15%)	33(16.7%)	0.79	11(27.5%)	1026(20.1%)	0.25	9(22.5%)	3095(19.8%)	0.67
1	33(27.5%)	7875(36.7%)	0.02	9(22.5%)	63(31.8%)	0.24	9(22.5%)	1472(28.9%)	0.37	15(37.5%)	6340(40.6%)	0.69
2	51(42.5%)	5936(27.6%)	0.006	19(47.5%)	56(28.3%)	0.02	15(37.5%)	1626(31.9%)	0.45	15(37.6%)	4254(27.2%)	0.14
3	6(5%)	2018(9.4%)	0.07	4(10%)	31(15.7%)	0.35	1(2.5%)	653(12.9%)	0.05	1(2.5%)	1334(8.5%)	0.17
4	5(4.2%)	928(4.3%)	0.92	1(2.5%)	15(7.6%)	0.24	4(10%)	321(6.3%)	0.34	0(0%)	592(3.8%)	0.21
Treatment§	n=120	n=35646		n=40	n=297		n=40	n=8688		n=40	n=26661	
Surgery	107(88.33%)	32570(91.4%)	0.3	35(90%)	265(89.2%)	0.88	33(80%)	7676(88.4%)	0.09	38(95%)	24629(92.4%)	0.54
Radiation	56(46.7%)	13203(37%)	0.02	18(45%)	103(34.7%)	0.2	18(45%)	3210(36.9%)	0.29	20(50%)	9890(37.1%)	0.09
Chemo	59(48.3%)	11072(31.1%)	< 0.0001	25(62.5%)	119(40.1%)	< 0.007	15(35%)	3310(38.1%)	0.69	19(47.5%)	7643(28.7%)	0.008
Hormone	63(50.8%)	15936(44.7%)	0.18	22(55%)	75(25.3%)	0.0001	19(42.5%)	7814(89.9%)	< 0.0001	22(55%)	8047(30.2%)	0.0006
Mortality‡ Alive	106(88.3%)	28717(77.5%)	0.004	37(92.5%)	212(71.4%)	0.004	31(77.5%)	6313(72.7%)	0.5	38(95%)	21145(79.3%)	< 0.0001

‡status noted at the end of the study period; §patients may have had more than one treatment.

Table 3. Comparisor	n of our data to	o SEER histology data*
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	Total			Hispanic			Black			White		
	Our Study n=120	SEER n=326,122	p-value	Our Study n=40	SEER n=29,852	p-value	Our Study n=40	SEER n=33,504	p-value	Our Study n=40	SEER n=262,766	p-value
DCIS	19(15.8%)	59,497(18.2%)	0.5	5(12.5%)	5,405 (15.1%)	0.65	9(22.5%)	6,423 (19.2%)	0.59	6(15%)	47,669 (18.1%)	) 0.61
LCIS	8(6.7%)	8,399(2.6%)	0.005	2(5%)	687 (10.8%)	0.24	3(7.5%)	650 (8.8%)	0.77	3(7.5%)	7,062 (12.3%)	0.36
IDC	86(71.8%)	205,645(63.1%)	0.05	33(82.5%)	19,880 (73.0%)	) 0.18	25(62.5%)	22,253 (75.1%)	0.07	28(70%)	163, 512 (71.5%	6) 0.83
ILC	3(2.5%)	25,025(7.7%)	0.03	0(0%)	1,940 (7.1%)	0.08	2(5%)	1,967 (6.6%)	0.68	1(2.5%)	21, 118 (9.2%)	0.14
Mixed‡	4(3.3%)	27,556(8.4%)	0.04	1(2.5%)	1,940 (7.1%)	0.26	1(2.5%)	2,211 (7.5%)	0.23	2(5%)	23,405 (10.2%)	) 0.28

\*SEER 18 data used as a comparison; †Totals include only the races compared in this study; ‡mixed histology indicates IDC and ILC.

more LCIS overall, as well as in each race cohort. This suggests that breast cancer at our institution is identified earlier in progression, which may partially explain the better outcomes for all races at our institution compared to the state. This pattern is repeated when comparing our data to the national SEER histology. These data may reflect pathology expertise in identifying early lesions in cooperation with dedication to a multidisciplinary management of breast cancer in conjunction with the state's Best Chance Network (scdhec.gov) referral network.<sup>34</sup>

Hormone receptor status also portends treatment options and outcomes.31 Incidence of ER+/PR+ lesions for Hispanic women were between Black and White rates, with Black women having the lowest ER+/PR+ incidence.<sup>22, 32</sup> Likewise, the incidence of ER-/PR-/Her-2- (triple negative) breast cancer in Hispanic women was found to be between Black and White women in our study, reflecting the national findings.<sup>22, 33</sup> Overall, this supports the plethora of literature indicating that hormone receptor negative lesions are more frequent in non-white patients<sup>19,20,3</sup> a phenomenon postulated to be associated with genetic/environmental factors related to estrogens and, even, country of birth.<sup>35</sup> While this may not be true for Black women<sup>34</sup>, this is plausible regarding the lower ER+/PR+ and higher ER-/PR-/Her-2- rates in Hispanic women, given the more recent immigration of this population.<sup>26,27</sup> While these data were unavailable for our cohort, an analysis of these factors would be useful in determining risk and prognosis of Hispanic women longitudinally within the community as other environmental factors begin to potentially influence their breast cancer biology.

Regarding stage of diagnosis between different races, less Hispanics were diagnosed with stage I lesions than white women, supporting prior studies reporting later stages at diagnosis for Hispanic women.<sup>25, 36</sup> This observation has been attributed to lower utilization of mammography and delayed follow-up after an abnormal mammogram<sup>37-39</sup> suggesting barriers to health care access for this demographic. Conversely, the Best Chance Network, free clinics and low-income clinics which all refer to our institution indicate that the Hispanic population actually over-utilizes mammography services. Thus, in our Hispanic cohort, the later stage at diagnosis may reflect a lack of timely patient follow-up regarding an abnormal mammogram or the increased radiation exposure due to greater than yearly imaging, highlighting the importance of creative and culturally relevant educational relationships within this community to foster awareness and appropriate screening for this population. When comparing stage between our institution and the state registry, we had fewer Stage 0/I patients, but a significantly greater number of Stage II diagnoses; additionally, fewer Stage III/IV diagnoses were noted at our institution. These differences may reflect variation of demographics between the regions of the state.

Interestingly, all patients diagnosed with Stage 0 had a poorer prognosis initially when compared to later stages, potentially due to patient perception, causing neglect of appropriate follow-up and delay of suggested treatments. Additionally, Stage 0 patients may opt for limited treatment despite NCCN guidelines (nccn.org)<sup>40</sup> of lumpectomy or mastectomy. These perceptions may be overcome by enhanced patient education<sup>25</sup>, particularly for patients with lower levels of education and those with limited health literacy.<sup>25</sup> Additionally, language and cultural barriers may hinder the appropriate transmission of health information<sup>41,42</sup>, including breast health.<sup>34,41</sup>

All patients at our institution were treated with the standard of care as noted in no significant differences among races. This is in contrast to the literature, which reports that Hispanic and Black women are often less likely to receive guideline concordant treatment.<sup>32,43,44</sup> When comparing our treatments to the state and SEER data, our institution more significantly utilizes radiation, chemotherapy and hormone therapy than is reported by the state, potentially due to our wellcoordinated multidisciplinary approach to breast cancer treatment. Outcome data demonstrates that our approach appears to be effective, given that our mortality rate is significantly lower when compared to the state registry. It is important to note that our institutional data could not be separated from the state registry data, suggesting that there may even be a larger discrepancy than the comparison reflects.

Finally, in concordance with the current literature, the Hispanic group had significantly more uninsured patients than any other race.<sup>36,44</sup> This may be attributed to fear due to residency status, the language barrier, or other general lack of understanding of the process to sign up for government insurance. Our institution has multiple measures to facilitate insurance procurement, assisting with registration for Medicare/Medicaid and robust language services providing translation and interpretation. Additionally, through the Best Chance Network, free screenings are provided to underserved women. Interestingly, while Hispanics had higher rates of being uninsured in our group, there was no statistical difference in outcome, suggesting equality in treatment.

Overall, despite noted discrepancies among Hispanic, Black and White women, no deleterious impact on outcomes was observed. While our findings were concordant with both state and national data, we report a better survival. While this is encouraging, cultural norms of Hispanic women need to be further examined to eliminate barriers to access, increase education and optimize breast health in Hispanic women. Additionally, genetic variables should be evaluated to elucidate differences that could portend alternate standards in screening guidelines, lifestyle management and potential



Hispanic BC evaluation

targeted therapies. Ultimately, our hope is to open dialogue which would initiate larger studies regarding these issues to better serve the population of our state and region.

## Conflicts of Interest

None.

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## **Ethical disclosure**

The authors state that they have obtained appropriate institutional review board approval and have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved through institutional universal consent (Health Sciences South Carolina Pro00006780).

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# Breast Cancer Cell Lines, HER2/Neu Phenotype, and a Higher Propensity to Reactive Oxygen Species Production

Zahra Mohammadi Abgarmi<sup>a</sup>, Abbas Sahebghadam Lotfi<sup>a</sup>, Saeid Abroun<sup>a</sup>, Masoud Soleimani<sup>b</sup>, Shahla Mohammad Ganji<sup>\*</sup>, Parvaneh Baktash<sup>c</sup>, Arash Moradi<sup>c</sup>

<sup>a</sup> Department of Clinical Biochemistry, Faculty of Medical Science, Tarbiat Modares University, Tehran, Iran

derivatization spectrophotometrically at 360-385 nm.

levels induced by pro-oxidant anticancer agents.

MDA-MB-231 and MCF-7 cell lines.

Background: The reactive oxygen species (ROS) generated in the biological

Methods: Intracellular ROS was determined using (2'-7'dichlorofluorescin

**Results:** The oxidative stress induces ROS (p<0.05), MDA (Malondialdehyde) (p<0.05), and protein carbonylation (p<0.01) was significantly higher in HER2-positive BT-474, SK-BR-3, and MDA-MB-453 compared to the HER2-negative

**Conclusion:** We hypothesized that increased oxidative stress in HER2-positive

cell lines is due to the oncogenic function of the HER2 and PI3K/Akt signaling

activation, resulting in glycolysis induction. It is assumed that HER2-positive cell

lines with high ROS levels are more vulnerable to further damage by increased ROS

systems play an important role in pathological conditions and specific normal

diacetate) DCFH-DA fluorimetric probe, malondialdehyde as lipid peroxidation

index detected by the Thiobarbituric acid reactive substances method. Cells' protein carbonyl contents were assessed with 2,4-Dinitrophenylhydrazine (DNPH)

cellular processes, such as signaling pathways and drug sensitivity.

<sup>b</sup> Department of Hematology, Faculty of Medical Science, Tarbiat Modares University, Tehran, Iran

<sup>°</sup> Department of Molecular Medicine, National Institute of Genetic Engineering and Biotechnology,

ABSTRACT

Tehran, Iran

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#### Introduction

Caused by the reactive oxygen species (ROS), oxidative stress is defined as an imbalance between generation and elimination of the ROS<sup>-1</sup>. ROS is commonly produced in the cells as a consequence of aerobic respiration as well as intermediates in the various enzymatic and non-enzymatic processes<sup>-2, 3</sup>. ROS damages the number of macromolecules such as nucleic acids, proteins, and fatty acids, resulting in a change in their structure and function<sup>-3</sup>. Cells apply

Shahla Mohammad Ġanji

Address: 17Km Tehran- Karaj Highway, Pajohesh Blvd, National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran. PO Box: 14956/161 Tell: +98-21-44 787 466 Fax: +98-21-44 787 399 Email: <u>shahla@nigeb.ac.ir</u> both enzymatic and non-enzymatic mechanisms for neutralizing the produced free radicals<sup>4</sup>. The imbalance between ROS generation and antioxidant scavenging systems induced by oxidative stress<sup>5</sup> is involved in the etiology of various diseases and conditions such as neurodegenerative disorder (Alzheimer, Parkinson), cardiovascular disease (atherosclerosis), inflammatory disease (arthritis, lupus erythematosus), diabetes mellitus, aging, and carcinogenesis<sup>6</sup>. ROS damages cell membrane lipids such as polyunsaturated fatty acids (PUFAs) and initiate lipid peroxidation<sup>7</sup>. Malondialdehyde (MDA) and 4-hydoxynonenal (4-HNE) are important markers of the lipids oxidative damage<sup>8</sup>. ROS's most important destructive effects on DNA are double-strand breaks and purine/pyrimidine modification resulting in the mutation, genomic instability, and an altered gene expression<sup>9</sup>. The

<sup>\*</sup> Address for correspondence:

accumulation of 8-hydroxy guanosine (8-OHdG) is an index of the DNA oxidative damage<sup>10</sup>. The altered redox imbalance toward peroxidants also affects proteins, causing protein fragmentation and production of the carbonyl (CO) groups in lysine, arginine, threonine, and proline residues proteins. Other oxidative changes include converting the cysteine residues in the proteins to disulfide bonds, methionine to methionine sulphoxide, tryptophan to kynurenine formyl kynurenine<sup>11</sup>.

Despite such destructive effects, ROS has many biological functions in normal cells<sup>12</sup>. Recent studies have suggested that ROS act as the second messenger in numerous cellular processes<sup>13</sup>. Disturbance in the balance between ROS concentration and antioxidant systems, resulting in oxidized redox-sensitive proteins activation and triggering signaling pathways <sup>4</sup>. It is also well known that a modest increase in the ROS is involved in the multiple specific signaling pathways in cancer cells, such as survival, proliferation, angiogenesis, and metastasis. At the same time, higher levels of ROS can trigger cell death signaling pathways such as death receptor – and mitochondria-mediated apoptosis, necrosis, and autophagy<sup>15, 16</sup>. The target proteins for ROS in signaling pathways have thiol groups that act as redox sensors<sup>17</sup>. The ROS targeted signaling proteins are receptor tyrosine kinases (RTKs) such as PDGF- $\alpha$ , PDGF- $\beta$ , VEGF, and EGF receptors, non-receptor tyrosine Kinases (NRTKs) such as Src- kinase and Janus kinase (JAK), protein tyrosine phosphatases (PTPs), serine/threonine kinases such as Akt and MAPK (mitogen-activated protein kinase) family, NF- $\kappa\beta$ , AP-1 transcription factor, p21 and p53<sup>13,18</sup>.

Because of the double-edged sword property of the ROS and its role in the survival or cell death, ROS manipulating therapy may be a useful strategy for eliminating cancer cells<sup>18</sup>. This study aims to measure the oxidative stress markers in the breast cancer cell lines (BT-474, SK-BR-3, MDA-MB-453, MDA-MB-231, and MCF-7) as the first step in order to study the drug sensitivity in HER2 positive breast cancer cells.

#### Methods

#### Materials

The human breast cancer cell lines (BT-474, SK-BR-3, MDA-MB-453, MDA-MB-231, and MCF-7) were obtained from the Iranian Biological Resource Centre (IBRC, Tehran, Iran). DMEM and RPMI-1640 cell culture media, fetal bovine serum (FBS), and penicillin-streptomycin were purchased from Gibco, BRL (Life technology, Paisley, Scotland). Trichloroacetic acids (TCA) and phenylmethylsulfonyl fluoride (PMSF) were obtained from Sigma Chem. Co (Germany). Also, 2,4-Dinitrophenylhydrazine (DCFH-DA) was obtained from a Molecular probe (Eugene, Oregon, USA). Dimethyl sulfoxide (DMSO) and thiobarbituric acid (TBA) were obtained from Merck (Darmstadt, Germany).

#### Cell culture

BT-474, MDA-MB-453, and MDA-MB-231 cells were maintained in RPMI-1640 medium with 10% fetal bovine serum (FBS). SK-BR-3 and MCF-7 cells were maintained in the Dulbecco's minimum Eagle's media (DMEM) with 10% FBS. BT-474 and SKBR3 media were supplemented with 2mM L-Glutamine, as well. Penicillin (100U/ml) and streptomycin (100 $\mu$ g/ml) were used to control bacterial contamination in all cell lines culture media. The cells cultured in the T-25 flask were maintained at 37°C in the humidified atmosphere with 5% CO2 and passaged every two days.

#### Cell lysate preparation

For preparation of the cell lysate, the cells were collected, washed with cold PBS (pH 7.4), and were lysed in the ice-cold lysis buffer [50 mM Tris–HCl, pH 7.4, 0.5% (v/v) Triton X-100, 0.1% (w/v) SDS, 150 mM NaCl, 1 mM EDTA] containing 1mM PMSF as protease inhibitor. Cells were kept on ice for 30 min while vortexed every 10 min. The resulting mixture was centrifuged at 14,000 ×g for 15 min at 4°C. The obtained supernatant protein concentration was assayed using Bradford protein assay.

#### Determination of intracellular ROS

Generation of ROS was detected with 2'-7'dichlorodihydrofluorescein diacetate (DCFH-DA) fluorimetric nonpolar probe, which was taken up by the cells and deacetylated by cellular esterases to the polar 2',7'-dichloro-dihydro fluorescin (DCFH) which was oxidized by ROS and other peroxides to highly fluorescent 2',7'- dichlorofluorescein (DCF). Therefore, the intensity of the fluorescence was correlated with the amount of ROS. The experiments were carried out using a stock solution of DCFH-DA (20mM w/v) prepared in DMSO and stored in the dark at -20 °C. For the determination of ROS,  $2 \times 10^4$ cells were seeded per 96-well plate. After 24 h, 100 µL DCFH-DA solution (final concentration of 10 µM) was added to each well and incubated at 37 °C for 45 min in the dark. The fluorescence readings were taken at excitation and emission wavelengths of 480 and 530 nm, respectively, in a BioTek Synergy HT multi-detection microplate reader.

#### Determination of malondialdehyde

Malondialdehyde (MDA) is the end product of polyunsaturated fatty acids (PUFAs) peroxidation in the cells. This reactive aldehyde is among the important indicators of oxidative stress. MDA production was quantified by the thiobarbituric acid reactive substances (TBARS) method. Briefly, cells were seeded in  $3 \times 105$  per well of a 6-well plate, and 500 µL of cell lysates were mixed with 1 mL of 10% (w/v) cold TCA to precipitate proteins. 1 ml 0.67 % (w/v) thiobarbituric acid (TBA) was added to the supernatant and was heated at 95°C for one hour. The pink-colored

product (MDA– TBA complex) absorbance was determined at 532nm spectrophotometrically. The lysates' protein concentration was determined by the Bradford method, and the MDA concentration of the samples was calculated using the following formula: Absorbance at 532nm /  $1.56 \times 105 - 1 \text{ cm}^{-1}$  and expressed as nmol/mg protein.

#### Determination of the protein carbonylation

The protein carbonyl (PCO) content is a common index of oxidative modification of the proteins. Protein carbonyl contents were quantified through evaluating the reaction between 2,4-dinitrophenylhydrazine (DNPH) and protein carbonyls producing Schiff base to convert hydrazone derivatives as determined by applying spectrophotometer at 360-385 nm. Briefly,  $3 \times 10^{\circ}$  cells were seeded per well in a 6-well plate, trypsinized, and lysed with proper lysis buffer at 4°C. A volume of 900 µL of the cell lysate was mixed with 100 µL 10%(w/v) streptomycin solution. Following 15 min incubation, centrifugation was carried out at 5000 rpm  $\times 10$  min, and the supernatant was used to assay protein content. 0.2 ml of dinitrophenylhydrazine (10 mM in 2N HCl) was added to the supernatant. After 1 hr. of incubation at room temperature and vortexing every 10 min, 2 ml of TCA (10 % w/v) was added and centrifuged (3000 rpm, 10 min). The precipitate was washed twice with 4 ml of ethanol/ethyl acetate (1:1, v/v), then dissolved in 1 ml of guanidine hydrochloride (6M, pH = 2.3) and vortexed. Protein carbonyl content was determined at 360nm, and results were reported as nmol applying the Beer-Lambert formula ( $\varepsilon_{\text{DNPH}}$  $=2.29\times10^{4} \,\mathrm{cm}^{-1}\mathrm{M}^{-1}$ ).

#### Statistical analysis

Each experiment was carried out in triplicate, and

all experiments were performed three times. Statistical analysis was performed using SPSS (version 16) and the results are expressed as means  $\pm$  standard deviation (SD). The differences between the two groups were tested using Student's t-test, and a P-value = 0.05 was considered statistically significant.

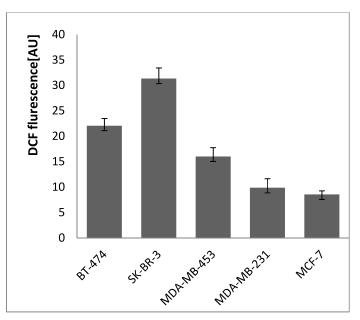
#### Results

*HER2-positive cells and reactive oxygen species production* 

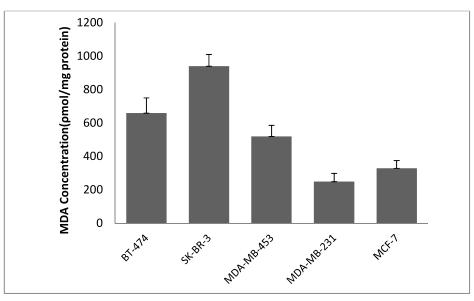
The DCFH-DA probe determined the concentration of intracellular reactive oxygen species (ROS), and the fluorescence intensity was measured in a 96 well plate using a spectrofluorometer. DCF fluorescence intensity correlated with ROS concentration of the cells. Figure 1 shows the relative DCF-fluorescence intensity (mean  $\pm$  SD, N=3) in BT-474, SK-BR-3, MDA-MB-453, MDA-MB-231, and MCF-7 breast cancer cell lines, respectively. A significant difference was detected between ROS levels in HER2-positive BT-474, SK-BR-3, and MDA-MB-453 cells compared with the HER2-negative MDA-MB-231 MCF-7 cells (p<0.05).

#### Evaluation of lipid peroxidation

Malondialdehyde, as a major degradation product of the lipid peroxidation, was determined by the thiobarbituric acid (TBA) test. This spectrophotometric method is based on the reaction between MDA and the two TBA molecules, resulting in a pink-colored complex formation (TBA2-MDA) with maximum absorption at 535nm. The level of malondialdehyde in the HER2-positive BT-474, SK-BR-3, and MDA-MB-453 was significantly higher (p<0.01) than that in HER2-negative MDA-MB-231 and MCF-7 cells, correspondingly (p<0.05). (Figure 2)



**Figure 1.** ROS level of HER2-positive (BT-474, SK-BR-3, and MDA-MB-453) and HER2-negative (MDA-MB-231 and MCF-7) breast cancer cell lines. Each column represents the mean value ± standard deviation of the three independent experiments. (p<0.01). As control, DCFH-DA solution without cells was used in this experiment. The ROS level in the HER2-positive cells was significantly higher as compared with the HER2-negative cells. (p<0.05)



**Figure 2.** Malondialdehyde level of HER2 positive (BT-474, SK-BR-3, and MDA-MB-453) and HER2 negative (MDA-MB-231 and MCF7) breast cancer cell lines. Each column represents the mean ± standard deviation of three individual experiments. (p<0.01). The malondialdehyde level in the HER2 positive-cells was significantly higher as compared with the HER2-negative. (p<0.05)

#### Determination of protein carbonyl content of cells

As a consequence of reactive oxygen species production, cellular protein oxidation results in the inactivation of protein functions. Direct oxidation of lysine, arginine, proline, and threonine (i.e., the primary protein oxidation) amino acids or addition of the reactive aldehyde to the amino acids side chains (secondary protein oxidation) causes the formation of reactive ketones that react with 2,4dinitrophenylhydrazine (DNPH) to form detectable hydrazones. The obtained results indicate that protein carbonyl levels in HER2-positive cells, BT-474, SK-BR-3, and MDA-MB-453 are significantly higher (p<0.01) than HER2-negative cells, including MDA-MB-231 and MCF-7 cells (p<0.05). (Figure 3)

#### Discussion

Breast cancer is the most common type of cancer among women worldwide and the second leading cause of cancer mortality among women in developing countries, and a leading cause of death even in the developed countries.<sup>19-21</sup> Almost one among the eight women in the US will develop invasive breast cancer during their lifetime<sup>22</sup> The HER2 transmembrane receptor tyrosine kinase (RTK) gene has been found to be amplified in approximately 15–30% of invasive breast cancers. Breast tumors with HER2 overexpression are more invasive and resistant to anticancer therapies than breast tumors negative for this gene.<sup>23</sup>

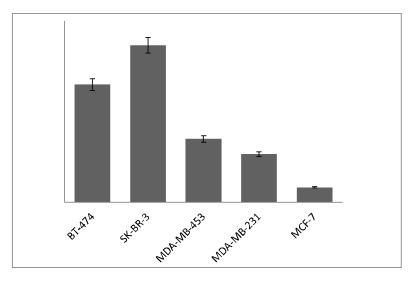


Figure 3. Protein carbonyls levels of BT-474, SK-BR-3, MDA-MB-453, MDA-MB-231, and MCF7 breast cancer cell lines. Each column represents the mean value ± standard deviation of three individual experiments (p<0.01). The protein carbonyl level in the HER2 positive cells (BT-474, SK-BR-3, and MDA-MB-453) was significantly higher than HER2 negative (MDA-MB-231 and MCF7) cells. (p<0.05)</p>

While there is considerable evidence suggesting that cancer cells have elevated levels of reactive oxygen species, ROS's status in HER2 positive cells has not received much research attenttion. The elevated levels of ROS in cancer cells act as a second messenger involved in the induction of signaling pathways, resulting in many biological responses, such as proliferation, apoptosis, cell survival, etc. In the present study, we evaluated the level of ROS, malondialdehyde, and protein carbonyl content as the markers of the oxidative stress in the HER2 positive cells (i.e., BT-474, SK-BR-3, and MDA-MB-453) as well as HER2 negative breast cancer cell lines (i.e., MDA-MB-231, MCF-7). Our findings indicate that as the oxidative stress markers increase, the levels of ROS (Figure 1), malondialdehyde(Figure 2), and protein carbonylation(Figure 3) are significantly increased in the HER2 positive breast cancer cells (i.e., BT-474, SK-BR-3, and MDA-MB-453) as compared with the levels of such markers in the HER2 negative cells including MDA-MB-231 and MCF-7 cells.

There is evidence indicating an association between the HER2 receptor and oxidative stress signaling. Several ROS, in particular, hydrogen peroxide can act as the modulator of PI3K/Akt and p38 MAPK pathways. These pathways are activated by the HER2 receptor<sup>25</sup>. Akt or protein kinase B is one of the important down stream signaling proteins of the HER2 receptor. Up-regulation and aberrant activation of the Akt have been identified in many cancerous cells, such as breast cancer cells<sup>26,27</sup>. Moreover, recent studies have shown that Akt is involved in regulating intracellular reactive oxygen species by stimulating glycolysis and oxidative phosphorylation in the mitochondria via phosphorylation of FoxO transcription factor, ERK1/2, and Rac signaling pathway<sup>28,29</sup>. This evidence supports our finding of the increasing levels of ROS in the HER2 positive breast cancer cells.

Gupta et al., in 2012, showed the positive roles of ROS in tumorigenesis, prevention, and therapy, and by comparing our results with them, we may assume that at lower ROS levels, cell growth and antioxidant production are stimulated. However, at the threshold and higher ROS levels, the cells' antioxidant defense would be exhausted and lead the cells to programmed cell death. In HER2 positive cell lines with high ROS levels, the threshold levels are lower than those in the HER2 negative breast cancer cells<sup>30</sup>.

In conclusion, the findings of this study would predict that HER2 positive breast cancer cell lines including HER2-positive BT-474, SK-BR-3, and MDA-MB-453 with high ROS levels should be more sensitive to the induction of apoptosis by ROS generative agents and may also provide a novel chemotherapeutic means for developing drugs to eliminate HER2 positive breast cancer cells (p<0.05).

## Acknowledgment

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## **Conflict of Interest**

None.

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# Pre-Operative Localization of Axillary Lymph Nodes with Tattoo Ink: A Preliminary Report

Kelly S Myers\*<sup>a</sup>, Sachin Aggarwal<sup>a</sup>, Eniola T Oluyemi<sup>a</sup>, Mehran Habibi<sup>b</sup>, Emily B Ambinder<sup>a</sup>, Armina Azizi<sup>a</sup>, Parvinder Sujlana<sup>a</sup>, Jessica Hung<sup>a</sup>, Nagi Khouri<sup>a</sup>, David Eisner<sup>a</sup>, Philip A Di Carlo<sup>a</sup>, Ashley M Cimino-Mathews<sup>c</sup>, Melissa S Camp<sup>b</sup>

<sup>a</sup> Department of Radiology, Johns Hopkins School of Medicine, Baltimore, Maryland, USA

<sup>b</sup> Department of Surgery, Johns Hopkins School of Medicine, Baltimore, Maryland, USA

<sup>c</sup> Department of Pathology, Johns Hopkins School of Medicine, Baltimore, Maryland, USA

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ABSTRACT

**Background:** Pre-operative localization options in the axilla are limited. This study aimed to explore the utility of pre-operative localization of axillary lymph nodes using tattoo ink with multidisciplinary correlations.

**Methods:** In this prospective, Institutional Review Board (IRB)-approved study, 19 lymph nodes in 17 patients underwent pre-operative localization with ultrasound-guided injection of Spot tattoo ink. The success rate of intraoperative identification of the tattooed node as well as the frequency in which the tattooed node was also a sentinel node were recorded. Radiologic, surgical and pathologic images were collected.

**Results:** Tattoo ink localization was successful in 16/17 (94.1%) of patients. Tattoo ink did not hinder pathologic evaluation in any cases but was taken up by additional adjacent nodes in 1/17 successful localizations (5.9%). Successful sentinel lymph node biopsy (SLNB) occurred in 13 patients in whom 14 lymph nodes underwent pre-operative tattoo ink localization. Nine of the 14 (64.2%) tattooed lymph nodes were also a sentinel node.

**Conclusion:** In this study, pre-operative localization of axillary lymph nodes with tattoo ink was highly successful In patients undergoing SLNB, a significant number of the tattooed nodes were not sentinel nodes (35.8%), suggesting the importance of targeted lymph node excision in addition to SLNB.

## Introduction

Pre-operative localization of axillary lymph nodes is often desired. For example, localization may ensure that a previously biopsied lymph node is excised, which is especially important in patients undergoing neoadjuvant chemotherapy in order for pathologic

\* Address for correspondence: Kelly S Myers, MD Johns Hopkins School of Medicine, Department of Radiology 601 N Caroline St, Baltimore, MD, 21287, USA Tel: 410-955-7095 Fax: 410-614-7663 Email: <u>kmyers25@jhmi.edu</u> response to be fully assessed. However, options for pre-operative localization in the axilla are limited. Historically at our institution, in the absence of preoperative localization of previously biopsied lymph nodes, the excised lymph nodes are evaluated with an intraoperative specimen radiograph to confirm the presence of a biopsy clip. This method, however, is limited as it dose not provide targeted localization. While hook wires are commonly used for localization for findings in the breast, there is only a small amount of data regarding the safety and reliability of hook wires in the axilla.<sup>1-3</sup> Other options such as radioactive <sup>125</sup>I seeds, MagSeed and Tag localizations have additional limitations, as they require special equipment and are costly.<sup>3-7</sup> Ultrasound-guided injection of tattoo ink has emerged as a safe, widely-available, cost-effective technique for labeling specific lymph nodes for which excision is desired.<sup>8-15</sup>

Multiple previous studies have reported experience with ultrasound-guided injection of tattoo ink into targeted axillary lymph nodes using Charcotrace (Phebra, Sydney, Australia) or Spot endoscopic marker (GI Supply, Camp Hill, PA) with sample sizes ranging from 20 to 75 patients.<sup>8-15</sup> All of these studies report a 100% technical success rate of the injection procedure and no adverse outcomes. Additionally, all studies report a high success rate of visualization of the tattooed lymph node intraoperatively, with success rates ranging from 96.4-100%. Several studies report that the tattoo ink spilled to several additional axillary lymph nodes; however, this was predominantly in studies where the injection procedure occurred weeks to months prior to surgery.<sup>9-11</sup> Among three studies in which the injection was performed on the day of or the day prior to surgery, two did not report this finding<sup>13,14</sup>, and one reported occasional spilling of tattoo ink into lymph nodes along the injection track.<sup>12</sup> In previous studies using a tattoo ink in patients also undergoing sentinel lymph node biopsy (SLNB), the tattooed lymph node was also a sentinel node in 58-98.5% of cases.<sup>8</sup>

The aim of this prospective study was to report the success rate of pre-operative localization of axillary lymph nodes using ultrasound-guided injection of tattoo ink and to provide direct surgical and pathologic correlation. Additionally, in patients undergoing SLNB, the frequency in which the tattooed lymph node corresponded to a sentinel lymph node was also evaluated.

#### Methods

In this prospective, Health Insurance Portability and Accountability Act (HIPAA)-compliant, Institutional Review Board-approved study, 17 patients with 19 axillary lymph nodes participated after providing written informed consent from August 1, 2018 to March 10, 2020. All patients aged 18 years or older with one or more axillary lymph nodes for which pre-operative localization with tattoo ink was desired by the breast surgeon were eligible. At our institution, Hydromark biopsy clips are routinely placed at the time of ultrasound guided biopsy of axillary lymph nodes in order to guide future ultrasound-guided localization if needed. Ultrasoundguided injection of 0.1-0.5 ml Spot endoscopic marker (GI Supply, Camp Hill, PA) into the cortex of the target lymph node was performed on the day of surgery (n=17) or the day before surgery (n=2) by one of nine radiologists specialized in breast imaging. With the patient in the surgical position, the skin overlying the inked lymph node was marked with a sterile surgical marker.

In the 14 patients undergoing sentinel lymph

node biopsy (SNLB), dual tracing was used (both Tc<sup>99</sup> and blue dye). For the Tc<sup>99</sup> tracer, approximately 1 mCi was injected subdermally at the areolar margin 2-24 hours prior to surgery. For the blue dye, 5 cc of Lymphazurin blue dye was injected into the retroareolar location of the breast after induction of anesthesia. After entry into the axillary space, using a gamma probe and visual inspection, all identifiable hot, blue and inked nodes were resected. Intraoperative specimen radiograph of the resected lymph nodes was performed to evaluate for presence of a previously placed biopsy clip, if indicated.

Tattoo ink localization was considered technically successful if the breast surgeon was able to visually identify the black tattooed lymph node intra-operatively. In those patients undergoing SLNB, the breast surgeon reported whether the tattooed node was also a sentinel node (hot and/or blue in addition to containing tattoo ink). All lymph nodes and associated surgically resected breast specimens were sent to surgical pathology for routine, standard of care processing, histologic evaluation, and diagnosis. At the initiation of this study, the pathology slides of the tattooed lymph nodes from the first several study patients were reviewed by the study pathologist (board-certified pathologist specializing in breast pathology) to confirm that the tattoo ink did not interfere with the diagnostic interpretation, before additional patients were accrued. A representative sample of at least four tattooed lymph nodes was also evaluated histologically at the conclusion of the study.

Descriptive statistics including success rate of pre-operative localization with the tattoo ink and the percent of tattooed nodes that were also sentinel nodes were calculated using SPSS version 26 (IBM, Armonk NY).

## Results

#### Study Population

Seventeen patients with 19 axillary lymph nodes for which pre-operative localization was desired by the breast surgeon gave written informed consent and were included in the study. While most patients underwent tattoo ink injection for a single specific lymph node, two patients underwent tattoo ink injection into two lymph nodes. Mean patient age was 56 years (standard deviation 14.1 years, range 28 to 81). Mean body mass index (BMI) was 28.5 (standard deviation 7.3). Sixteen of the 17 patients (94.1%) were female. Fifteen of the 17 patients (88.2%) had a diagnosis of breast cancer, one patient had a diagnosis of melanoma and one patient had a core biopsy of an axillary lymph node suspicious for B cell lymphoma. Of the 15 breast cancer patients, 11 (73.3%) underwent neoadjuvant chemotherapy or neoadjuvant immunotherapy prior to the localization procedure and SNLB. Sixteen of the 19 (84.2%) targeted lymph nodes had previously

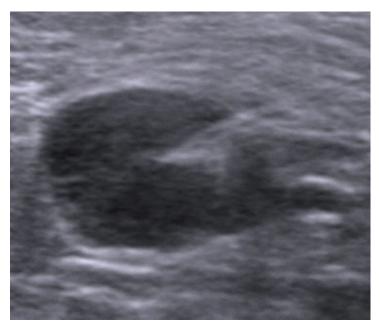


undergone an ultrasound-guided core biopsy.

#### Success Rate of Tattooed Node Localization

Ultrasound-guided injection of tattoo ink into the cortex of the target lymph node was successful for all 19 nodes (Figure 1). There were no reported complications from the localization procedure. Seventeen of the 19 lymph nodes (89.5%) were identified intra-operatively (Figure 2). The two lymph nodes that were not identified intra-

operatively were in the same patient and were not identified due to diffuse seepage of tattoo ink in the adjacent tissues. In one patient, the ink had diffused into the surrounding tissues with three lymph nodes containing tattoo ink (including the targeted lymph node). This localization was considered successful as the targeted node could be identified and excised. Therefore, tattoo ink localization was successful in 16/17 (94.1%) of patients.



**Figure 1.** Pre-operative localization with tattoo ink: 67-year-old female with right breast cancer and biopsy proven metastasis to a right axillary lymph node. Ultrasound image demonstrates ultrasound-guided injection of Spot tattoo ink into the cortex of the previously biopsied lymph node. The tattooed lymph node was visually identified as a black lymph node intra-operatively, and surgical pathology confirmed metastatic carcinoma involving the lymph node with biopsy site changes. However, the tattooed node was not a sentinel node. The patient also underwent sentinel lymph node biopsy which yielded 0/1 positive lymph nodes.

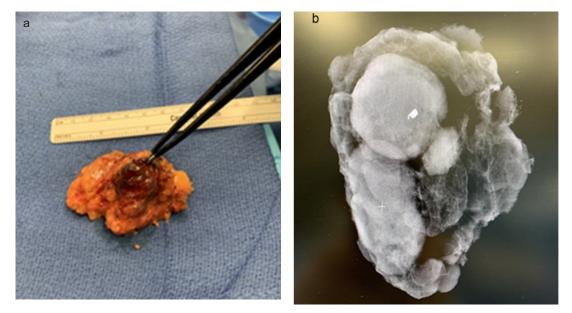
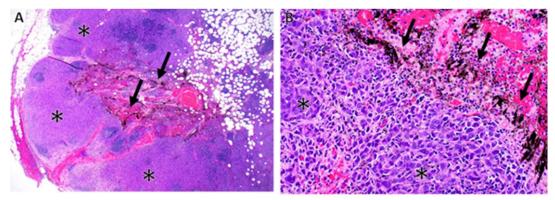


Figure 2. Successful surgical excision of a lymph node localized with tattoo ink: 47-year-old female with inavsive lobular carcinoma and biopsy proven metastasis to an axillary lymph node. Following neoadjuvant chemotherapy, ultrasound-guided tattoo ink localization of the previously biopsied lymph node was performed on the day of surgery. Intraoperatively, (A) the lymph node was visually identified as black, with (B) a specimen radiograph confirming the biopsy clip within the node. Sentinel lymph node biopsy (SNLB) was also performed, and the previously biopsied node was also the sentinel node and was malignant at surgical excision.



**Figure 3.** Histologic confirmation of tattoo ink within localized lymph node: A previously biopsied, radiographically abnormal axillary lymph node was spot ink localized on the day of surgery and was identified intraoperatively to be a sentinel lymph node. Upon histologic review, the black tattoo ink (arrows) was identified within the medullary sinuses of the lymph node, which was extensively involved by metastatic lobular carcinoma (asterisks) (A & B, H&E, X20 and X200).

#### Sentinel Lymph Node Biopsy Outcomes

Sentinel lymph node biopsy (SLNB) was attempted in 14/15 (93.3%) of the breast cancer patients and was successful in 13/14 (92.9%). SLNB was not performed in one breast cancer patient, who was an 81 year-old female with an 11 year history of stage 4 breast cancer. Although there was no progression in distant metastasis, there was increased size of her left breast mass and a single left axillary lymph node, and, therefore, targeted excision was performed without SLNB. Among the 13 successful SLNB, the average number of excised lymph nodes was 2.8 (standard deviation 1.6, median 2.5, range 1-6). In one patient in whom SLNB was unsuccessful, intra-operative identification of the previously biopsied lymph node containing tattoo ink was successful. Among the 13 patients with successful SLNB, there were 14 lymph nodes which underwent pre-operative tattoo ink localization. Nine of the 14 (64.2%) tattooed lymph nodes were also a sentinel node. Of the 5 excised tattooed nodes that were not a sentinel node, all 5 had a previous ultrasound guided biopsy, 3/5 (60%) were in patients who underwent neoadjuvant chemotherapy prior to surgery and 4/5(80%) contained malignancy at surgical excision. In 1/5 (20%) of these patients, the sentinel node was benign but the localized lymph node was malignant.

#### Surgical Pathology

The presence of tattoo ink did not interfere with surgical pathology interpretation (Figure 3). Histologically, the tattoo ink was visualized within the cortical or medullary sinuses of the lymph nodes, and occasionally in the adjacent mature adipose tissue.

The patient with melanoma had tattoo ink localization of two borderline-appearing lymph nodes, one of which had undergone core biopsy with findings consistent with benign nevus cells. However, in the setting of a history of axillary melanoma, excision was recommended. Tattoo ink localization of both lymph nodes was successful and surgical pathology was benign for both lymph nodes at excision.

The second non-breast cancer patient had increased size of axillary lymph node with core biopsy demonstrating atypical lymphoid proliferation concerning for B-cell non-Hodgkin lymphoma on flow cytometry. Tattoo ink localization of the biopsied lymph node was successful, and surgical pathology and flow cytometry at excision were consistent with a reactive lymph node.

#### Discussion

Pre-operative localization of axillary lymph nodes is often desired, but current options for imageguided localization are limited. Ultrasound-guided tattoo ink has emerged as a potential technique to safely and accurately localize specific axillary lymph nodes. This study aimed to prospectively evaluate the success rate of preoperative localization of axillary lymph nodes using tattoo ink, as well as to assess the frequency with which tattooed nodes corresponded to sentinel lymph nodes.

In this study, 17/19 (89.5%) tattooed lymph nodes were successfully identified intra-operatively, with successful identification occurring in 16/17 (94.1%) of patients. Although this is minimally lower than what was observed in previous studies, which report success rates ranging from 96.4-100%<sup>8.15</sup>, this is likely due to the small sample size in this study, as the procedure was successful in all but one patient. This study provides further evidence that ultrasoundguided injection of tattoo ink is a safe, reliable, and highly successful method for pre-operative localization in the axilla. Furthermore, tattoo ink is widely available, inexpensive and does not require specialized equipment, such that it may be implemented easily on a large-scale in the community, including in areas with limited resources.

Among patients also undergoing SLNB in this study, the tattooed node was also a sentinel node in 9/14 (64.2%) of patients, which is within the range of previous studies.<sup>8-15</sup> For our study patients, if only the

sentinel lymph nodes had been excised without additional targeted localization and excision of the tattooed node, the localized lymph node would have remained in situ for 5/14 (35.7%) of patients undergoing SNLB. Of the five tattooed nodes that were not sentinel nodes, 4/5 (80%) were malignant. Furthermore, 3/5 (60%) of these lymph nodes had undergone ultrasound guided biopsy with malignant result prior to neoadjuvant chemotherapy, such that the pathologic response to neoadjuvant chemotherapy in these nodes would not have been known if only SLNB was performed without excision of the localized node.

This study has several limitations. The sample size of 17 lymph nodes in 19 patients is relatively small. Additionally, the tattoo injection procedure was performed by one of nine breast radiologists and surgical excision was performed by one of the five surgeons specializing in breast surgery and/or with expertise in surgical management of the axilla, adding potential variability to procedural technique. However, this study provides direct multidisciplinary correlations with the intra-operative and pathologic appearance in addition to supporting the technique as a successful and easily accessible method for localization. Additionally, to our knowledge, all previous studies have included only breast cancer patients, and this study included all patients in whom axillary lymph node localization was desired, including melanoma and possible lymphoma.

Existing methods for pre-operative localization in the axilla such as radioactive <sup>125</sup>I seeds, MagSeed and Tag localizations have limitations including high cost and the requirement of specialized equipment.<sup>1-7</sup> This study supports previous reports that ultrasound guided injection of tattoo ink is emerging as a safe, inexpensive, widely available technique for preoperative localization in the axilla with a high success rate of intraoperative visualization of tattooed nodes. Future larger studies may further define specific protocols for the successful application of this technique.

**Conflicts of Interest** None.

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# Preliminary Report of Partial-breast Irradiation Following Neoadjuvant Chemotherapy from COMBAT-NEO: Clinical Outcome of Multicatheter BrAchyTherapy after NEOadjuvant Chemotherapy

Kazuhiko Sato\*<sup>a</sup>, Hiromi Fuchikami<sup>a</sup>, Naoko Takeda<sup>a</sup>, Takahiro Shimo<sup>b</sup>, Masahiro Kato<sup>b</sup>, Tomohiko Okawa<sup>c</sup>

<sup>a</sup> Department of Breast Oncology, Tokyo-West Tokushukai Hospital, Tokyo, Japan

<sup>b</sup> Department of Radiation Oncology, Tokyo-West Tokushukai Hospital, Tokyo, Japan

<sup>°</sup> Preventive Medical Center, Sano City Hospital, Tochigi, Japan

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#### ABSTRACT

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## Key words:

Breast cancer, breast conserving therapy, partial breast irradiation, neoadjuvant chemotherapy, multicatheter interstitial brachytherapy **Background:** Breast-conserving therapy (BCT) with partial-breast irradiation (PBI) has become a standard alternative to whole-breast irradiation. Recently, neoadjuvant chemotherapy (NACT) has been widely performed for early breast cancer. Although BCT using perioperative PBI decreased invasiveness and geographic miss, risks of adverse events and local recurrence remain a concern for patients receiving NACT. Thus, a prospectively registered study, the Clinical Outcome of Multicatheter BrAchyTherapy after NEOadjuvant chemotherapy (COMBAT-NEO), was conducted.

**Methods:** Patients who underwent BCT using multicatheter-interstitial brachytherapy (MIB) by intraoperative catheter implant were analyzed. Early and late adverse events (AEs) including higher grade skin toxicities and wound complications, and tumor control of patients receiving NACT were evaluated in comparison with adjuvant chemotherapy (ACT) and no chemotherapy (no-CT).

**Results:** Between April 2017 and February 2020, 265 consecutive patients who received single-stage BCT were evaluated, including 13 NACT (4.9%), 68 ACT (25.7%), and 184 no-CT (69.4%). The median follow-up time and age were 30.0 months and 59.0 years, respectively. All patients were followed up for at least 12 months. Although AEs in NACT, ACT, and no-CT were observed in 1 (7.7%), 5 (7.4%), and 11 (6.0%) patients, respectively (p = 0.91) and there was no acute AE in NACT patients. Overall, 3 (1.1%) ipsilateral and 1 (0.4%) contralateral breast tumor recurrences were observed in no-CT patients. There were no regional and distant recurrences.

**Conclusion:** Although this pilot study was based on a small sample size with short follow-up, these preliminary results support the study of a single-stage BCT with MIB-PBI following NACT.

Introduction

In recent years, partial-breast irradiation (PBI) has become a standard adjuvant radiation treatment for

\* Address for correspondence: Kazuhiko Sato, MD, PhD Address: Department of Breast Oncology, Tokyo-West Tokushukai Hospital, 3-1-1 Matsubara, Akishima, 196-0003, Tokyo, Japan; Tel: +81-42-500-4433; Fax: +81-42-500-4432; Email: <u>kazsato.boc@gmail.com</u> breast-conserving therapy (BCT), acting as an alternative method to whole-breast irradiation (WBI) in patients with low risk breast cancer.<sup>1</sup> Among established PBI techniques, multicatheter-interstitial brachytherapy (MIB) has been supported by randomized controlled trials.<sup>2,3</sup> It is a useful technique especially for patients with small breasts because of a high conformity to the cavity and a limited normal tissue exposure.<sup>4</sup> Careful patient selection combined with modern imaging studies and perioperative



partial-breast brachytherapy by intraoperative catheter implant could allow single-stage BCT.<sup>5, 6, 7</sup> Despite the advantages of decreased invasiveness and less geographic miss, some concerns regarding this single-stage BCT include radiation toxicity and surgical complications because this may produce a negative impact on wound recovery.<sup>8</sup>

In general, systemic therapy using cytotoxic chemotherapy has been incorporated as a multidisciplinary approach, not only to improve breast cancer-specific survival, but also to reduce local recurrences.<sup>9</sup> The indication and therapeutic regimen of chemotherapy after surgery were considered on the basis of the postoperative pathology as an adjuvant chemotherapy (ACT). When ACT was conducted before surgery as a neoadjuvant chemotherapy (NACT), more patients could undergo BCT with better cosmesis due to tumor shrinkage.<sup>10</sup> Recently, NACT has been increasingly performed for patients in order to obtain the desired therapeutic response and consider an additional ACT.<sup>11,12,13</sup>

In our institution, single-stage BCT using MIB-PBI has been performed by intraoperative catheter implant, and has showed excellent local control with adequate toxicity.<sup>14</sup> Although it was not clear whether there was an increased risk of local recurrence or adverse events on BCT using WBI after NACT<sup>15</sup>, clinical outcomes with PBI remain unknown. Therefore, a single-center prospective registered trial, Clinical Outcome of Multicatheter BrAchyTherapy for partial-breast irradiation after NEOadjuvant chemotherapy in breast cancer patients (COMBAT-NEO), was conducted to investigate the toxicity, tumor control, and cosmetic outcomes for patients receiving perioperative MIB-PBI after NACT. Here, the preliminary results of early and late adverse events (AEs) and tumor control in 13 patients were reported in comparison to patients undergoing MIB-PBI during the same period of time.

#### Methods

#### Study design and patient eligibility

Patients with histologically confirmed breast cancer of stages I and II were eligible when meeting the following criteria: female, age of 40 years or older, candidates for breast-conserving surgery (BCS) without NACT, and a 3 cm or less maximum tumor diameter after NACT. Patients with positive axillary nodes before NACT were included, but axillary dissection was conducted in all patients irrespective of the response to NAC. Written informed consent was obtained from all patients who wished to participate in this study after completion of NACT. After MIB-PBI, patients were allowed to receive an additional WBI based on the final pathology, who were included for the analysis. Firstly, 20 patients were enrolled for the detailed analysis of early AEs from the perspective of safety

and more candidates were included for the evaluation of late AEs and tumor control. Preoperative patient workup including standard laboratory parameters, mammography, and ultrasonography as well as contrast-enhanced magnetic resonance imaging (MRI) of the breast and axilla was included. In general, an ultrasound visible clip (HydroMARK<sup>™</sup>: Mammotome, Cincinnati, OH) was placed for patients who planned to receive NAC. Patients with an axillary node suspected for metastasis underwent axillary fine-needle aspiration. When the nodal metastasis was confirmed by fine-needle aspiration, an axillary dissection was performed with or without NACT.

This study was designed as a prospective singleinstitutional phase II trial. After approval by the Central Ethics Committee of the Tokushukai Medical Group, registration started in April 2017.

#### Technique of single-stage BCT using MIB-PBI

BCS was performed by the removal of the tumor with a 1.0-cm gross margin using a moving incision to prevent direct radiation exposure to the wound.<sup>16</sup> After confirmation of a negative surgical margin by specimen mammography, rigid steel needles were placed to act as a reference in dosimetric planning for preoperative contrast-enhanced computed tomography (CT).<sup>17</sup> Plastic tubes were replaced by steel needles to introduce the Iridium-192 brachytherapy source. After surgery, patients received a postoperative CT for treatment planning of MIB-PBI with Oncentra Brachy (ver. 4.5.1. Elekta, Stockholm, Sweden).

The clinical target volume (CTV) was created with 1.0-cm margins beyond the delineated cavity. Planning target volume (PTV) was equal to the CTV. The PTV (PTV EVAL) was set at 5 mm under the skin and the surface of the pectoral muscle as superior and deep margins, respectively. A total dose of 32 Gy in 8 fractions was delivered on 4 consecutive working days, twice a day with at least 6-hour intervals. At least 90% of the PTV EVAL was covered with 90% of the prescribed dose (PD). The dose limits were as follows: volume receiving 150% of PD (V150%)  $\leq$ 70 cm<sup>3</sup>, V200%  $\leq$ 20 cm<sup>3</sup>, maximal skin and chest wall dose ideally <75% PD, and strictly <100% PD in our protocol.<sup>14</sup> All patients received antibiotics during catheter implantation. Catheters were removed immediately after the final radiotherapy.

#### Chemotherapeutic regimens

In general, NACT regimens were implemented from the standard regimens of ACT. Most patients started with a chemotherapeutic regimen consisting of AC (adriamycin/cyclophosphamide: 60/600 mg/m<sup>2</sup>) x4 intravenously (IV) every 2 weeks followed by paclitaxel (80 mg/m<sup>2</sup>) x12 IV every week<sup>18</sup>, or TC (docetaxel/cyclophosphamide: 60/600 mg/m<sup>2</sup>) x4 IV every 3 weeks.<sup>19</sup> Trastuzumab (8 mg/kg loading dose followed by 6 mg/kg every 3 weeks) and pertuzumab (840 mg loading dose followed by 420 mg every 3 weeks) was administered with the first paclitaxel cycle for patients with human epidermal growth factor receptor 2 (HER 2)-positive disease. At the completion of paclitaxel, patients were then scheduled to undergo single-stage BCT using MIB-PBI. Trastuzumab and Pertuzumab were administered every 3 weeks to complete the oneyear duration.

#### Study population and outcome assessment

In the COMBAT-NEO study, the primary outcome was early AEs, and the secondary outcomes were late AEs, ipsilateral breast tumor recurrence (IBTR), and long-term cosmetic outcome (UMIN000026976). Early and late AEs were prospectively assessed at the completion of brachytherapy and in one month, with a follow-up every 3 to 4 months until 60 months and then every 12 months. In this study, physician-assessed AEs including higher grade skin toxicity, hemorrhage, symptomatic seroma, and breast infection were evaluated as clinically significant complications.<sup>20</sup> Grade 3 or more skin toxicity using the Common Terminology Criteria for Adverse Events (CTCAE, version 4.0) was defined as a higher-grade skin toxicity.<sup>21</sup> Postoperative hemorrhage was defined as any surgical procedures for hemostasis at the time of catheter removal. The definition of symptomatic seroma was one that requires multiple aspirations or leads to temporary drainage of the content from the wound. Breast infection was considered to be a surgical site infection (SSI) as defined by the Centers for Disease Control and Prevention National Nosocomial Infections Surveillance System criteria, including purulent drainage from the incision, organisms isolated from an aseptically obtained culture, and wound dehiscence.<sup>22</sup> Early AEs were defined as those occurring 3 months from the date of surgery, and those occurring later were defined as late AEs.

The evaluation of locoregional recurrence was performed using mammography and contrastenhanced breast MRI every year. All locoregional, distant failure, and survival outcomes were evaluated on the date of the diagnosis. Bilateral tumors treated with MIB-PBI were counted as representing two patients. IBTR was divided into marginal miss and elsewhere failure, depending on the distance between the original tumor site and the recurrence site.<sup>23</sup>

All AEs and clinical outcomes were reported to the central office of Tokushukai Group Ethical Committee at 1, 3, 12 months, and every 12 months thereafter until 60 months following entry. In this preliminary report, the AEs and tumor control efficacy in patients undergoing MBI-PBI after NACT were compared with those in patients receiving MIB-PBI without NACT during the same period.

#### Statistical analysis

To estimate the proportion of complications over time and to compare complications among NACT, ACT and no-CT groups, contingency table analyses were performed. Differences between continuous variables and proportions were analyzed with ANOVA and Fisher's exact test, respectively. The Kaplan–Meier estimate was performed to evaluate the likelihood for IBTR. All p-values less than 0.05 were considered to be statistically significant. The analyses were conducted using SPSS software, version 27 (IBM SPSS Statistics for Windows, Armonk, NY).

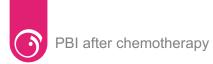
## Results

Patients and tumor characteristics and treatment variables

Between April 2017 and February 2020, 13 patients were received MIB-PBI after NACT. The clinical characteristics of all patients are shown in Table 1. In the NACT cohort, 12 patients (92.3%) received anthracycline and taxane-based therapy and 5 patients (38.5%) received anti-Her2 therapies. The medium time interval between the last dose of NAC and the surgery was 11 days (interquartile range [IQR], 8 - 17.5). There were 4 patients (30.8%) who achieved pathological complete response (pCR) and no patients were reported to be marginally positive (0%). During the same period of time, a total of 265 patients consequently received perioperative MIB-PBI including 13 NACT (4.9%), 68 ACT (25.7%), and 184 no-CT (69.4%). Table 2 summarizes patient and tumor characteristics, and treatment variables. At the time of this interim analysis, the median follow-up time was 30.0 months (IQR: 21.9 - 37.8), and all patients were followed up for at least 12 months. The median patient age and the average pathologic tumor size were 59.0 years (IQR: 49.0 - 69.0) and 10.0 mm (IQR: 6.0-15.0), respectively. Dosimetric valuables are shown in Table 3. The median volumes of the cavity and CTV equivalent to PTV were 11.4 cm<sup>3</sup> (IQR, 7.9 - 17.1) and 34.9 cm<sup>3</sup> (IQR, 21.5 - 48.3), respectively. The medium numbers of catheters and planes were 5 (IQR, 4 - 7) and 2 (IQR, 1 - 2), respectively. The target coverage of the protocol was achieved for all patients. Maximum fractional dose to the skin and the chest wall were 2.7 Gy (IQR, 2.5 -2.9) and 2.4 Gy (IQR, 1.8-2.9), respectively.

#### Early adverse events

Physician-assessed AEs are summarized in Table 4. During the follow-up in the first 3 months, two grade  $\geq$ 3 skin toxicities (0.8%), two hemorrhages (0.8%), six symptomatic seromas (2.3%), and three



Age, years	Pretreatment clinical stage	Baseline IHC	Type of NACT	Axillary surgery	Pathological stage	Additional radiotherapy
45	cT1N1	ER+HER2-	AC-P	Dissection	ypT0N0	
52	cT2N0	ER+HER2-	AC-P	SNB	ypTisN0	
66	cT1N1	ER+HER2-	AC-P	Dissection	ypT1N0	
50	cT2N1	ER+HER2+	AC-P+Tr	Dissection	ypT1N1	WBI
71	cT2N1	ER+HER2-	AC-P	Dissection	ypT1N1	WBI
41	cT2N1	ER-HER2-	AC-P	Dissection	ypT1miN0	
63	cT2N0	ER-HER2+	D+Tr+Per	SNB	ypT1N0	—
63	cT2N1	ER+HER2+	AC-P+Tr	Dissection	ypT1N1	WBI
74	cT2N1	ER-HER2+	AC-P+Tr+Per	Dissection	ypTisN0	
48	cT2N0	ER-HER2-	AC-P	SNB	ypTisN0	—
40	cT2N0	ER-HER2-	AC-P	SNB	ypT1N0	
72	cT1N0	ER+HER2-	AC-P	SNB	ypT1N0	
50	cT2N1	ER+HER2+	AC-P+Tr+Per	Dissection	ypT1N1	WBI

Abbreviations: NACT; neoadjuvant chemotherapy, IHC; immunohistochemistry, ER; estrogen receptor, HER2; human epidermal growth factor receptor 2, AC; adriamycin and cyclophosphamide, P; paclitaxel, DOC; docetaxel, Tr; trastuzumab, Per; pertuzumab, SNB; sentinel-node biopsy, WBI; whole-breast irradiation

Table 2. Patient and tumor characteristics receiving MIB-PBI among three different systemic treatment cohorts
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Variables	NACT (n = 13)	ACT (n = 68)	no-CT (n = 184)	Р
Median follow-up, months	33.6 (22.8–37.2)	29.5 (22.6–38.3)	30.1 (21.6–37.4)	0.96
Median age, years	52.0 (47.3-67.3)	62.0 (53.5-69.0)	58.0 (48.0-69.5)	0.54
*Median invasive diameter, mm	10.0 (1.5–14.5)	15.0 (10.0-20.0)	10.0 (4.0–15.0)	0.27
Margin status				
Negative/close	13 (100)	65 (95.6)	168 (91.3)	0.30
Positive	0 (0)	3 (4.4)	16 (8.7)	
**Lymph node status				
Negative/micrometastasis	5 (38.5)	64 (94.1)	181 (98.4)	< 0.0001
Positive	8 (61.5)	4 (5.9)	3 (1.6)	
Axillary surgery				
SNB/no surgery	5 (38.5)	66 (97.1)	182 (98.9)	< 0.0001
Axillary dissection	8 (61.5)	2 (2.9)	2 (1.1)	
Additional WBI				
Yes	4 (30.8)	1 (1.5)	1 (0.5)	< 0.0001
No	9 (69.2)	67 (98.5)	183 (99.5)	
Adjuvant endocrine therapy				
Yes	9 (69.2)	45 (66.2)	171 (92.9)	< 0.0001
No	4 (30.8)	23 (33.8)	13 (7.1)	
(Neo-) adjuvant chemotherapy				
Anthracyclines or taxanes	1 (7.7)	50 (73.5)		< 0.0001
Anthracyclines and taxanes	12 (92.3)	18 (26.5)		
Anti-Her2 therapy				
Yes	5 (38.5)	26 (38.2)		0.99
No	8 (61.5)	42 (61.8)		

\*Assessed for invasive tumor only. \*\*Assessed by axillary surgery only. Abbreviations: NACT; neoadjuvant chemotherapy, ACT; adjuvant chemotherapy, CT; chemotherapy, SNB; sentinel-node biopsy, WBI; whole-breast irradiation, HER2; human epidermal growth factor receptor 2 Continuous variables are reported as medians (interquartile ranges). Categorical variables are reported as numbers (%).

Table 3. Dosimetric variables of	patients receiving MIB-PBI	among three systemic treatments

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Variables	NACT $(n = 13)$	ACT (n = 68)	no-CT (n = 184)	Р
Cavity volume, cm <sup>3</sup>	12.5 (8.3–19.6)	13.0 (9.3–17.4)	11.2 (7.6–16.1)	0.65
PTV, cm <sup>3</sup>	40.3 (15.8-61.7)	43.2 (27.1–52.2)	31.7 (21.0-46.0)	0.37
V100, cm <sup>3</sup>	37.4 (12.2-63.3)	45.9 (28.4–56.3)	36.6 (24.3–53.3)	0.33
V150, cm <sup>3</sup>	11.6 (5.5-28.0)	21.3 (11.8-29.6)	16.0 (9.7–26.2)	0.32
V200, cm <sup>3</sup>	9.1 (5.2–12.5)	10.5 (6.2–15.3)	7.9 (5.7–14.6)	0.27
Maximum skin dose, Gy	2.6 (2.4–2.8)	2.7 (2.6–2.9)	2.7 (2.5–2.9)	0.90
Maximum chest wall dose, Gy	2.6 (1.4-3.7)	2.3 (1.6-2.9)	2.3 (1.8–2.9)	0.75

Abbreviations: NACT; neoadjuvant chemotherapy, ACT; adjuvant chemotherapy, CT; chemotherapy, PTV; planning target volume Continuous variables are reported as medians (interquartile ranges).

	Onset	NACT (n = 13)	ACT (n = 68)	no-CT (n = 184)	Total (n = 265)	Р
Skin toxicity	Early	0	2 (2.9)	0	2 (0.8)	
	Late	0	0	0	0	
Hemorrhage	Early	0	0	2 (1.1)	2 (0.8)	
	Late	0	0	0	0	
Symptomatic seroma	Early	0	1 (1.5)	5 (2.7)	6 (2.3)	
	Late	1 (7.7)	1 (1.5)	0	2 (0.8)	
Surgical site infection	Early	0	0	3 (1.6)	3 (1.1)	
	Late	0	1 (1.5)	1 (0.5)	2 (0.8)	
Total		1 (7.7)	5 (7.4)	11 (6.0)	17 (6.4)	0.91

Table 4. Early and late adverse events receiving MIB-PBI among three different systemic treatment cohorts

Abbreviations: NACT; neoadjuvant chemotherapy, ACT; adjuvant chemotherapy, CT; chemotherapy Categorical variables are reported as number (%).

SSIs (1.1%) were observed in the entire cohort. The cases of high-grade skin toxicities occurred as severe dermatitis on the area of radiation exposure with the first TC dose which could be diagnosed as a radiation recall reaction. The incidence of early AEs was observed in 10 ACT patients (5.4%) and three no-CT patients (4.4%). There were no clinically significant complications during the first 3 months in the NACT patients (0%).

## Late and overall adverse events

After the 3 month follow-up, one symptomatic seroma each in the NACT (7.7%) and ACT (1.5%) patients and one SSIs each in the ACT (1.5%) and no-CT (0.5%) patients were observed. A rib fracture, telangiectasias, and other frequently reported major toxicities did not develop. Therefore, there were two grade  $\geq 3$  skin toxicities (0.8%), two hemorrhages (0.8%), eight symptomatic seromas (3.0%), and five SSIs (1.9%) observed in the entire cohort at all time points. The distribution of those adverse events had no significant coherence among three different systemic treatment cohorts (p=0.91).

## Tumor control outcomes

Overall, three IBTRs (1.1%) and one contralateral breast tumor were observed in the no-CT cohort. Both of the cases with IBTR occurred as a recurrence elsewhere. Based on a 2-year actual analysis of NACT, ACT, and no-CT patients, IBTRfree survival rates were 100%, 100%, and 98.8%, respectively. Because neither regional nor distant recurrences developed, breast cancer specific survival rate was 100%. One patient receiving NACT died from gastric cancer 24.2 months after MIB-PBI.

## Discussion

NACT has been implemented in a multidisciplinary local treatment for early breast cancer. Although this interim report was based on a retrospective analysis with a small sample size and a short follow-up period, single-stage BCT with MIB-PBI following NACT did not demonstrate any early AEs, as well as any additional negative impact on wound complications and locoregional recurrences.

NACT was originally performed in patients with inoperable locally advanced disease for surgery and has been incorporated into operable disease in order to undergo BCT because of downsizing. According to the recent advances in systemic therapies, NACT has been performed not only to widen availability of BCT but also to investigate the pathological reaction to consider additional systemic treatment. For patients with residual disease in the breast after NACT (non-pCR), additional ACT using capecitabine and trastuzumab emtansine (T-DM1) have been considered for patients with high-risk luminal and triple-negative breast cancer, and HER2-positive disease, respectively.<sup>11,12</sup> The introduction of an immune-checkpoint inhibitor into NACT has also been considered for patients with triple-negative breast cancer to improve outcomes.<sup>24</sup> Those with increasing NACT use with extra AE were investigated because the cytotoxic agent may have a negative impact on the surgical wound recovery. However, evidence showing no extra risk of surgical complications and IBTR in selected patients has been reported.<sup>15</sup>

A deescalating local treatment needs to be considered for patients previously receiving NACT. For example, the ongoing RESPONDER trial aims to develop a precise approach for the assessment of pCR using a vacuum-assisted biopsy without surgical management following NACT.<sup>25</sup> PBI should be carefully considered after NACT as well. With various techniques and fractionation regimens available for PBI, efficacy of tumor control, AEs, and cosmesis can be obtained by adequate patient selection, technique, and dose delivery.<sup>26</sup> Although partial-breast brachytherapy was widely available with the longest follow-up, unique side effects, such as symptomatic seroma and breast infection from indwelling catheters and a higher gradient radiation dose may be observed. In the registry of partialbreast brachytherapy with an intracavitary device, 13% and 8.2% of symptomatic seroma and breast infection, respectively, were identified.<sup>27</sup> In our



series, there were eight (3.0%) and five (1.9%) patients who experienced symptomatic seroma and SSI, respectively. The low rate of infectious complications may in part be reflective of the prophylactic use of antibiotics, the reduced total time of indwelling brachytherapy catheter, and the utilization of the moving incision technique. Since NACT may defer wound healing, the risk for wound complications may increase. Similarly, because radiotherapy was started before wound recovery, perioperative PBI may be a concern. However, the incidence rate of the AEs in the NACT cohort was acceptable in our series.

There are some limitations to this study that should be noted. We acknowledge that this is an interim report based on a small number of patients and a short follow-up period. The data from the follow-up period was only sufficient to evaluate the early adverse event. Potential benefit of NACT for patients with small tumor has not been widely accepted, which may result in slow patient accrual. Second, the efficacy of PBI after NACT was based on a retrospective comparison to ACT and no-CT patients with different backgrounds. Finally, the generalizability of these results is limited to a singleinstitute with specific techniques. However, this study is very unique, and no other reports investigating the efficacy of PBI after NACT exists, and preliminary results support this study. We expect to be able to report on the safety and the possible efficacy of single-stage BCT using MIB-PBI at the completion of our trial.

#### **Conflict of Interest**

The authors declare that they have no conflict of interests.

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# DOI: 10.32768/abc.202182156-161 Autoimmune / Inflammatory Syndrome Induced by Adjuvants (Asia Syndrome) Associated with Silicone Breast Implant Rupture

Erica Araujo Santiago\*<sup>a</sup>, Ivie Braga de Paula<sup>a,b,c,d</sup>

<sup>a</sup> Department of Radiology, Felicio Rocho Hospital, Belo Horizonte, Minas Gerais, Brazil <sup>b</sup> Department of Radiology, Orizonti Hospital, Belo Horizonte, Minas Gerais, Brazil <sup>c</sup> Department of Radiology, Conrad Clinic, Belo Horizonte, Minas Gerais, Brazil <sup>d</sup> Department of Radiology, Hermes Pardini Institute, Belo Horizonte, Minas Gerais, Brazil

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#### ABSTRACT

**Background:** Autoimmune/inflammatory syndrome (ASIA) constitutes a set of related immune mediated diseases that share a common clinical picture and a history of a previous exposure to an adjuvant agent. From a clinical standpoint, patients present with none specific manifestations such as myalgia, arthralgia, chronic fatigue and dry mouth as well as neurological manifestations such as cognitive disturbances, memory loss and neurologic disabilities.

**Case presentation:** A previously healthy 25-year-old patient who underwent breast augmentation 3 years ago, with an asymptomatic rupture of the silicone breast implant, presented with three major criteria of ASIA, and improved after bilateral implant removal. She also had pleuritis and pericarditis, rarely described in such disease. A literature review on complications related to breast implants, their questionable relationship to the onset of autoimmune pathologies, and basic aspects of the diagnosis and management of ASIA was carried out.

**Conclusion:** The silicone presented in breast implants should be considered as an adjuvant, with the potential to cause chronic stimulation to the immune system. This can lead to systemic manifestations that can be severe in patients genetically predisposed and potentially not reversible even after surgical removal of the implants. When facing patients with breast implants and systemic clinical symptoms, lymph node disorders, neurological manifestations, or serositis as in the case presented, without other defined etiology, the possibility of ASIA should be considered in the differential diagnosis.

#### Introduction

Silicone breast implants have been used for reconstructions and cosmetic purposes since 1960.<sup>1</sup> Breast augmentation mammoplasty using silicone implants is the most commonly performed cosmetic surgery in the United States, with approximately 300,000 procedures performed per year.<sup>2</sup> This is also observed in Brazil, according to the biannual

\* Address for correspondence:

Erica Araujo Santiago, MD Address: Av. do Contorno, 9530 - Barro Preto, Belo Horizonte- MG, 30110-934 MG, Brazil Tel +55 31988646224 Email: <u>erica.1487@hotmail.com</u> consensus of the Brazilian Society of Plastic Surgery of 2018.<sup>3</sup> The initial generations of breast implants had high rupture rates and the suspicion of their relationship with collagen diseases caused the US Food and Drug Administration to suspend its use in 1992. This decision was revoked in 2006, after improvements made by manufacturers and the absence of conclusive evidence of the association between implants and collagen diseases.<sup>4-6</sup> However, the association of silicone implants with systemic pathologies, such as lymphoma, ASIA and other autoimmune diseases, has been questioned in some studies.<sup>7</sup>

Autoimmune/Inflammatory syndrome, induced by adjuvants (ASIA), is a disease that was first introduced by Shoenfeld et al. in 2011.<sup>8</sup> It constitutes

#### Table 1. Suggested criteria for ASIA diagnosis

#### MAJOR CRITERIA

1. Exposure to external stimuli (infection, vaccine, silicone, other adjuvants), before the onset of symptoms.

- 2. Presentation of at least one of the following symptoms:
  - Muscle weakness, myalgia, or myositis;
    - Arthritis or arthralgia;
    - Chronic fatigue, poor restorative sleep, or other sleep disorders;
    - Neurological manifestations (especially those associated with demyelination);
    - Cognitive or memory deficit;
    - Fever, dry mouth.
- 3. Improvement of symptoms after removal of the adjuvant.
- 4. Typical biopsy of the organs involved.

#### MINOR CRITERIA

- Presence of autoantibodies or antibodies directed to the adjuvant.
- Other clinical manifestations (e.g., irritable bowel syndrome)
- Specific HLA (e.g., HLA, DRB1, HLA DQB1)
- Presentation of an autoimmune disease (e.g., multiple sclerosis, systemic sclerosis)

a set of closely related immune mediated diseases that share a common clinical picture as well as a history of previous exposure to an adjuvant agent, silicone being one of them.<sup>9</sup> Shoenfeld et al. have proposed several major and minor criteria that may aid in the diagnosis of ASIA syndrome (Table 1).

The diagnosis of the disease is made by the presence of at least 2 major criteria or 1 major criterion and two minor ones.<sup>8</sup>

From 2011 until 2016, more than 4000 documented cases of ASIA syndrome were reported with various clinical severity and diverse history of adjuvant exposure.<sup>10</sup> There is little known about geographical distribution<sup>11</sup>, although many of the autoimmune diseases are more prevalent in populations that live further away from the equator as it is believed that limited exposure to sun, and therefore the lack of production of vitamin D, may be associated with ASIA.<sup>12</sup> In this report, we present the case of a 25-year-old patient who underwent breast augmentation 3 years ago, with an asymptomatic rupture of the silicone breast implant, who presented three of the major criteria for ASIA (exposure to external stimulus (silicone), symptoms of fever and fatigue and complete improvement after removal of the adjuvant).

A literature review was conducted using PubMed, MEDLINE and Cochrane databases by searching for the keywords. Subsequently, additional papers were located by bibliography review and through manual searching until February 2021 when the review ended. Papers were included if they specifically discussed ASIA in the setting of silicone breast implants, Shoenfeld's syndrome, Breast prosthesis syndrome, Silicone and implant incompatibility syndrome (SIIS). Only articles written in English in the adult population were included. The relevant literature was then analyzed to determine whether consensus existed on the topics discussed in the present case.

We consider it worthwhile to highlight that there is a certain heterogeneity in the terminology used for

autoimmune/inflammatory reactions related to silicone implants. While "siliconosis" is one of the diseases included in ASIA by Schoenfeld et al.<sup>13</sup>, the term "Silicone implant incompatibility syndrome (SIIS) is defined as having symptoms or signs of silicone allergy, capsular contracture, and/or systemic manifestations such as chronic fatigue, arthralgia, myalgias, asthenia, and/or fever, which do not fulfilled Shoenfeld's criteria for ASIA.<sup>13</sup> Also, "Breast prosthesis syndrome" is defined as a specific immune disease related to breast implants, a mixed autoimmune disease or non-specific immunological disease, characterized by clinical findings very similar to ASIA, such as arthralgia, chronic fatigue, myalgia, fever, sleep disturbances, among others, but without well-defined diagnostic criteria.

#### **Case Presentation**

A previously healthy 25-year-old patient who underwent breast augmentation with the placement of silicone implants in November 2018, sought hospital care 11 months after the surgical procedure, complaining of persistent fever, severe chest pain, which used to get worse at bedtime, besides dyspnea, general malaise, and fatigue for 15 days. Physical examination revealed painful axillary and infraclavicular lymph node enlargements on the left.

Initial blood work showed an increase in CRP (167mg / dL) and the WBC count was normal (4.900/mm3). In the posterior investigation for rheumatological conditions, the tests for Antinuclear Antibodies (ANA), the erythrocyte sedimentation rate and rheumatoid factor were negative. The hepatitis and HIV serology were also negative. Echocardiogram showed slightly increased refringence of the posterior pericardium, with a small associated pericardial effusion, without segmental changes in contractility. Chest tomography in addition to pericardial effusion (Figure 1) showed axillary, infraclavicular, and parasternal lymph nodes on the left, some with a necrotic center, associated with inflammatory changes in the adjacent soft tissues (Figure 2). Despite the

limitation of the tomography in viewing the breast implants, due to the irregularity of their contours on the left, the hypothesis of rupture of the breast implant on this side was suspected (Figure 3).

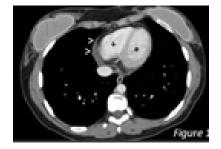


Figure 1. CT image of the chest after injection of the contrast medium, at the level of the right (\*) and left (+) ventricles, showing a hypodense image, with a lentiform aspect, in the topography of the pericardium, compatible with a small pericardial effusion (>).

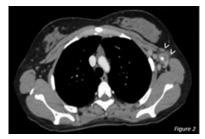
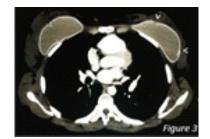


Figure 2. CT image of the chest after injection of the contrast medium, showing axillary lymph nodes enlargement (<), one of them with a hypodense and hypocaptant center, suggestive of necrosis (\*).



**Figure 3.** CT image of the chest, after injection of the contrast medium, showing irregularities in the contours of the capsule of the left breast implant (<).

Breast ultrasonography showed regularities in left implant contours and collection surrounding it, suggestive of intracapsular rupture (Figure 4). Axillary, infraclavicular, and parasternal lymph nodes were also observed on the left, with a "snow storm" shadow (Figure 5), indicating extracapsular silicone.

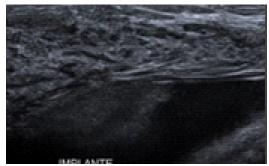


Figure 4. US image of the left breast, showing poorly defined collection of the implant, an indirect sign of rupture.

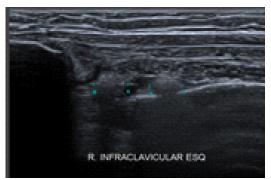


Figure 5. US images of the infractavicular region, showing lymph nodes with "snowstorm" shadow (+), indicative of silicone-associated lymph node disease.

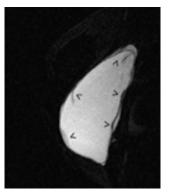


Figure 6. MRI image of the left breast, weighted in sequence for silicone, showing curvilinear hypo signal lines inside the implant (>), characterizing the linguini sign, suggestive of intracapsular rupture.

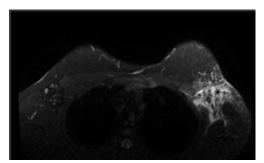


Figure 7. MRI image of the breasts and armpits, weighted in T2WI with fat suppression, showing an extensive inflammatory process in the left axilla, characterized by heterogeneous hypersignal (\*) in this topography.

MRI of the breasts was performed, which showed an intracapsular rupture of the left breast implant (Figure 6) and axillary lymphadenopathies on this side with adjacent edema (Figure 7).

The patient underwent an excisional biopsy of the largest left axillary lymph node, which demonstrated necrotizing lymphadenitis associated with foreign body material, compatible with extracapsular silicone. Bilateral surgical extraction of the breast implants was performed, thirty-five days after the beginning of the symptoms, followed by cleaning of the surgical stores and biopsy of the implant capsules. During the surgical procedure, it was observed that the right implant was intact, but with non-compliant liquid, compatible with silicone. While the left implant had an intracapsular rupture, with a large amount of intracapsular silicone around the implant and a fibrotic capsule. Complete resolution of symptoms was reported in clinical control one month after the surgical procedure. The patient was symptom-free 16 months after surgery.

Pathologic examination of the capsule of the right implant showed synovial metaplasia, characterized by fibrohistiocytic cells covering one of the faces, polarized perpendicularly to the surface. The biopsy of the left capsule also showed synovial metaplasia, in addition to several foci of foreign body reaction to silicone-compatible material. Immunohistochemistry (IHC) was not done.

Three major criteria for ASIA were fulfilled, including exposure to external stimulus (silicone), symptoms of fever and fatigue, in addition to symptoms improvement after removal of the adjuvant, and no identification of other causal factors for the symptoms, even after investigation.

## Discussion

Silicone breast implants have been used for reconstructions and cosmetic purposes since 1960.<sup>1</sup> They are composed of a silicone elastomer envelope and filled with gel-shaped silicone.<sup>15</sup> Although silicone does not have a direct effect on the immune system, it has been suggested that it may have an effect as an adjuvant. The agent whose function is to direct the immune system to the production of antibodies (humoral response) or to the stimulation of T lymphocytes (cell-mediated reaction) is called adjuvant, in order to intensify the immune reaction. Thus, even if the immune system does not react specifically to silicone, this substance can favor an immune reaction to other antigens, triggering autoimmune, and connective tissue diseases, in genetically predisposed patients.<sup>16</sup> The initial generations of breast implants had high rupture rates and the suspicion of their relationship with collagen diseases caused the US Food and Drug Administration to suspend its use in 1992. This decision was revoked in 2006, after improvements made by manufacturers and the absence of conclusive evidence of the association between implants and collagen diseases.<sup>4-6</sup>

There are still controversies today about the possibility of silicone implants being related to autoimmunity reactions.<sup>17</sup> Decades after the

description of the first case of the syndrome described as "Breast disease by adjuvant"<sup>18</sup>, a pathology that did not fit into the typical connective tissue disease and that was called adjuvant-induced autoimmune syndrome (ASIA) was recognized, related to silicone<sup>13</sup>. Resembling other autoimmune disease entities, the etiopathogenesis of these conditions involves a multifactorial interplay between environmental factors and genetic predisposition as noted by the association with certain HLA haplotypes.<sup>11</sup> In part, the mechanism involves the chronic stimulation of the immune system, which may then lead to the release of inflammatory cytokines including interferon  $\gamma$ , interferon α, interleukin (IL)-1, IL-6, tumor necrosis factor (TNF) $\alpha$  and so forth. Therefore, this syndrome can, in part, be induced by this cascade of cytokines released in response to the chronic stimulation.<sup>1</sup>

Patients who develop ASIA may present a series of specific and non-specific manifestations, with very heterogeneous clinical manifestations, which can be more severe in patients with breast implant rupture. The interval between implantation/rupture and the presentation of symptoms is quite variable. The diagnosis of the disease is made by the presence of at least 2 major criteria or 1 major criterion and two minor ones, detailed in Table 1.<sup>8</sup>

In the case presented in this report, the patient presented a silent intracapsular unilateral silicone breast implant rupture. Intracapsular rupture of implants is the most common type, accounting for 77-89% of all ruptures.<sup>19</sup> Extracapsular rupture, on the other hand, is less frequent and is characterized by the leakage of silicone through the fibrous capsule, involving the adjacent mammary parenchyma.<sup>20</sup> Ultrasonography detects ruptures in silicone implants with a sensitivity of about 50-77% and specificity between 55 and 84%. The most specific ultrasound signal is the visualization of multiple elongated, hyperechogenic, linear, or curvilinear images, inside the implant, called the stair sign.<sup>21</sup> Magnetic resonance imaging has high sensitivity and specificity for detecting implant ruptures, approximately 72-94% and 85-100%, respectively.<sup>22</sup> The most specific finding of intracapsular rupture is called the "linguine sign", which is characterized by layers of the silicone elastomer capsule, collapsed inside the silicone gel, contained by the fibrous capsule.<sup>23</sup>

Caravantes et al.<sup>14</sup> published a review in which they recommend performing, as part of the preoperative evaluation, the determination of family and pathological clinical history, immunological markers (antithyroid antibodies, rheumatoid factor, ESR, immunoglobulins, antinuclear antibodies and C-reactive protein) and mammography or ultrasound, depending on whether the patient's age is greater than or less than 40 years. In the case of patients without any risk factor, the follow-up after silicone breast implant placement consists of assessment every two years for ten years with ultrasound and immunological markers. In the case of positive history or immunological markers, rheumatology assessment is confirmed and the follow-up consists of ultrasound once a year, MRI after 5 and 10 years and immunological markers in the first year and every two years for 10 years.

Fuzzard et al.<sup>24</sup> published a literature review where they suggest an algorithm that begins with a clear informed consent process and identification of risk factors for SIIS development prior to implantation. Subsequently, if SIIS develops, they advocate early multidisciplinary input. The first line treatment strategy should be centered around patient education and acknowledgement of their symptoms. If this fails to alleviate the condition, medical management should be trialed under the guidance of an autoimmune specialist. In the cohort of patients who have additional ongoing issues, explantation is recommended.

Mizuno, Y. et al.<sup>25</sup> conclude that when patients with silicone breast implants present with pleuritis or pericarditis, physicians should consider the possibility of implant rupture and explanation. Shaik IH et al.<sup>26</sup> presented a case of recurrent pleural effusion related to breast implants where dramatic improvement of pain and effusion after removal of implants was helpful in confirming the diagnosis and treatment. Dagan A. et al.<sup>27</sup>, described a case of adultonset Still's disease (AOSD) associated with breast augmentation as part of autoimmune syndrome induced by adjuvants (ASIA), manifested with pleuritis and pericarditis, developed after breast mammoplasty, concluding that, since some patients do recover from the AOSD with medical treatment only, this should be the first line of action. If the patient fails to recover with medical treatment, as was in the present case, an imaging study, preferably MRI of the breasts, should be done to detect leakageand surgery should be discussed with the patient.

In conclusion, the silicone present in breast implants should be considered as an adjuvant, with the potential to cause chronic stimulation to the immune system. This can lead to systemic manifestations that can be severe in genetically predisposed patients and is potentially not reversible even after surgical removal of the implants. Among patients with breast implants and systemic clinical symptoms, lymph node disorders, neurological manifestations, or serositis as in the case presented, without other defined etiology, the possibility of ASIA should be considered in the diagnosis.

#### **Conflicts of Interest**

The authors declare that they have no conflict of interest related to the publication of this manuscript.

#### **Ethical Consideration**

The description and publication of the case was

authorized by the patient through informed consent.

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## DOI: 10.32768/abc.202183162-173 Marine Cyclic Dipeptide Cyclo (L-Leu-L-Pro) Protects Normal Breast Epithelial Cells from tBHP-induced Oxidative Damage by Targeting CD151

Deepak KGK<sup>a</sup>, Seema Kumari<sup>a</sup>, RamaRao Malla\*<sup>a</sup>

<sup>®</sup> Cancer Biology Lab, Department of Biochemistry and Bioinformatics, GIS, GITAM (Deemed to be University), Visakhapatnam-530045, Andhra Pradesh, India

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#### ABSTRACT

**Background:** Oxidative stress plays a key role in breast carcinogenesis. Cyclo (L-Leu-L-Pro) (CLP) is a homodetic cyclic dipeptide with 2,5-diketopiperazine scaffold isolated from marine actinobacteria. This study aimed to evaluate the protective activity of CLP and linear - (L-Leu-L-Pro) (LP) from tert-butyl hydroperoxide (tBHP)-induced damage using normal breast epithelial cell line model (MCF-12A).

**Methods:** The cytoprotective activity was evaluated by detecting the changes in intracellular ROS, mitochondrial superoxide, hydroxyl radical, hydrogen peroxide, and lipid peroxidation detection assays as well as cytotoxic assays of MTT, LDH assays and phase contrast microscopy. Genoprotective activity was evaluated by (Apurinic/Apyrimidinic) AP site, alkaline Comet, and 8-hydroxy-2deoxyguanosine assays.

**Results:** The marine cyclic peptide, CLP, significantly protected MCF-12A cells by scavenging tBHP induced intracellular ROS such as super oxide, hydroxyl radicals and hydrogen peroxide, and by reducing the cytotoxicity and genotoxicity effect compared to LP. Moreover, the results showed that CD151 gene silencing by shRNA significantly reduced the overexpression of CD151, tBHP-induced ROS generation, cytotoxicity and genotoxicity in MCF-12A cells. The overexpression of CD151 caused increased levels of cytochrome P450, but was reduced following the application of CD151shRNA and CLP which led to elevated levels of intracellular ROS.

**Conclusion:** In the present study we noticed that CD151 gene silencing by shRNA and treatment with CLP have similar effects on reducing the intracellular ROS. This study uncovers the protective activity of CLP against a CD151-mediated oxidative stress-induced cellular damage. Our observations suggest that the anti-stress and anti-inflammation properties of CLP might have implications in cancer and are worth testing in cancer cell lines and tumor cells.

Key words: Actinobacteria, apoptosis, cytoprotective, DNA damage, MCF-12A cells.

## Introduction

Oxidative stress plays a key role in breast carcinogenesis.<sup>1</sup> The prooxidant, tert-butyl hydroperoxide (tBHP), is extensively used as an exogenous stress inducer.<sup>2</sup> tBHP mimics oxidative

\* Address for correspondence: Prof. Rama Rao Malla, PhD Department of Biochemistry and Bioinformatics, GIS, GITAM (Deemed to be University), Visakhapatnam-530045 Tel: 91-7386168249 Email: <u>dr.rrmalla@gmail.com</u> stress by resembling products of oxidative stressmediated lipoperoxidation<sup>3</sup> and has extensively been used as an inducer of ROS-mediated oxidative stress.<sup>4</sup> Typically, two pathways, cytochrome P450 (CYP), and glutathione oxidase are involved in tBHP metabolism, and tBHP metabolites are known to reduce mitochondrial function by inducing oxidative stress.<sup>5</sup> tBHP also induces oxidative stress through NFkB pathway.<sup>6</sup> Moreover, tBHP is a well-studied oxidative stress-mediated tumor promoter.<sup>7, 8</sup> The metabolites of tBHP are known to promote free radical mediated carcinogenesis in skin cells<sup>9</sup>, epidermal cells<sup>10</sup>, fibroblasts and keratinocytes.<sup>11</sup> Also, it promotes carcinogenesis by inducing the expression of oncogenes in epidermal cells<sup>10</sup>, breast cells<sup>12</sup>, and promote lung tumors.<sup>13</sup> tBHP promotes cytotoxicity and genotoxicity in various cell lines.<sup>14</sup>tBHP-mediated oxidative stress triggers EGFR activation by promoting phosphorylation at its tyrosine residues<sup>15</sup> which causes cellular transformation of epithelial cells.<sup>16</sup>

Our previous study<sup>17</sup>, and another study<sup>18</sup> demonstrated that CD151, a member of a well-known multicellularity-associated gene family, is associated with EGFR signaling. TSP-15, a human CD151, is linked with ROS generating dual oxidase.<sup>19,20</sup> A study has reported that ROS generation system requires tetraspanin and they co-occur during evolution.<sup>21</sup> Another study reported that tetraspanins are essential in the induction of  $H_2O_2$  by dual oxidase via forming a complex at the cell surface.<sup>19</sup> CD151 is one of the oncogenic members of the tetraspanin family that participates in cancer progression and metastasis by associating with ROS generating proteases, signaling enzymes, GPCRs, cadherins, and proteoglycans.<sup>2</sup> Recently, we demonstrated that CD151 interacts with EGFR in breast cancer cells.<sup>17</sup> EGFR is involved in the regulation of oxidative stress.<sup>23</sup> Using the Chip assay, Chen et al. demonstrated that EGF/EGFR complex directly binds to the promoter of cytochrome P450 (CYP).<sup>24</sup> The role of members of cytochrome P450 (CYP) has been well reported in the conversion of metabolites into potentially toxic compounds<sup>25</sup> which can damage cells by forming DNA/protein adducts or by generating ROS.<sup>26</sup> Also, CYP mediates cytotoxicity and cell death induced by toxic compounds in epithelial cells<sup>27, 28</sup> and drug induced cell death by increasing the LDH leakage in hepatocytes<sup>29</sup>, lipid peroxidation dependent cytotoxicity and apoptosis<sup>30</sup> as well as arachidonic acid induced cytotoxicity and apoptosis.<sup>31</sup> CYP genes are controlled by various regulatory networks and microRNAs<sup>32</sup>, receptors and transcriptional factors at basal levels<sup>33</sup>, heat shock proteins by stabilization<sup>34</sup>, protein-protein interaction<sup>35</sup> and organization in the lipid membranes.<sup>36</sup> We speculate that CD151 may be associated with TEM establishment to facilitate cytochrome P450 mediated ROS generation in tBHPinduced cytotoxicity in MCF-12A cells.

Natural products prevent disease progression by scavenging ROS and enhancing the body's natural defense by inhibiting lipid peroxidation or direct interaction with key stress-related signaling molecules. Moreover, they can also control the initiation of carcinogenesis by protecting against DNA damage.<sup>37</sup> Marine secondary metabolites find clinical applications owing to their antioxidant<sup>38,39</sup> or protective activity.<sup>40, 41</sup> Actinobacteria contribute to 70% of the known drugs.<sup>42</sup> Novel peptides with marine sources are under clinical trials due to their

antibacterial, anti-inflammatory, and anti-cancer properties.  $^{\!\!\!\!^{43}}$ 

Cyclic dipeptides are modest peptide derivatives, usually present in nature<sup>44</sup> and are normally more stable and potent than other cyclic peptides in terms of drug efficacy. Cyclic dipeptides with different biological activities were isolated from marine organisms.<sup>45-50</sup> They are known to exhibit antifungal<sup>51,52</sup>, antitumor<sup>53</sup>, antiviral<sup>54</sup>, and antibacterial activities.55, 56 For instance, cyclo (D-Tyr-D-Phe) exhibits antioxidant and anti-cancer properties.<sup>57</sup> Cyclo(His-Pro) was found to activate caspase-3 and enhance poly(ADP-ribose) polymerase (PARP) cleavage and DNA fragmentation.<sup>58</sup> The cyclic dipeptides, azonazine, cyclo (His-Pro), 2,5-diketopiperazines, petrocidin A are well known for targeting cancer cells.<sup>39</sup>Nilov et al. (2018) demonstrated that cyclo (L-Ala-L-Ala) and cyclo (L-Ala-D-Ala) had sensitize DNA damaging effects of chemotherapeutics in drug resistance cancer cells.6

Cyclo(L-Leu-L-Pro) (CLP), a homodetic cyclic peptide belongs to the class of 2,5-diketopiperazines, isolated from marine actinobacteria strain<sup>61,62</sup>, marine *Streptomyces*<sup>63</sup>, sponge<sup>64</sup> and halobacterium.<sup>65</sup> It is noted for vast biological actions including antimicrobial<sup>66</sup>, antifouling<sup>46</sup>, antioxidant<sup>67</sup>, cytotoxic<sup>68, 69</sup>, anti-mutagenic activities<sup>70</sup>, anticarcinogenic<sup>71</sup> and anti-migratory potential of TNBC cells.<sup>72</sup> CLP's biological role was reported as a nutrient by the human metabolic database (https://hmdb.ca/metabolites/ HMDB0034276), although cytoprotective activity was not observed. Therefore, we evaluated CLP's cytoprotective and genoprotective activities against tBHP-induced damages in MCF-12A cell line model targeting CD151-EGFR signaling pathway.

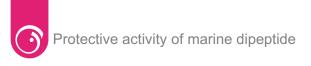
## Methods

#### Culture and Treatment

MCF-12A cells were obtained from ATCC and maintained in DME medium containing 10% FBS at 37 °C in an incubator under 5% CO<sub>2</sub> atmosphere. To evaluate radical scavenging activity, MCF-12A cells  $(1x10^4 \text{ cells/well})$  in a 96-well plate were pretreated overnight with tBHP (0.25mM) and then with CLP, ascorbic acid (AA) and linear L-Leucyl-L-Proline (LP) (0, 20, 40, 60, 80 and 100  $\mu$ M) for 24 hours.

tBHP, CLP, LP and AA were prepared in 0.1% DMSO in DMEM. To evaluate cytoprotective and genoprotective activities, MCF-12A cells were pretreated with CLP (0, 20, 40, 60, 80 and 100  $\mu$ M) overnight and then with tBHP (0.25 mM) for 24 hours.

The concentration of CLP that we used was based on our previous studies on TNBC's.<sup>17</sup> AA and LP were used as positive controls to compare the radical scavenging, cytoprotective and genoprotective activities of CLP.



## Intracellular ROS detection assay

The intracellular ROS was computed using DCF-DA by cellular ROS detection assay kit as per the manufacturer's instructions (Abcam, Cambridge, USA, ab113851). Briefly, the cell samples were rinsed with PBS, and stained with DCF-DA ( $20 \mu M$ ) for 30 min. After staining, the samples were washed again with PBS and the fluorescence emitted by the cells was measured at the Ex/Em wavelength 485/535 nm using a spectrofluorometer. The tBHPinduced intracellular ROS scavenging ability was reported as percentage of tBHP treated control.<sup>73</sup>

#### Mitochondrial superoxide (O2-) detection assay

The mitochondrial superoxide was measured with a mitochondrial superoxide detection assay kit according to the manufacturer's instructions (Abcam, Cambridge, USA, ab219943). The cells were rinsed with PBS and incubated with 100  $\mu$ L of MitoROS-580 staining solution for 1 hour. Next, the fluorescence intensity was measured as outlined above, at the Ex/Em wavelength 540/590nm, and tBHP-induced mitochondrial superoxide scavenging activity was expressed as percentage of tBHP treated control.<sup>74</sup>

## Mitochondrial hydroxyl radical detection assay

The mitochondrial hydroxyl radicals were measured using a mitochondrial hydroxyl radical detection assay kit (Abcam, Cambridge, USA, ab219931). The cells were incubated with assay buffer (100  $\mu$ L) and OH580 staining solution (100  $\mu$ L) for 1 hour at 37 °C after rinsing with PBS. Then, the fluorescence intensity was measured as outlined above. The scavenging ability of mitochondrial hydroxyl radical was expressed as percentage of tBHP treated control.

#### Intracellular hydrogen peroxide assay

Intracellular hydrogen peroxides was determined using an  $H_2O_2$  cell-based assay kit (Caymen, Michigan, USA, Cat# 600050). Next, the cells were rinsed with PBS and 10 µL of each assay buffer and enzyme reaction solution containing horseradish hydrogen peroxidase and hydrogen peroxide detector (ADHP) was added. Within 5 minutes, the fluorescence emitted by the cells was measured at Ex/Em of 530/590 nm. The tBHP-induced intracellular hydrogen peroxide scavenging activity was expressed as percentage of tBHP control.<sup>75</sup>

#### Lipid peroxidation (MDA) assay

The lipid peroxidation was evaluated using a lipid peroxidation (MDA) assay kit (Abcam, Cambridge, USA, ab118970). Two hundred  $\mu$ L of the treated cell lysis solution was added to each well, homogenized by placing on ice, and centrifuged at 13,000 rpm for 10 minutes. Then the cell lysate (200  $\mu$ L) was incubated with 200  $\mu$ L of TBA reagent for 1

h at 95°C. Next, the absorbance was measured at 532 nm using a microplate reader. The inhibition of tBHP-induced lipid peroxidation was expressed as percentage of tBHP treated control.<sup>76</sup>

## MTT assay

The treated cells were incubated with MTT reagent (5 mg/mL of PBS) for 2 hours. Next, the formazan crystals formed were solubilized by adding 200 $\mu$ L of DMSO. The absorbance was computed using an ELISA reader at 595 nm. The cytoprotective effect was expressed by comparing cytotoxicity with tBHP treated control.<sup>72</sup>

## Lactate dehydrogenase (LDH) assay

Treated cells were rapidly homogenized in LDH assay buffer on ice and cell debris was removed by sedimentation at 10,000 rpm for 15 minuets at 4 °C. The soluble fraction was used for measuring LDH activity by LDH activity assay Kit (Sigma-Aldrich, USA). The protective effect was expressed by comparing the LDH levels with tBHP treated control.<sup>77</sup>

## Phase-contrast microscopy

Cells (3x103/well) in a 96-well plate were treated with tBHP (0.25mM) alone or in combination with CLP (84.21  $\mu$ M) or LP (176  $\mu$ M) for 24 hours. After treatment, the cells were washed thrice with serum-free media, and images were captured at 40X resolution under the inverted phase-contrast microscope.

## Detection of AP Sites

The cellular DNA was extracted from treated and untreated cells. The reaction mixture [1  $\mu$ g of DNA, 200  $\mu$ l of 10 mM Tris (pH 9), 15  $\mu$ l of 5 M NaCl] was incubated with 30  $\mu$ l of avidin-HRP for 60 min at room temperature (RT). The DNA HRP was extracted by treating with 65  $\mu$ l of 1 mM DAPER for 5 minutes followed by centrifugation for 5min at 12,500 × g at 4 °C. The pellet was washed with 1.4 ml of wash buffer and suspended in 500  $\mu$ l ice-cold 50 mM Na-citrate (pH 5.3) by sonication. The HRP activity was determined showing AP sites in DNA–HRP by ELISA method.

## Detection of 8-hydroxy-2'-deoxyguanosine (8-OHdG)

Competitive ELISA was performed using an 8-OHdG ELISA kit, by the protocol of the Cayman Chemicals (USA). The purified DNA was subjected to enzymatic digestion using nuclease P1 at 50 °C for 1 h and with alkaline phosphatase at 37 °C for 30 minutes. The digested DNA was boiled for 10 minuets and placed on ice for 5 minutes. The hydrolyzed DNA was measured by reading the absorbance at 412 nm. The levels of 8-OHdG were measured and expressed as  $\mu$ g of 8-OHdG/ml.<sup>78</sup>

### Alkaline Comet assay

The treated and untreated cells were plated on a slide previously coated three times with low melting agarose (0.75 %). The slide was then placed in lysing solution at 4 °C for 1 hour followed by electrophoresis by setting the voltage to 20 V for 20 min. The slide was then soaked in neutralizing buffer followed by ethanol for 5 minutes. Then the slide was stained with ethidium bromide (40  $\mu$ l). The tail length was measured, and the olive tail movement (OTM) was computed as: (head mean) x tail % DNA/100.

## Cell death by Annexin VELISA method

After treatment, cell samples were washed with PBS, and apoptotic cells were determined using Annexin V ELISA method. Briefly, cells were incubated with 50  $\mu$ l of annexin V antibody at 25 °C for 1 hour. The apoptosis rate was determined as per the manufacturer's instructions (Abcam, USA).<sup>79</sup>

## Cell-based ELISA for quantification of Cd151

After treatment, the cells were washed with PBS and incubated with CD151 primary antibody overnight at 4 °C. After washing off the unbound primary antibody, cells were treated with HRP-conjugated secondary antibody specific to CD151 (100  $\mu$ L) and incubated at 37 °C for 1h. Following washing the unbound secondary antibody, the cells in each well were incubated with TMB One-Step Substrate Reagent (100  $\mu$ L) at 37 °C in the dark for 30 min. After stopping the reaction by adding stop solution (50  $\mu$ L), the absorbance was computed using a microplate reader set to 450 nm.

#### CD151 gene silencing using shRNA

After overnight seeding of MCF-12A cells (5x104) in a 6-well plate, the cells were transfected with CD151 shRNA overnight as described by Gayatri et al.<sup>80, 81</sup> After transfection, the cells were treated with tBHP (0.25 mM) for 24 hours and intracellular ROS was measured using DCF-DA using cellular ROS detection assay kit, extracellular LDH using LDH assay kit and DNA damage using alkaline comet assay as described above.

## CD151 overexpression in MCF-12A cells

After overnight seeding of MCF-12A cells  $(5x10^4)$  in a 6-well plate, the cells were transfected with 10 µg untagged PrecisionShuttle mammalian plasmid encoding CD151(CAT#: SC319271, OriGene, USA) using TurboFectin Transfection Reagent (TF81001, OriGene) following the manufacturer's protocol. After 24 hours of transfection, the cells were treated with CLP and LP for 24 hours. Then CD151 expression levels were determined by cell-based ELISA, and intracellular ROS, LDH and DNA damage were determined using their specific methods outlined above.

# Protein-protein interaction by ClusPro and PyDOCK webserver

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The ClusPro is a widely used fully automated protein-protein docking server, which uses direct docking of two interacting proteins (https:// cluspro.bu.edu).<sup>82</sup> It uses PIPER16 for the rigid body docking program based on the Fast Fourier Transform (FFT) correlation approach. PyDOCK web server, which uses rigid-body docking orientations generated by FTDock and evaluation is based on electrostatics, de-solvation energy and limited van der Waals interactions.<sup>83</sup> Using ClusPro and PyDOCK webserver, the interaction between CD151 and cytochrome p450 was predicted.

#### Measurement of cytochrome P450 levels by ELISA

After treatment, cell samples were collected by scraping into ice-cold PBS, sedimented by centrifugation at 3000 rpm for 5min. The cell pellet was suspended in a cell lysis buffer, and the cells were lysed by pipetting up and down 5-10 times. Then the lysate was cleared by removing cell debris using centrifugation at 10,000 rpm for 5min at 4 °C. Next, cytochrome P450 levels in the cell lysate were determined by human quantitative sandwich cytochrome P450 ELISA kit (CUSABIO Technology LLC, USA) using cytochrome p450 antibody precoated microplate following the manufacturer's instructions. After removing the unbound substances by washing, the plate was incubated by adding cytochrome P450 antibody conjugated to biotin, followed by HRP conjugated avidin. Finally, the color was developed by incubating with TMB substrate solution. After stopping the color development using a stop solution, the optical density was measured at 450 nm using a microplate reader.

#### Statistical analysis

The data from the experiments were analyzed statistically and represented graphically using Microsoft Excel. To ensure the consistency of the results, each experiment was performed three times, and values were represented as mean  $\pm$  SD (n+3). One-way variance analysis was performed to calculate the means of the dependent variable (response) and independent variable (concentration) using NumPy (v 1.1.2) and Google Colab. Student t-test was performed to compare mean values. The statistical significance was set with a confidence level of 95% and p value <0.05. IC<sub>50</sub> values were calculated using MS Excel ProPlus (Version 2016).

## Results

#### Intracellular ROS scavenging ability of cyclo(L-Leu-L-Pro)

tBHP (0.25mM) stimulates augmented oxidative stress in MCF-12A cell line which helps to understand the protective role of CLP in regulating CD151-mediated oxidative stress. The scavenging



activity of CLP applied at increasing concentrations (0-100 µM) against tBHP-induced intracellular ROS production was compared with that of linear dipeptide (LP) and radical scavenger, AA (Figure 1a). The prooxidant tBHP triggered considerable oxidation of DCFH<sub>2</sub> to DCF and enhanced intracellular fluorescence intensity in controls. However, CLP treatment decreased the tBHPinduced fluorescence intensity in a dose-dependent manner. Cyclic dipeptide CLP diffuses into the cells through the cell membrane, where it may prevent the generation of ROS required to oxidize intracellular DCFH2 to the fluorescent DCF. tBHP stimulated intracellular ROS generation, scavenged by treatment with CLP. The percent of radical scavenging activity are 17.8±2.3, 32.6±2.2, 41.7±2.3, 54.2±3.4 and 70.2±3.3% with CLP, and the positive control AA exhibited 21.6±2.5, 39.4±2.3, 52.4±2.4, 59.3±3.4 and 75.3±3.2% of radical scavenging activity, while for another positive control LP, the radical scavenging activity was  $8.2\pm1.2$ ,  $12.2\pm1.3$ ,  $21.2\pm1.3$ ,  $32.2\pm2.4$  and 38.1±2.4% at 20, 40, 60, 80 and 100µM, respectively. The IC<sub>50</sub> values of CLP, AA and LP for scavenging intracellular ROS were 70.6, 130 and 60.5µM, respectively.(Figure 1a) This study demonstrates the concentration-dependent intracellular ROS scavenging activity of the CLP like ascorbic acid, which is higher than that of LP.

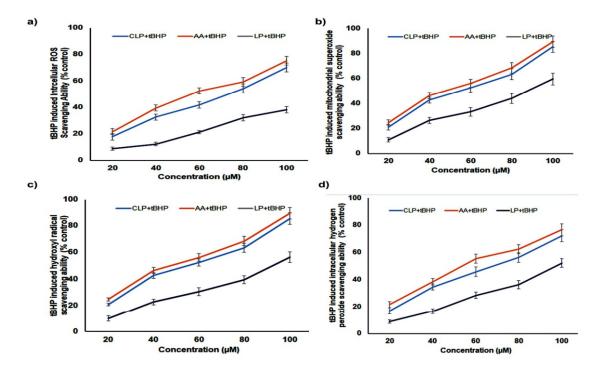
Superoxide ion  $(O_2)$  is the most acceptable candidate for causing oxidative stress damage to the cell.<sup>84-86</sup> The oxidative stress causes the escape of electrons from mitochondrial ETC and directly generates the O<sub>2</sub> by reacting with molecular oxygen.<sup>87</sup> The present study observed that tBHP-induced intracellular O<sub>2</sub>.- was scavenged by 20.8±2.3, 42.6±2.3, 52.5 ±3.4, 63.4±3.3 and 85.4±4.3% with CLP and 24.6±2.2, 46.3±2.4, 56.2±3.3, 68.5±4.3 and 89.4±4.4% with AA and 10.8±1.8, 26.3±2.4, 33.2±3.4, 43.9±4.1 and 59.6±4.8% with AA at 20, 40, 60, 80 and100µM as shown in Figure 1b. The IC<sub>50</sub> of the CLP was 56.0 µM, LP was 86.4 µM and AA was 50.7 µM, indicating the potential O<sub>2</sub> scavenging ability of CLP close to AA.

Among the reactive oxygen centered species, hydroxyl radicals (OH) cause serious damage to proteins, polyunsaturated fatty acids and DNA and are implicated in radical-mediated pathology. The intracellular OH scavenging action of CLP was related to intracellular antioxidant ability. The results depicted in Figure 1c show that tBHP- induced intracellular OH scavenging activity of the CLP was 19.2±2.3, 28.3±2.3, 41.4±2.3, 55.4±3.4 and 81.5±4.4% whereas AA showed 21.4±2.3, 32.2±2.3, 48.4±3.3, 62.4±3.4 and 82.8±4.4% and LP exhibited 9.8±1.8, 22.6±2.1, 30.5±2.9, 39.4±3.1 and 56.4±3.8% at 20,40,60,80 and 100µM, respectively. The IC<sub>50</sub> of CLP was 66.3  $\mu$ M, LP was 93.1  $\mu$ M and AA was 60.68µM, indicating potential intracellular OH scavenging activity of CLP, similar to AA.

One of the interesting developments in the free radical-mediated pathology is the formation of hydroxyl radicals by the interaction between  $O_2^-$  and  $H_2O_2$ . Hence, we evaluated the intracellular  $H_2O_2$ scavenging activity of the CLP. The study observed that tBHP-induced intracellular H<sub>2</sub>O<sub>2</sub> scavenging activity with the CLP was  $16.9\pm2.3$ ,  $34.6\pm2.3$ , 45.7±3.3, 56.2±3.4 and 72.1±4.3%, whereas 21.6±2.5, 38.4±2.3, 55.4±3.5, 62.3±3.4 and 76.4±4.3% with AA, and 8.6±1.2, 16.6±1.8, 28.5±2.1, 36.3±2.9 and 52.1±3.2% with LP at 20, 40, 60, 80 and 100µM, respectively, indicating that intracellular  $H_2O_2$  scavenging activity of CLP is similar to that of AA as shown in Figure 1d. The significant radical scavenging ability may recommend CLP for the treatment of stress associated cancers.

## *Cytoprotective activity of CLP against tBHPmediated cytotoxicity*

The current study evaluated the protective ability of the CLP on tBHP stimulated lipid peroxidation in MCF-12A cells. Malondialdehyde (MDA) is the most widely cited lipoperoxidation product instigating from unsaturated lipids of membranes during oxidative stress. The protective effect of CLP was confirmed by lipid peroxidation assay. The percentage of inhibition of tBHP-induced lipid peroxidation by serial concentrations of CLP increased in a dose dependent manner to a maximum of 89.8±5.3%. This figure stood at 91.8 $\pm$ 7.3 % with AA and 57.8 $\pm$ 4.1% with LP (Figure 2a). This study also evaluated the impact of the CLP on tBHP-mediated cytotoxicity in MCF-12A cells. The results show that percent of viable cells increased with increasing concentration. Treatment with CLP showed 68.6±3.2% viability, which indicates that CLP efficiently enhanced tBHP treated MCF-12A cells viability. Treatment with positive controls like AA and LP showed maximum viability of 74.9±4.3 and 33.4±2.8%, respectively (Figure 2b). Further, the CLP's cytoprotective activity was evaluated by measuring LDH leakage from cell, an indicator of the cell membrane damage. The CLP efficiently blocked tBHP-induced LDH release from MCF-12A cells. The percentage of inhibition of LDH release increased with increasing concentration from 20 to 100µM. The maximum inhibition of LDH release was measured with CLP (62.8±3.4%), AA  $(71.5\pm4.3\%)$  and LP  $(28.4\pm2.1\%)$  with IC<sub>50</sub> of 82.9  $\mu$ M, 72.7  $\mu$ M and 174  $\mu$ M, respectively (Figure 2c). Further, the effect of CLP on tBHP-induced morphology of MCF-12A cells was monitored under phase-contrast microscopy (Figure 2d). The results show that tBHP (0.25mM) induced morphological changes in MCF-12A cells. However, pretreatment with CLP (84.2µM) significantly protected the MCF-12A cells from tBHP-induced morphological changes better than LP (174  $\mu$ M) and AA (76.6 $\mu$ M). These results suggest the potential cytoprotective activity of the CLP. Table 1 shows the data related to



**Figure 1.** Protective effect of CLP from tBHP-induced intracellular ROS levels in MCF-12A. Cells  $(1x10^4/well)$  in a 96-well plate were pretreated overnight with tBHP (0.25 mM) and subsequently with serial concentrations of CLP (0, 20, 40, 60, 80 and 100  $\mu$ M) for 24 h. Only tBHP treated cells were served as untreated control. The fluorescence generated upon conversion of DCFH2 to DCF due to the CLP scavenging activity was measured at the Ex/Em wavelength 485/535 nm using a spectrofluorometer and expressed as % control. The percent control is defined as: Fluorescence change in CLP treated cells/ tBHP treated cells x100. The same concentrations of ascorbic acid (AA) and linear dipeptide, L-Leucyl-L-Proline (LP) were used as positive controls. The graphs show the protective effect of CLP on tBHP-induced a) intracellular ROS production, b) mitochondrial superoxides, c) mitochondrial hydroxyl radicals and d) intracellular hydrogen peroxide. The cumulative data of each assay were collected from three independent experiments and shown as means ±SEM (n=3).

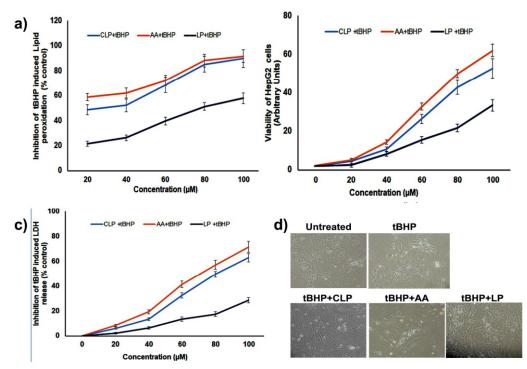


Figure 2. Protective activity of CLP against tBHP-induced cytotoxicity in MCF-12A cells.

Cells were pretreated overnight with CLP (0, 20, 40, 60, 80 and 100  $\mu$ M), subsequently with tBHP (0.25mM) and incubated for 24 h. Only tBHP treated cells were served as untreated control. AA and LP were used as positive control under similar concentrations. a) Protective effect of CLP on tBHP-induced lipid peroxidation in MCF-12A cells. b) Protective effect of CLP on the tBHP-induced cytotoxicity of MCF-12A cells. The results were expressed as % viability of tBHP treated control. c) Protective effect of CLP on tBHP induced cell membrane damage. The results were expressed as % inhibition of LDH leakage. d) Protective effect of CLP on tBHP induced morphological changes by phase-contrast microscopy. The cumulative data of each assay were obtained from 3 independent experiments and shown as means ±SEM (n=3).



No	Concentration Inhibition of lipid peroxidation (%)			Cell viability (%)			LDH leakage (%)			
	(µM)	CLP+tBHP	AA+ tBHP	LP+ tBHP	CLP+tBHP	AA+ tBHP	LP+ tBHP	CLP+tBHP	AA+ tBHP	LP+ tBHP
1	20	48.8±3.3	58.8±4.3	21.6±1.9	4.5±0.9	5.2±0.9	2.4±0.8	2.4±0.8	8.4±0.9	2.2±0.8
2	40	52.2±4.2	62.2±5.2	26.2±2.1	$10.8 \pm 1.1$	14.4±1.2	$8.2 \pm 1.1$	8.2±1.1	19.3±1.5	6.5±1.2
3	60	$68.6 \pm 4.1$	72.6±6.1	39.6±2.9	26.4±1.9	32.8±2.3	15.6±1.7	15.6±1.7	41.3±2.6	13.4±1.6
4	80	85.20±5.2	88.2±6.2	51.2±3.1	42.8±2.1	49.9±3.3	21.7±1.9	21.7±1.9	57.1±3.7	$17.8 \pm 2.1$
5	100	89.8±5.3	91.8±7.3	$57.8 \pm 4.1$	68.6±3.2	74.9±4.3	33.4±2.8	33.4±2.8	71.5±4.3	$28.4 \pm 2.1$

Table 1. Cytoprotective effect of CLP on MCF-12A cells

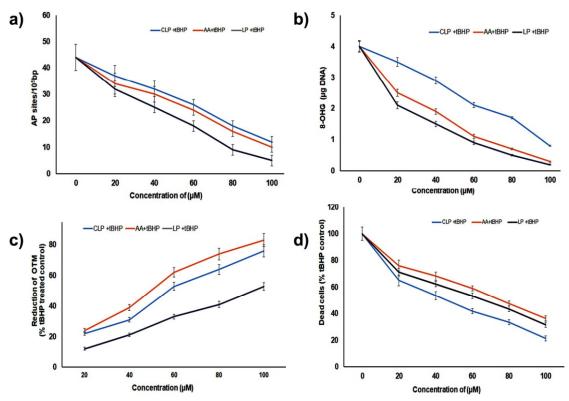
Cytoprotective activity of CLP (Cyclo(L-Leu-L-Pro)) was measured in terms of inhibition of lipid peroxidation, cell viability and inhibition of LDH leakage against tBHP-induced cell damage in MCF-12A cells. AA (Ascorbic acid) and LP (L-Leucyl-L-Proline) were used as control. The results were expressed in percent of inhibition of lipid peroxidation, percent cell viability and percent of inhibition of LDH release. Each experiment was performed three times, and values were represented as mean  $\pm$  SD (n+3)

cytoprotective activities of CLP, LP or AA at different concentrations (20-100  $\mu$ M).

## *Genoprotective ability of CLP on tBHP-induced* DNA damage

DNA is one of the most prominent biological targets of oxidative stress and is the most significant cancer development contributor. The CLP's genoprotective ability against tBHP-induced stress-mediated DNA damage in MCF-12A cells was determined, as shown in Figure 3a. tBHP treated cells found 44±5 AP sites/10<sup>5</sup>bp, but cells treated with the CLP show fewer AP sites in concentration dependent manner. The AP sites were decreased to  $12\pm 2$  AP sites/10<sup>5</sup>bp with CLP,  $5\pm 2$  AP sites/105bp with LP and  $10\pm 2$  AP sites/10<sup>5</sup>bp with AA. Also, 8-OHdG, a product of oxidatively damaged DNA, was quantified by monitoring the

formation of abasic sites using competitive ELISA. This assay employs an 8-OHdG-coated plate and an HRP-linked antibody to recognize oxidative stressmediated damage of DNA with high sensitivity. The results showed that 8-OHdG concentration was significantly high in tBHP treated control  $(4\pm0.19\mu g/ml)$ , but its levels were decreased with CLP to 0.80±0.02, AA (0.3±0.01) and LP (0.2±0.01) µg /ml in concentration dependent manner as shown in Figure 3b. The concentration of 8-OHdG was significantly less with 100µM of CLP compared to tBHP treated control, indicating the DNA damage protecting activity. In vitro comet assay is a sensitive and frequently used method to confirm the genoprotective activity.<sup>9</sup> Therefore, DNA damage protecting activity of CLP, AA and LP in tBHP treated MCF-12A cells was assessed using in vitro Comet assay, and the results



**Figure 3.** Genoprotective activity of CLP against tBHP-induced DNA damage in MCF-12A cells. Cells were pretreated overnight with CLP (0, 20, 40, 60, 80 and 100  $\mu$ M), subsequently with tBHP (0.25 mM) and incubated for 24 h. AA and LP under similar conditions were used as a positive control. The genoprotective activity of CLP against tBHP-induced DNA damage in MCF-12A cells in terms of (a) Abasic (AP) sites/1x106bp, (b) 8-OHdG formation ( $\mu$ g/ml), (c) Olive Tail Moment (%tBHP treated control). (d) Apoptotic cell death (%tBHP treated control) was determined by Annexin V ELISA method. The cumulative data of each assay were obtained from 3 independent experiments and shown as means ±SEM (n=3).

Table 2. Genoprotective	effect of CLP on	MCF-12A cells
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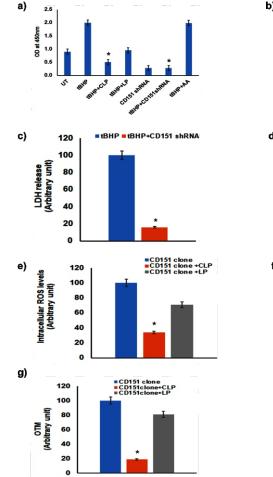
No	Concentration	oncentration AP sites/10 <sup>s</sup> bp		8-OHdG (µg /ml)		Decrease in OTM (%)		Dead cells (%)					
	(µM)	CLP+ tBHP	AA+ tBHP	LP+ tBHP	CLP+ tBHP	AA+ tBHP	LP+ tBHP	CLP+ tBHP	AA+ tBHP	LP+ tBHP	CLP+ tBHP	AA+ tBHP	LP+ BHP
1	20	38±4	34±4	32±3	3.5±0.4	2.5±0.11	2.1±0.11	22±1.8	24±1.1	12±0.9	65.1±4.3	76.2±4.1	71.3±3.2
2	40	32±3	30±3	25±2	$2.9{\pm}0.1$	$1.9{\pm}0.09$	1.5±0.09	34±1.9	39±2.4	21±1.1	53.6±3.2	86.3±3.6	62.3±3.1
3	60	26±2	24±2	18±2	$2.1\pm0.1$	$1.1 \pm 0.06$	$0.9 \pm 0.02$	53±2.7	62±2.8	33±1.4	41.8±2.1	59.2±3.2	53.5±2.9
4	80	18±2	16±2	9±2	$1.7\pm0.03$	$0.7{\pm}0.03$	$0.5\pm\!\!0.01$	64±2.8	74±2.9	41±2.1	33.4±2.1	47.5±3.1	43.5±1.8
5	100	12±2	$10\pm 2$	5±2	$0.80 \pm 0.02$	0.3±0.01	$0.2\pm0.01$	76±3.3	83±3.1	53±2.9	21.2±1.9	36.3±2.2	31.6±1.6

Genoprotective activity of CLP (Cyclo(L-Leu-L-Pro)) was measured in terms of AP sites/105bp, inhibition of 8-OHdG, decrease in OTM (%) and percent of dead cells against tBHP-induced cell damage in MCF-12A cells. AA (Ascorbic acid) and LP (L-Leucyl-L-Proline) were used as control. Each experiment was performed three times, and values were represented as mean  $\pm$  SD (n+3)

were expressed as the Olive Tail Moment (OTM) as shown in Figure 3c. The OTM was increased by 80% in MCF-12A cells treated with tBHP compared to the negative control. However, CLP decreased the tBHPinduced OTM to 3±2.9% compared to tBHP positive control. Finally, DNA damage protecting activity of CLP, AA, and LP was confirmed by Annexin V assay in tBHP treated MCF-12A cells (Figure 3d).

2.5

The results showed that the number of dead cells decreased with CLP was 31.6±1.6% and with positive controls AA (36.3±2.2%) and LP (31.6±1.6%), respectively with an increase in concentration to 100 µM compared to tBHP control, which is considered as 100%. Table 2 lists the data of genoprotective effect of CLP, LP or AA in MCF-12A cells.



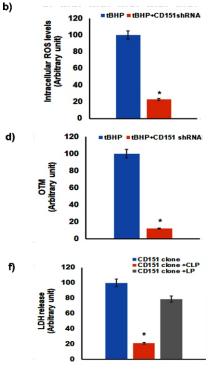
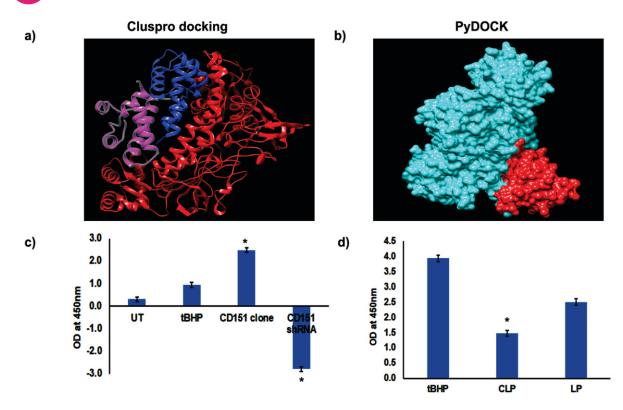


Figure 4. CLP protects tHBP treated MCF-12A cells by targeting CD151.

tBHP-induced CD151 levels were reduced with CLP and CD151shRNA (a). MCF-12A cells were pretreated overnight with CLP (70.6  $\mu$ M) and ascorbic acid (60.5 $\mu$ M) or transfected with CD151shRNA, subsequently with tBHP (0.25 mM) and incubated for 24 h. Untreated cells were served as control. The expression of CD151 was determined by cell-based ELISA assay. The results were expressed in OD at 450nm. After silencing the CD151 gene using shRNA, the intracellular ROS (b), extracellular LDH levels (c) and OTM (d) were evaluated in tBHP treated MCF-12A cells. Means ±SEM (n=3), p<0.05. MCF-12A cells (5x104) in a 6-well plate were transfected with 10 µg untagged Precision Shuttle mammalian plasmid encoding CD151 using Turbo Fectin Transfection Reagent. After 24h of transfection, cells were treated with CLP or LP for 24h. The intracellular ROS was measured using DCF-DA by cellular ROS detection assay kit (e), extracellular LDH using LDH assay kit (f) and DNA damage by alkaline comet assay (g) as described above. The cumulative data of each assay were obtained from 3 independent experiments, and results were shown as means ±SEM (n=3),\* p<0.05



#### Figure 5. In Silico interaction of CD151 with P450.

a) *In silico* docking of CD151 (receptor) with P450 (ligand) was performed using ClusPro web server. The receptor and ligand were represented as ribbon form. The binding energy of CD151 with P450 was calculated as -990 kcal/mol. b) *In silico* docking of CD151 (receptor) with P450 (ligand) was performed using PyDOCK webserver. The receptor and ligand were represented as a solid model. The binding energy of CD151 with P450 was calculated as -38.3 kcal/mol. c) The impact of tBHP, CD151 shRNA and CD151 clone on cytochrome p450 levels was determined using quantitative sandwich ELISA. d) Effect of CLP and LP on tBHP-induced cytochrome p450 levels. The cumulative data of each assay were obtained from 3 independent experiments, and results were shown as means  $\pm$ SEM (n=3), \*p<0.05

Pathway analysis of CD151 mediated t-BHP induced cytotoxicity

Kučera et al. reported that t-BHP is metabolized by cytochrome P450 and glutathione peroxidase mediated pathways.<sup>5</sup> CD151-dependent tetraspaninenriched microdomains (TEM) correlated with hepatocarcinoma.<sup>91</sup> Previously, we showed that CLP reduced the CD151 expression and its interaction with EGFR.<sup>17</sup> In the current study, we found that tBHP stimulated a 10-fold increase in the expression of CD151 compared to untreated control in MCF-12A cells. However, CLP and LP treatment reduced the 4.0 (p>0.05) and 2.1-folds of tBHP-induced expression of CD151, respectively, and CD151shRNA treatment 7.1-folds(p>0.05) in MCF-12A cells (Figure 4a), but AA did not affect the CD151 expression (Data not shown). CD151 gene silencing using shRNA reduced the intracellular ROS (4.3-folds) (Figure 4b), extracellular LDH levels (6.2-folds) (Figure 4c), and OTM (8.3-folds) (Figure 4d) in tBHP treated MCF-12A cells. Further, CLP and LP treatment reduced the intracellular ROS levels 2.9 and 1.4-folds, respectively (Figure 4e), LDH levels 4.7 and 1.3-folds, respectively (Figure 4f) and OTM levels 5.2 and 1.2-folds, respectively (Figure 4g) in CD151 overexpressed MCF-12A cells, indicating that CLP protects the MCF-12A

cells by targeting Cd151.

Protein-protein interactions are important for understanding cellular function and organization. To find the downstream mediator of CD151 in tHBPinduced cytotoxicity, we analyzed the interaction between CD151 and cytochrome P450, which is an important mediator of tBHP metabolism and membrane damage. By using Cluspro docking method, we interpreted that CD151 interacts with cytochrome P450 strongly (Figure 5a) with docking score of –993 k cal/mol. We have verified the results using another docking study, PyDOCK webserver (Figure 5b). The docking score of CD151 with P450 was -38.39k cal/mol. Thus, P450 shows a high binding tendency for CD151 protein.

To further focus our investigation on the protection exerted by CLP in CD151 mediated cytotoxicity in tBHP treated MCF-12A cells, we determined the cytochrome p450 levels in MCF-12A cells treated with tBHP, CD151 shRNA and CD151 clone using quantitative sandwich ELISA (Figure 5c). The results showed that CD151 clone enhanced the cytochrome P450 levels by 8-folds, and CD151 shRNA reduced the cytochrome P450 levels by 9-folds. However, tBHP-induced cytochrome P450 levels by 9-folds. Further, treatment with CLP and LP significantly reduced the tBHP-induced

cytochrome P450 levels (Figure 5d), but not considerably by AA (Data Not shown). These results indicate that CD151 is an upstream mediator of tBHP-induced cytotoxicity in MCF-12A cells.

## Discussion

In normal physiological conditions, the homeostasis of ROS is maintained by cellular antioxidant defense system but during oxidative stress this homeostasis is lost which causes disturbance in the metabolism of free radicals and their detoxification.<sup>92</sup> Excessive generation of radicals causes oxidative damage in proteins, fatty acids, and DNA which leads to inflammation and cancer. The stimulation of the antiradical mechanism is one of the most significant determinants of cytoprotective ability against oxidative stress-induced diseases.<sup>93</sup>

CLP is a biologically active homodetic cyclic peptide, which we reported to exhibit significant inhibition of tBHP-induced oxidative stress in MCF-12A cell line. CLP's scavenging potential against intracellular ROS and mitochondrial superoxide and hydroxyl radicals was comparable to that of Ascorbic acid, a well-known antioxidant. This study emphasizes the significant cytoprotective as well as genoprotective activity of the CLP against tBHPinduced cellular stress in MCF-12A cells and results were compared to linear dipeptide (LP). Previously, a large amount of data pertaining to dipeptides, including aspartame (L-aspartyl-L-phenylalanine methyl ester) as well as L-alanyl-L-glutamine as cytoprotective agents was documented.<sup>94</sup>Kaul et al. provided evidence suggesting that proline (Pro) has the ability to scavenge free radicals *in vitro*.<sup>95</sup> Besides the role of proline as antioxidant, ameliorating metal toxicity has also been reported recently.<sup>96,97</sup> Research by Phang et al. emphasized proline as a "stress substrate" and suggested it as a potential anti-cancer agent.<sup>98</sup> Also, Pro has wound healing potential.<sup>99</sup> In addition, Pro was reported as an activator of mTOR signal pathway in concert with leucine."

Cyclic dipeptides can bind to diverse targets due to conformationally constrained scaffolds and a vast number of tailoring enzymes.<sup>101</sup> Yan et al. reported that hydrophobic amino acids and proline are critical for cyclic dipeptides' biological activity.<sup>102</sup> The peptide bonds in proteins normally have a planar trans configuration. However, due to neighboring substituents' steric requirements, the cis form is more stable than the trans form when the Pro (imino acid) occurs at the bond's carboxyl-terminal side.<sup>103</sup> Further, the Pro ring's restricted mobility might be related to cyclic dipeptides.<sup>104</sup> The cis-trans isomerism of the N-alkyl amide bond in the Pro was found to be involved in receptor-mediated biological activity.<sup>55</sup> The connection between the configuration of Pro and its physical activity might be important in explaining the mechanism of the inhibitory activity

of cyclodipeptides.

Because of these properties, cyclic dipeptides consist of hydrophobic amino acids like leucine, and terminal amino acid proline are products of rational drug design. CLP significantly improved the viability of tBHP treated MCF-12A cells in a concentration-dependent manner. Further, CLP's cytoprotective effect in tBHP-induced cellular leakage of LDH was significantly high compared to other dipeptides like  $\beta$ -alanine-histidine and carnosine.<sup>105</sup> Besides, pretreatment with CLP protected MCF-12A cells from tBHP-induced morphological changes better than LP. These results indicate the efficacy of marine natural product, the CLP as a cytoprotective agent.

Later, we studied CLP's effect on genoprotective activity against tBHP-induced stress in MCF-12A cell line. The results showed that CLP exhibited remarkable protection against DNA damage in terms of levels of 8-OHdG and tailing in comet assay. Limited reports are available on genoprotective activity of dipeptide. Thus, we have made an attempt to study the effect of CLP as a cytoprotective and genoprotective agent against stress-induced model. The present study showed that CLP is an efficient dipeptide, which can regulate cytoprotective cellular mechanism in addition to providing protection to DNA damage caused by oxidative stress.

Apart from metastatic activity, CD151dependent TEM mediates hepatocarcinoma<sup>91</sup> and liver fibrosis<sup>106</sup>, and has also been proposed as CD151 potential therapeutic target of liver fibrosis.<sup>107</sup> CD151 is a membrane protein that transduces intracellular signaling and regulates cellular functions. Previously, we showed that CLP reduced the CD151 expression and its interaction with EGFR.<sup>17</sup> Bae et al. demonstrated that EGF induces H<sub>2</sub>O<sub>2</sub> generation via EGFR in cancer cell lines.<sup>108</sup> The present study observed increased levels of CD151 in tBHP treated MCF-12A cells. However, tBHP-induced CD151 levels were reduced with CD151shRNA and CLP treatment which are comparable. However, LP did not affect CD151 expression. In addition, CD151 gene silencing decreased the tHBP- induced ROS and LDH levels in tHBP treated MCF-12A cells, indicating the involvement of CD151 in tHBP- induced cytotoxicity. In addition, CD151 gene silencing reduced the tHBP induced OTM in MCF-12A cells. Further, CLP reduced the intracellular ROS, extracellular LDH levels, and genotoxicity (OTM) in CD151 overexpressed MCF-12A cells.

High docking score in the interaction study of CD151 with cytochrome P450 by Cluspro docking method and PyDOCK web server indicated strong interaction CD151 with cytochrome P450. Further, overexpression of CD151 increased the CYP levels but reduced with CD151 shRNA with a moderate increase with tBHP. Also, treatment with CLP and

LP significantly reduced the tBHP induced cytochrome P450 levels, but not AA. Additionally, CD151 gene silencing using CD151 shRNA reduced the cytochrome P450 levels in tBHP-induced MCF-12A cells. These results indicate that CD151 is an upstream mediator of tBHP-induced cytotoxicity in MCF-12A cells and CLP protecting the MCF-12A cells by targeting Cd151.

In the present study, we have reported the cytoprotective role of CLP against various ROS types using MCF-12A cell line model. CLP increases the cellular antioxidant status in MCF-12A cells and scavenges the tBHP-induced intracellular ROS levels. This study also observed that the CLP protected MCF-12A cells from tBHP-induced lipid peroxidation, LDH leakage, and morphological changes. Moreover, a significant genoprotective activity of the CLP was observed against tBHPinduced DNA damage in MCF-12A cell line compared to positive control LP. Furthermore, CLP also reduced the tBHP-induced DNA damage by decreasing AP sites, 8-OHdG levels, and OTM in a concentration-dependent manner. There was a significant effect of CLP on expression of CD151 which can be compared to CD151 shRNA mediated gene silencing. Protein-protein interaction study by Cluspro docking method and PyDOCK web server indicated strong interaction CD151 with cytochrome P450. An increase in cytochrome P450 levels with CD151 overexpression and reduction with CD151 gene silencing by shRNA, as well as reduction with CLP and LP, indicates the involvement of CD151 gene via cytochrome P450 in tBHP-induced cytotoxicity in MCF-12A cells. These results also suggest that CLP protects the MCF-12A cells more significantly by targeting CD151 compared to its linear counterpart, LP. In conclusion, based on the intracellular ROS scavenging ability, cytoprotective, and genoprotective activities, CLP can be used as an efficient agent against oxidative damage mediated pathological diseases like cancer and inflammation.

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## **Ethical statements**

None.

## **Conflict of Interest**

Authors declare no potential conflicts of interest.

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## Practice and Outcome of Exercise Intervention on Breast Cancer-Specific Quality of Life of Breast Cancer Survivors in Nigeria

Linda C. Odikpo\*<sup>a</sup>, Edith N. Chiejina<sup>a</sup>

<sup>a</sup> Department of Nursing Science, Nnamdi Azikiwe University Awka, Nigeria

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## ABSTRACT

**Background:** Lifestyle modification like exercise is an essential rehabilitation measure that improves the quality of life (QoL) of women with breast cancer and helps in preventing cancer related complications. This study assessed the practice and outcome of exercise interventions on breast cancer-specific quality of life of survivors in Delta State, Nigeria.

**Methods:** Experimental design was applied with intervention (47) and control (47) groups. This design involved a pre-test, an intervention, and a post-test. Exercise intervention (aerobic, resistance and flexibility exercises, including warm-up with Swiss ball and dance, climbing of stairs, treadmill, stationary exercise bicycle, shoulder, and arm exercises) was administered to the intervention group for twelve weeks. The assessment of breast cancer survivors' specific quality of life in the two groups was done with the English version of The European Organization for Research and Treatment of Cancer Breast Cancer-Specific Quality of Life Questionnaire (QLQ-EORTC-BR23) before and after the exercise intervention.

**Results:** The overall pre and post-intervention breast cancer-specific functional quality of life was  $65.4\pm22.7$  (intervention group);  $71.3\pm23.4$  (control group) and  $75.05\pm10.4$  (intervention group);  $58.65\pm12.9$  (control group) while the pre and post breast cancer-specific symptoms QoL was  $22.2\pm6.2$  (intervention group);  $24.1\pm9.6$  (control group) and  $11.8\pm13.0$  (intervention group);  $30.9\pm21.2$  (control group), respectively. All the women in the intervention group practiced exercise only at mild/moderate intensity and no notable side effects were observed during the practice by many of them (n=39). Significant differences existed in the overall post-intervention breast cancer-specific functional and symptoms QoL between the two groups (p<0.001) and no significant differences were observed among most of the specific QoL scales in relation to age, duration of diagnosis, and stage of the cancer diagnosis.

**Conclusion:** Exercise remains beneficial to women with breast cancer and has proven to be one of the necessary means to improve their overall well-being. Therefore, healthcare providers that manage these patients in different hospitals should always counsel and support them to initiate the recommended exercises for cancer survivors to enhance their survival.

\*Address for correspondence: Linda C. Odikpo, RN, B.NSC, PGDE, MSC Address: Department of Nursing Science, Nnamdi Azikiwe University Awka, Nigeria. Tel: +2348135705878 Email: <u>Ic.odikpo@unizik.edu.ng</u>

#### Introduction

Breast cancer is responsible for about 19.5% of all cancer cases and 16% of all cancer-related deaths in Nigeria with a severe negative effect on the women's breast cancer-specific quality of life issues.<sup>1</sup> With the improvements in early detection and treatment, the number of cancer survivors has continued to increase,

Key words:

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Practice.

with women with breast cancer accounting for 22% of total cancer survivors globally in 2012.<sup>2</sup> One of the means to enhance this survival and rehabilitation includes lifestyle modifications like exercise. The benefits of exercise cannot be overemphasized. It is associated with the reduction of comorbid chronic diseases such as type 2 diabetes and cardiovascular diseases. Exercise equally promotes body weight management<sup>3,4</sup>, and breast cancer-specific quality of life (QoL) of survivors<sup>5</sup>

Breast cancer-specific quality of life considers the functional and symptom scales with other subscales including body image, sexual functioning, sexual enjoyment, future perspectives and other symptoms subscales that are peculiar to breast cancer patients alone and could be assessed using the European Organization for Research and Treatment of Cancer (EORTC) Breast Cancer-Specific Quality of Life Questionnaire (QLQ-BR23).<sup>6-10</sup> Thus, EORTC has developed several site-specific questionnaires including one for breast cancer (QLQ-BR23) in order to collect more relevant patient-reported outcomes in studying quality of life in this group of cancer patients.<sup>11</sup>

Few studies have measured breast cancer specific QoL<sup>12-14</sup>, and results have showed improvement in the specific QoL outcome variables of women after a breast cancer diagnosis when subjected to exercise.<sup>5,15</sup> In a meta-analysis of randomized controlled clinical trials examining physical activity in breast cancer survivors, physical activity improved QoL.<sup>15</sup> Thus, exercise is a central component and a necessary tool for specific QoL improvement.<sup>16</sup> Imran et al. assessed QoL of breast cancer patients using BR 23 and discovered functional scales in most of the domains were high, while symptom scales were moderate-to-low for most items, showing better QoL.<sup>17</sup> However, this was not the case in a study by Saleha et al.<sup>18</sup>, where the QoL index in breast cancer patients was poor, especially for body image.<sup>19</sup>

Few Nigeria-based studies among breast cancer patients have considered exercise, and breast cancerspecific QoL among the breast cancer population. Hence, the current study is needed, especially in Nigeria, where the major care give to breast cancer patients is limited to only chemotherapy, radiotherapy, surgery and other therapies with little attention to exercise which is an important rehabilitation measure to enhance the breast cancer patient's survival. With the poor health-related quality of life seen in breast cancer patients, there is a need for exercise, especially in rehabilitation and aftercare. Thus, focus should be on reducing and improving cancer and treatment-related side effects that do not subside even after the end of the therapy.<sup>21</sup> Therefore, this study focused on the practice and outcome of an exercise intervention in breast cancerspecific QoL.

#### Methods

#### Research design

The study adopted an experimental design involving an intervention and a control group. This design involved two-phase work using pre-test and post-test. The pre-test collected baseline information on breast cancer-specific quality of life of women with breast cancer in Delta State. It was taken before implementing the exercise intervention. After twelve weeks, there was a post-test to assess the effect of exercise uptake on the specific quality of life among women who participated in the study.

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#### Study setting

The study area was included two tertiary hospitals, Federal Medical Centre (FMC), Asaba and Delta State University Teaching Hospital (DELSUTH) Oghara that manage breast cancer patients in Delta State. The sample for the study was selected out of the 128 women recruited and consisted of breast cancer survivors in the control (n=47) and intervention (n=47) groups. Purposive sampling technique was used to select the women with breast cancer who completed their primary treatments and still maintained follow up with the two hospitals. Purposive sampling was applied purely based on the judgment of the researchers.<sup>21</sup> No randomization was done.

#### Inclusion criteria

• Breast cancer patients who completed their primary treatments.

• Willingness to participate in the study by the women.

#### Exclusion criteria

• Women who had breast reconstruction and those not willing to participate were excluded from the study.

#### Instrument for data collection

The instrument for data collection in the study included a questionnaire which has two sections. Section A contains six items used to elicit information on the respondents' demographic data. Section B contains 23 items of EORTC-BR23 used to elicit information on the women's breast cancerspecific quality of life before and after exercise intervention. EORTC BR23 was developed in 1996 to assess specifically QoL of breast cancer patients.<sup>3</sup> The breast cancer disease-specific section starts from the functional scales. Items 9-12 elicited information on body image; items 14 and 15 elicit information on sexual enjoyment; item 16 is related to sexual functioning and 13 elicit information on future perspectives. For the symptoms scales, items 1-4, 6, and 7 elicit information on systemic therapy side effects; items 20-23 elicit information on breast symptoms; items 17, 18, and 19 elicit information on arm symptoms, while item 5 elicits information on an upset by hair loss in breast cancer clients. The QoL instrument is designed on a four-point scale with Not at all = 1, Little = 2, Quite a bit = 3, and very much = 4. A checklist for exercise uptake designed by the researcher to observe patients' exercise was also used in the study. It guided the recording of exercise by women with breast cancer. It contained items on weeks/days of exercise, type of exercise, exercise intensity, time covered during the exercise (in minutes), and notable side effects on the patients.

#### Validity and reliability of instrument

The EORTC-BR23 is a standardized instrument and, therefore, was not validated. However, the researchers did a reliability test to ascertain its usability in our environment since it is an international instrument. A reliability coefficient of 0.80 was obtained, which showed that the instrument is reliable.

#### Ethical consideration

Health Research Ethics Committee of FMC Asaba and DELSUTH Oghara respectively gave ethical approval for the study. Informed consent was also obtained from the patients before data collection.

#### Data collection techniquess

The pre-test data collection and recruitment of participants lasted for three months. The exercise for the intervention group for 12 weeks was with the fitness professional's assistance. The fitness professional also acted as a physiotherapist who was knowledgeable on the recommended exercise guidelines for cancer survivors. The exercises included aerobic, resistance and flexibility exercises including warm-up with Swiss ball and dance, climbing stairs, treadmill, stationary exercise bicycle, shoulder, and arm exercises. After the exercise uptake, the researcher collected post-test data on the two groups' breast cancer-specific QoL.

#### Data analysis

Data analysis was done with the Statistical Package for Social Sciences (SPSS), version 17. The average of the items contributing to the scale, the raw score, was calculated for the data collected with EORTC BR23 QoL before using the linear transformation to standardize the raw score. The researchers did this to make scores range from 0 to 100 before coding them for analysis. The scores for each of the scale in the domains were added and divided to get the mean score for each domain's subscales. A high functional scale score signified a high level of functioning, while a high symptom scale score directly related to a significant increase in symptomatology and problems. The scores obtained were compared to the standard reference point/values of the EORTC BR23 QLQ. Descriptive statistics of mean and standard deviation were used and the results are presented in tables. Test of the hypotheses was done by using an independent sample t-test and linear regression.

#### Results

Table 1 shows that the minimum and maximum ages of the respondents from control group were 19

Variables	Intervention group(%)	Control group(%)
Age Maximum	64	63
Minimum	30	19
Mean±SD	$44 \pm 8.0$	45.0±9.0
Marital status		
Married	33(70.2)	31(66.0)
Single	6(12.8)	8(17.0)
Widow	5(10.6)	4(8.5)
Divorced/separated	3(6.4)	4(8.5)
Level of education		
No formal	3(6.4)	4(8.5)
Had primary	2(4.3)	6(12.8)
Secondary	15(31.9)	15(31.9)
Bachelors/Masters/Doctorate	27(57.4)	22(46.8)
Religion	42(89.4)	45(95.7)
Christian religion	5(10.6)	1(2.1)
Islamic religion	0(0.0)	1(2.1)
Traditional religion		-()
Occupation		6(12.7)
Unemployed	0(0.0)	17(36.2)
Self-employed	14(29.8)	24(51.1)
Employed	33(70.2)	21(01.1)
Stage of being diagnosed with breast cancer		
Stage 1 being diagnosed with breast cancer Stage1	27(57.4)	20(42.6)
Stage 2	5(10.6)	12(25.5)
Stage 3	1(2.1)	0(0.0)
Stage 4	11(23.4)	13(27.7)
Not sure	3(6.4)	2(4.3)
1101 5410	5(0.1)	2(1.5)

**Table 1.** Socio-demographic characteristics of participants

Domain/scales		vention ±SD	Co M=	Reference poin	
	Pre	Post	Pre	Post	
Functional domain					
Body image	64.3±26.4	89.5±16.9*	63.1±20.0	56.2±24.7	> 66.7
Sexual functioning	69.1±33.5	71.0±12.1	78.4±29.6	60.6±16.1	0-33.3
Sexual enjoyment	68.1±34.7	72.3±24.3*	76.8±30.5	66.7±29.5	33.3-66.7
Future perspective	53.2±40.3	67.4±23.5	63.8±41.6	51.1±23.9*	≥66.7
Overall functional domain score	65.4±22.7	$75.05 \pm 10.4$	71.3±23.4	58.65±12.9	_000.7
Symptom domain					
Systemic therapy side effects	22.1±13.8	10.2±13.8*	23.0±12.0	28.1±18.7	4.8-23.8
Breast symptoms	36.6±16.2	7.6±10.2*	$40.9 \pm 40.9$	31.7±23.0	0-25
Arm symptoms	17.0±20.6	17.7±15.1*	22.2±23.8	33.1±22.0	0-33.3
Upset by hair loss	5.2±12.4	5.5±5.9	9.3±24.5	33.3±21.8	0-0
Overall symptom domain score	22.2±6.2	11.8±13.0	24.1±9.6	30.9±21.2	-

Table 2. Pre and post-intervention breast cancer-specific QoL of cancer survivors

Body image Q9-12, sexual functioning Q14, 15, sexual enjoyment Q16, future perspective Q13, systemic therapy side effects Q1-4, 6-8, breast symptoms Q20-23, arm symptoms Q17-19, upset by hair loss Q5. reference points adapted from EORTC reference point for BR23

years and 63 years, respectively, while those of the intervention group were 30 years and 64 years. Both groups were mostly Christians [Intervention (89.4%); Control (95.7%)], and were married [intervention (70.2%); control (66.0%)]. Most of the patients had tertiary education [intervention (57.4%), control (46.8%)], and were employed [intervention (70.2%); control (51.1%)]. The more common stage of disease at diagnosis was stage1 [intervention group (57.4%); control group (42.6%)].

Table 2 shows the pre and post-intervention functional and symptom status of the breast cancerspecific quality of life among breast cancer survivors in control and the intervention groups. For the pre-test functional domain, sexual functioning and sexual enjoyment was higher for the intervention group [Sexual functioning (69.1±33.5); sexual enjoyment  $(68.1\pm34.7)$ ] and likewise for the control group [sexual functioning  $(78.4\pm29.6)$ ; sexual enjoyment (76.8±30.5)]. Body image was below the reference value for both groups [intervention  $(64.3\pm26.4)$ ; control  $(63.1\pm20.0)$ ]. Regarding the symptom domain, breast symptoms were higher in both groups [intervention  $(36.6\pm16.2)$ ; control  $(40.9\pm40.9)$ ]. The overall breast cancer-specific functional quality of life was 65.4±22.7 (intervention group) and 71.3±23.4 (control group). The overall breast cancer-specific symptoms were 22.2±6.2 (intervention group) and  $24.1\pm9.6$  (control group). For the post-intervention, the functional domain, and body image, the values [89.5±16.9 (intervention), 71.0±12.1 (sexual functioning), 72.3±24.3 (sexual enjoyment), 64.1±11.5 (future perspective)] were all higher for the intervention group post-intervention than the control group [56.2±24.7 (body image), 60.6±16.1 (sexual functioning), 66.7±29.5 (sexual enjoyment), 51.1±23.9 (future perspective)]. As for the symptoms domain, the symptoms experienced by those in the intervention group reduced more post-intervention than those in the control group. Their overall breast cancer-specific functional quality of life was 75.05±10.4 and 58.65 $\pm$ 12.9, respectively, for intervention and control while the overall breast cancer-specific symptoms were 11.8 $\pm$ 13.0 and 30.9 $\pm$ 21.2t, respectively, for intervention and control group.

Table 3 shows the observed practice of exercise by women with breast cancer in the intervention group. Modality of exercise practiced was aerobic (100%), resistance (100%), and flexibility (100%). Majority (70.2%) practiced for a period of 12 weeks, and the time contributed by aerobic was 20-60 minutes (100%), resistance was 10-60 seconds (100%), and flexibility was 5-20 seconds (100%). The intensity of exercise practiced was mainly moderate (100%). The majority (83.0%) did not have any side effects due to the exercise.

Table 4 shows there is a significant difference between post-intervention disease specific functional and symptoms QoL of breast cancer women. For the functions, significant differences exist among body image (p<0.001), sexual functioning (p=0.003), future perspective (p=0.007), and the overall functional domain (p<0.001). For the

 Table 3. Observed exercise by breast cancer survivors

 in intervention group using the observation checklist.

Observed items	Intervention group(n=47)%
Number of Weeks covered	
<12 weeks	14(29.8)
Up to 12 weeks	33(70.2)
<3 days per week	18 (38.3)
Up to 3 days per week	29 (61.7)
Time covered In minutes per day	
Aerobic 20-60 munites	47(100)
Resistance 10-60 seconds	47(100)
Flexibility 5-20 seconds	47(100)
Modality of exercise practiced	
Aerobic, resistance and flexibili	ty 47(100)
The intensity of exercise	
Mild/Moderate	47(100)
Notable side effects	
Headache	3(6.4)
Mild syncope	1(2.1)
Dizziness	4(8.5)
None	39(83.0)

Variables	Difference in pre and post intervention scores							
	Intervention M±SD	Control M±SD	Т	p-value				
Functions								
Body image	25.2±11.7	-6.9±19.3	3.671	0.001				
Sexual function	2.8±30.2	-17.7±34.9	3.043	0.003				
Sexual enjoyment	4.2±21.2	$-10.9 \pm 43.3$	2.235	0.028				
Future perspective	14.2±45.9	-12.8±47.9	2.782	0.007				
Overall specific functions	9.7±27.3	$-12.6\pm38.1$	2.514	0.001				
Breast cancer-specific symptoms								
The systemic therapy side effect	-11.9±20.0	5.1±22.3	-3.870	0.001				
Breast symptoms	-28.8±10.1	-9.2±32.2	-3.556	0.001				
Arm symptoms	0.71±24.2	$10.9 \pm 32.8$	-1.709	0.091				
Upset by hair loss	0.3±-17.2	24±21.4	-3.944	0.001				
Overall symptom domain score	$-10.4 \pm 12.1$	6.8±16.9	-2.325	0.001				

**Table 4.** Independent sample t-test of differences in specific functional and symptom breast cancer-specific quality of life among breast cancer survivors who did exercise and those who did not

symptoms domain, significant differences exist among systemic therapy side effects (p<0.001), breast symptoms (p<0.001), arm symptoms (p=0.091), upset by hair loss (p<0.001), and the overall symptoms domain (p<0.001).

Table 5 shows no significant differences among most of the specific QoL scales in relation to age, duration of diagnosis, and stage of the cancer diagnosis. However, there were significant differences among sexual function which was negatively correlated with age for the control group (p=0.043); sexual function was positively correlated with duration of diagnosis for the intervention group (R=-0.449, p=0.002), while sexual enjoyment was positively correlated with duration of diagnosis for the control group (R=-0.283, p=0.022); future perspective was positively correlated with stage of the cancer diagnosis for both groups [intervention (R=0.333, p=0.022); control (R=0.347, P=0.017)]; breast symptoms was positively correlated with duration of diagnosis (R=-0.473, p=0.001) for the intervention group, while arm symptoms had a positive correlation with duration of diagnosis (R=0.347, p=0.017) and stage of cancer at diagnosis (R=-0.352, P=0.015) for the control group.

Table 5: Linear regression model of demographic and clinical variables on breast cancer specific symptom scales

Variables	Difference in pre and post intervention scores						
	Interver	ntion	Control				
	Standardized coefficient Beta	P-value	Standardized coefficient Beta	p-value			
Body image vs age	0.038	0.804	0.075	0.618			
Body image vs DOD	0.223	0.140	0.091	0.542			
Body image vs SOCAD	-0.093	0.540	0.126	0.398			
Sexual function vs age	0.287	0.051	0.297	0.043			
Sexual function vs DOD	-0.449	0.002	0.145	0.331			
Sexual function vs SOCAD	-0.031	0.834	0.227	0.125			
Sexual enjoyment vs age	0.237	0.196	-0.037	0.805			
Sexual enjoyment vs DOD	-0.283	0.022	0.211	0.155			
Sexual enjoyment vs SOCAD	0.162	0.076	-0.056	0.709			
Future perspective vs age	-0.134	0.368	0.226	0.126			
Future Perspective vs DOD	0.172	0.252	0.097	0.515			
Future perspective vs SOCAD	0.333	0.022	0.347	0.017			
Breast cancer-specific symptoms							
The systemic therapy side effect vs age	-0.089	0.551	0.089	0.554			
The systemic therapy side effect vs DOD	-0.193	0.198	0.274	0.063			
The systemic therapy side effect vs SOCAD	-0.076	0.611	0.029	0.848			
Breast symptoms vs age	0.107	0.480	0.054	0.719			
Breast symptoms vs DOD	-0.473	0.001	0.235	0.111			
Breast symptoms vs SOCAD	0.049	0.746	0.003	0.982			
Arm symptoms vs age	0.067	0.656	0.006	0.970			
Arm symptoms vs DOD	-0.130	0.389	0.347	0.017			
Arm symptoms vs SOCAD	0.118	0.430	-0.352	0.015			
Upset by hair loss vs age	.109	0.121	-0.123	0.082			
Upset by hair loss vs DOD	0.137	0.232	0.211	0.342			
Upset by hair loss vs SOCAD	0.041	0.573	0.092	0.241			

DOD-duration of diagnosis. SOCAD-stage of cancer at diagnosis

#### Discussion

Assessment of the pre-intervention functional and symptoms scale of breast cancer-specific quality of life of breast cancer survivors in the control and intervention groups using the EORTC BR23 QLQ showed that except body image for both groups which was below the reference point and future perspectives for the intervention group, other subscales of the instrument were far above the reference values. The women's breast symptoms and hair loss upset experienced by a few of them were very worrisome. This finding agrees with Haddou et al.<sup>13</sup> who discovered that the functional status of breast cancer patients was low while the status of the symptoms remained high.<sup>12</sup> In the present study, the same was discovered except for sexual functioning and sexual enjoyment. Nageeti et al. found that body image, breast symptom, and future perspective scored the lowest among women with breast cancer<sup>22</sup>, while the most distressing symptom was hair loss as discovered in this present study. Impaired body image is a serious issue that affects women with breast cancer.<sup>23-25</sup> In contrast, Imran et al. reported higher scores for body image and future perspective<sup>17</sup>, while the least score was for sexual functioning pre-assessment, which was not the case in this study where body image score was less than the reference value. However, sexual enjoyment and sexual functioning had higher scores.

The organized exercise intervention was carried out for women who survived breast cancer in the intervention group. This exercise was done to reduce their sedentary lifestyle and improve their physical activity level because it has been stated by Hormeber that most cancer populations are sedentary.<sup>26</sup> The exercise done consisted of endurance (aerobic), resistance, and flexibility exercises. The participants tolerated these exercises well. As observed during the practice, the exercises were judged to be safe, and the same was also seen in a similar study by David and Cynthia.<sup>27</sup> The exercise program was organized specifically for the women who participated actively. Therefore, organized specific programs are effective and may improve breast cancer-specific quality of life in breast cancer survivors.<sup>28</sup> There is an urgent need to implement programs like this in hospitals that manage cancer, especially breast cancer patients. This implementation will improve cancer patients' overall well-being, especially breast cancer-specific QoL issues.<sup>2</sup>

Regarding the post-intervention breast cancerspecific functional and symptoms quality of life of women with breast cancer in the control and intervention groups, the results showed that there was a remarkable improvement in the functional and symptoms status of women with breast cancer in the intervention group after the exercise intervention compared to the women in the control group who had lower scores in the functional domain and there was no noticeable decrease likewise in their symptoms compared to their pre-intervention scores. A similar result was reported in other studies where exercise intervention improved the women's overall wellbeing in the intervention group compared to the control group who did not exercise.<sup>27, 28</sup> Similar results were also reported by Volaklis who found that exercise improved body image and self-esteem<sup>30</sup>, and also Denlinger and Engstrom who discovered an improvement in breast cancer-specific quality of life among breast cancer patients after physical activity.<sup>15</sup> Another study also reported that exercise significantly affected specific quality-of-life issues, including body image/self-esteem, sexuality, and pain.<sup>30</sup>

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The results also showed that sexual functioning and sexual enjoyment were good before and after exercise intervention. This finding suggests that these women's disease condition did not influence the sexual functioning and sexual enjoyment in both groups. Sexuality and sexual functioning, assessed in this study, belong to a cardinal domain of BR23 quality of life in breast cancer patients. Previous research supports this result showing that women stay sexually active and functional with no elevated psychosocial disorders or sexual dysfunction and no decrease in sexual activity after mastectomy.<sup>31</sup> Two studies contradict this result, reporting a significant deterioration in sexual function and sexual disorders among breast cancer patients.<sup>32, 33</sup> These studies also stated that many cancer survivors are at risk for developing psycho-physiological symptoms, including sexual dysfunction. Contradicting this finding, another study reported no single pattern of sexual life after breast cancer.<sup>34</sup> A study by de la Cruz found that women with breast cancer might have reduced or interrupted sexual activity while receiving treatment, and many of them have an ineffective sexual function with changes in various areas of sexuality, and these changes differ among women receiving treatment and those who have completed their treatment.<sup>35</sup> It is essential to state that feeling of intimacy or sexual activity should be sustained even when there is a health challenge, including breast cancer, to enhance emotional stability. Hence, ACS stated that sexuality and intimacy have been shown to help people face cancer by dealing with feelings of distress and going through treatment.<sup>36</sup> Therefore, finding that participants in this study were still sexually functional is promising as it will help them remain emotionally stable despite the prevailing condition.

The results showed that significant difference existed concerning the breast cancer-specific QoL issues of women with breast cancer. For the functional domain, statistically significant improvement was observed across many functional scales for women who did exercise compared to those who did not. For the symptoms, a statistically significant decrease was observed between the scale and overall domain scores for symptoms among women who did exercise compared to those who did not. This finding is consistent with several reports that exercise has a significant influence on the breast cancer-specific QoL of the patients that do exercise despite some barriers that may influence its effective practice.<sup>29,30,37,38</sup>

Results also revealed there was no significant difference among most of the scales of the specific QoL concerning age, duration of diagnosis, and stage of cancer diagnosis except sexual function vs age for the control group, sexual function vs duration of diagnosis for the intervention group, sexual enjoyment vs duration of diagnosis for the control group, future perspective vs stage of the cancer diagnosis for both groups, breast symptoms vs duration of diagnosis for the intervention group, arm symptoms concerning the duration of diagnosis and stage of cancer at diagnosis for the control group. No known study has reported specific breast cancer QoL of life and age, duration of diagnosis, and cancer stage at diagnosis. However, a report by Rukshani et al. showed that higher QoL was associated with patients who were married, highly educated, employed, and had good family support.<sup>39</sup> Although the duration of illness was also significant, similar to our result, duration of illness specifically influenced sexual functioning and sexual enjoyment for the intervention and control group.

Similarly, educational status of college and above, being divorced, higher household income, higher scores of physical and social functioning were associated with significantly improved (better) quality of life rather than age, duration of illness or stage at diagnosis.<sup>40</sup> On the other hand, a relation was found in another report between the QoL (R = -0.19, p = 0.034) and the women's age.<sup>41</sup> With age, the respondents' QoL decreased, while in this study, age only influenced the sexual functioning of the women in the control group rather than the overall QoL. Thus, it is essential to know that women with breast cancer experience serious chronic health sequelae which affect breast cancer-specific QoL issues, and these adverse effects may be mitigated or reduced with the help of exercise. Breast cancer patients that come to the hospital usually have nurses as their first point of call, and nurses also remain one of the essential persons throughout their lives. Therefore, nurses, especially those in the oncology department, are encouraged to utilize every opportunity to educate the women with breast cancer about exercises they can do during and after treatment. They should also ensure the implementation and sustenance of the exercises through their support and other health care workers who manage these patients to enhance their well-being, especially breast cancer-specific quality of life issues.

In conclusion, exercise is beneficial in improving

the breast cancer-specific quality of life of women with breast cancer. Therefore, it is necessary that exercise becomes part of the treatment regimen for breast cancer patients. Shreds of evidence from the literature suggest that exercise may improve the overall well-being of women who survive breast cancer in particular and other cancer patients in general.

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## **Conflict of Interest**

The authors declare there is no conflict of interests associated with this study.

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## Average Absorbed Breast Dose (2ABD) to Mean Glandular Dose (MGD) Conversion Function for Digital Breast Tomosynthesis: A New Approach

Raffaele M Tucciariello<sup>a</sup>, Rocco Lamastra<sup>a</sup>, Patrizio Barca<sup>b</sup>, Marine Evelina Fantacci<sup>c</sup>, Antonio C Traino<sup>\*d</sup>

<sup>a</sup> Medical Physics Unit, St.Chiara University Hospital, Pisa, Italy

<sup>b</sup> Department of Medical Physics, St. Orsola-Malpighi University Hospital, Bologna, Italy

<sup>°</sup> Department of Physics, University of Pisa, Pisa, Roma, Italy

<sup>d</sup> National Institute for Nuclear Physics (INFN), Pisa Section, Pisa, Italy

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## ABSTRACT

**Background:** In this work a new method for the Mean Glandular Dose evaluation in digital breast tomosynthesis (DBT) is presented.

**Methods:** Starting from the experimental-based dosimetric index, 2ABD, which represents the average absorbed breast dose, the mean glandular dose MGD2ABD was calculated using a conversion function of glandularity f(G), obtained through the use of Monte Carlo simulations.

**Results:** f(G) was computed for a 4.5 cm thick breast: from its value MGD2ABD for different compressed breast thicknesses and glandularities was obtained. The comparison between MGD2ABD estimates and the dosimetric index provided in the current dosimetry protocols, following the Dance's approach, MGD<sub>Dance</sub>, showed a good agreement (<10%) for all the analyzed breast thicknesses and glandularities.

**Conclusion:** The strength of the proposed method can be considered an accurate mean glandular dose assessment starting from few and accessible parameters, reported in the header DICOM of each DBT exam.

#### Introduction

Digital breast tomosynthesis,

Breast cancer screening procedures are routinely performed in many countries to detect the most commonly diagnosed cancer among women. Early detection of cancer seems to reduce the breast cancer mortality with better survival rates.<sup>1</sup> Digital Mammography (DM) is the main X-ray technique used to detect breast masses and microcalcifications. Technological advancements have highly improved the DM technique over the years and today the best upgrade is represented by the Digital Breast Tomosynthesis (DBT)<sup>2,3</sup>, recently introduced in

\*Address for correspondence: Antonio C Traino, MD Address: Ref. Dr.A.C.Traino, U.O.Fisica Sanitaria, Azienda Ospedaliero-Universitaria Pisana, via Roma n.67, 56125 Pisa, Italy Tel: +39 050992957 Email: <u>c.traino@ao-pisa.toscana.it</u> clinical routine worldwide. DBT reduces the intrinsic tissues superimposition which characterizes the DM acquisitions: tissue overlapping can lead to lower sensitivity and specificity.<sup>4</sup>

The breast is mainly composed of adipose and glandular tissue. The latter is considered the radiosensitive tissue-at-risk. X-ray radiation dose absorbed by the glandular tissue must be accurately assessed to quantify the radio-induced cancer risk. International dosimetry protocols<sup>5-7</sup> suggest the formalism proposed by Dance<sup>8-12</sup> which provides c, g, s and T conversion factors from incident air kerma on the upper surface of the compressed breast to a mean glandular dose (named MGD<sub>Dance</sub> in this paper): c, g, s and T factors are computed via Monte Carlo (MC) calculations. These factors depend on beam quality, patient age, breast thickness and DBT scan angle; therefore, in order to compute MGD<sub>Dance</sub>,

mathematical interpolation from tabulate values are often required.

Recently a similar dosimetric index (Average Absorbed Breast Dose, 2ABD), based on experimental phantom measurements, has been described, which calculates the average absorbed breast dose in DM or DBT procedures.<sup>13–15</sup> It can be easily calculated for a specific anode/filter combination by knowing the exposure and geometric parameters reported in the DICOM header of each exam. Specifically, tube voltage, tube load, breast thickness, focus-to-surface distance and tube yield are needed to calculate 2ABD, which can be used in any clinical condition, i.e. for any employed mammographic device and for any analyzed breast thickness.

2ABD represents the average absorbed dose to the breast without considering its glandularity: nevertheless, the amount of glandular tissue within the breast (named glandularity) must be considered for a glandular dose assessment because different values of glandularity lead to different glandular dose estimates.

The mean glandular dose cannot be measured experimentally, and computer-based methods are used to compute challenging quantities. The Monte Carlo methods simulate radiation-matter interactions using artificially generated random variables to solve the mathematical problem under investigation, which is the radiation dose delivered to the gland. In this work, a function of glandularity has been introduced by Monte Carlo simulations, to convert 2ABD in the Mean Glandular Dose  $MGD_{2ABD}$ .  $MGD_{2ABD}$  values are finally compared with  $MGD_{Dance}$  estimates provided by the current formalism.

#### Methods

#### 2ABD Method

Recently a new dose index for DM and DBT procedures, named 2ABD, has been presented and described<sup>14,15</sup>. 2ABD allows the calculation of the value of the average breast dose in a simple way: it is easily computed for each anode/filter combination starting from the knowledge of tube voltage kVp, tube load mAs, breast thickness T, focus-to-surface distance FSD and tube yield Y<sub>th</sub>. kVp, mAs and T are characteristics of each DM or DBT exam and can be easily found in the DICOM header; FSD is a specific (and well known) characteristic of the mammographic device and, finally, Y<sub>tb</sub> can be measured once a time and periodically verified for all the employed devices. A complete description of the method is out of the scope of the present work and can be found in the previous publications<sup>14,15</sup> for both DM and DBT modalities.

2ABD can be calculated by the following equation:

$$2ABD \sim \frac{1}{T} \int_0^T k_{a,i} C e^{-mx} dx$$
<sup>(1)</sup>

where  $k_{a,i}$  is the incident air kerma on the breast/phantom surface;  $C \sim 0.77$  is a conversion factor from  $k_{a,i}$  to dose in phantom and m is a parameter which quantifies the beam attenuation in the breast/phantom, expressed as:

$$m = \frac{a}{kVp^b}$$
(2)

where a and b are fitting parameters (depending on the anode/filter combination, W/Al in our case), whose values are a =  $20.32 \pm 1.97$  kVp/cm and b =  $1.04 \pm 0.03$  respectively. The incident air kerma k<sub>a,i</sub> can be calculated by the following equation

$$k_{a,i} = \varepsilon \cdot (\alpha \cdot kVp^2 + \beta \cdot kVp + \gamma) \cdot mAs \cdot \frac{Y_{tb} FSD}{(FSD(-T)^2)}$$

where  $\varepsilon$  is a coefficient whose value is  $\varepsilon = 6.6033 \times [10]^{-4}$  mAs·cm<sup>2</sup>/mGy and Y<sub>tb</sub> (FSD) represents the yield (mGy/mAs) of the X-ray tube involved. Y<sub>tb</sub> (FSD) must be evaluated for a specific tube voltage (32 kVp in our case) as described in Traino et al.<sup>15</sup> Finally,  $\alpha$ ,  $\beta$ , and  $\gamma$  are fitting parameters depending on the particular anode/filter combination. For the W/A1 DBT anode/filter combination, the obtained values of  $\alpha$ ,  $\beta$ , and  $\gamma$  are  $\alpha = (5.70 \pm 0.86) \times 10^{-5}$  mGy/(kVp<sup>2</sup>·mAs),  $\beta = (3.77 \pm 0.56) \times 10^{-3}$  mGy/(kVp·mAs) and  $\gamma = (-8.44 \pm 0.89) \times 10^{-2}$  mGy/mAs, respectively. It is important to underline that these coefficients ( $\alpha$ ,  $\beta$ , and  $\gamma$ , a and b) can be used for all the mammographic devices whose anode/filter combination is W/A1.

To simulate the breast in experimental measurements, a homogeneous phantom with planar dimensions of  $16 \times 16 \text{ cm}^2$  and variable thickness was employed. The phantom is composed of polystyrene (C<sub>8</sub>H<sub>8</sub>) with an admixture of  $2.1 \pm 0.2$  % (mean  $\pm$  standard deviation) of TiO<sub>2</sub> and its density (very similar to the breast glandular tissue) is  $1.04 \pm 0.04$  g/cm<sup>3</sup>.

#### The Monte-Carlo model

Absorbed glandular dose estimates cannot be evaluated experimentally and MC simulations represent fundamental tools to assess the mean glandular dose by means of dedicated conversion factors obtained through the simulation of both MGD and  $k_{a,i}$  values. The MC code used in this work has been validated following AAPM TG 195 protocol<sup>16</sup> which defines the required geometry assumptions and the computational methods to adopt for obtaining MGD values. The validation procedure concerns the comparison between specified scoring data reported in the TG 195 protocol and those obtained using the MC code;

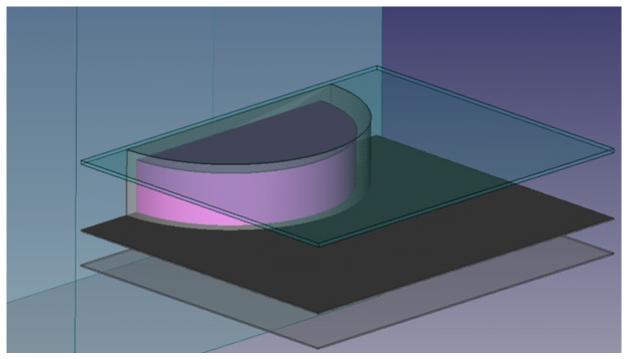


Figure 1. Representation of the digital breast phantom adopted for MC simulations. The breast tissue (in pink colour), in which the mean glandular dose estimates are computed, is surrounded by the 1.45 mm thick skin envelope; for visual purposes, the skin thickness is emphasized in this image. Compressed breast thickness is varied in the range 3-7 cm. Compression paddles (upper and lower) and the detector surface are represented.

MGD values (in mGy/photon) and energy deposit (in eV/photon) for a specified volume of interest have been compared for both monoenergetic and polychromatic beams, showing a maximum discrepancy of 0.35% for the MGD values and of 0.53% for the energy deposit. For the validation procedure, a publication of Dance et al.  $(2011)^{11}$  has been used for the comparison of the t-factors (i.e. the ratio between MGD values obtained at zero projection angles during the tomosynthesis investigation), with a maximum and a mean discrepancy respectively of 0.44% and 0.25% for all the analyzed data. A detailed description of the validation procedure has been fully described in the publication of Sarno et al.<sup>17</sup>, whose method we follow.

Glandular dose estimates are evaluated in terms of the MGD as historically defined by Wu and Boone through MC calculations in a homogeneous digital breast phantom.<sup>18-20</sup> The MC model involved in this work relies on a GEANT4-based MC code, which adopts a semi-cylindrical cross section breast phantom with radius of 10 cm with a homogeneous compound of glandular and adipose tissues forming a certain glandularity. Breast tissue is surrounded by a skin envelope of 1.45 mm thick (Figure 1), in line with the experimental findings derived from clinical breast CT (bCT) scans<sup>21</sup>, using the dedicated elemental composition provided by Boone.<sup>20</sup> This may be the principal aspect of novelty in MC dosimetry, in which the previously used skin depth was 5 mm adipose tissue.

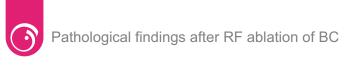
The effect of skin thickness on breast dosimetry has been investigated by many authors<sup>22,23</sup> and efforts in MC calculations have been made to obtain new dose conversion factors with the updated skin model.<sup>17,24,25</sup>

The MC code was designed to obtain mean glandular dose estimates, named  $MGD_{MC}$  in this work, using the methods already described in the literature.<sup>17,24–27</sup> MC calculations were employed to reproduce the experimental setup described in Traino et al.<sup>15</sup>, using W/Al spectra and a 15 degrees scan angle with 15 DBT projections. Moreover, incident air kerma estimates  $k_{a,i,MC}$  were computed following the formalism provided by Sarno and colleagues.<sup>27</sup> The number of histories launched in the MC calculations, 10<sup>8</sup> for the MGD scoring for each DBT projection angle and 10<sup>9</sup> for the air kerma scoring, were chosen to reduce the uncertainties under 0.2%.<sup>28</sup>

## From 2ABD to MGD2<sub>ABD</sub>

The experimental-based 2ABD method was developed in a homogeneous phantom, with 2ABD representing the mean absorbed dose in a homogenous phantom.

In the above-mentioned preliminary study,<sup>15</sup> it was found that the used phantom represented a good approximation of a homogenous breast whose glandularity was 1 (100% of tissue is glandular). Specifically, 2ABD matched MGD<sub>Dance</sub> within an accuracy of ~ 6 % for a phantom whose thickness was T=3 cm. The discrepancy between 2ABD and



 $\mathrm{MGD}_{\mathrm{Dance}}$  decreased if T increased and increased if the glandularity decreased.

In this work, a new approach to relating the 2ABD to a glandular dose  $MGD_{2ABD}$  is proposed. Specifically, the Mean Glandular Dose  $MGD_{2ABD}$  was calculated starting from 2ABD, obtained by:

$$MGD_{2ABD} = 2ABD \cdot f(G) \tag{4}$$

where f(G) is a function which depends on the glandularity G of the breast. f(G) is evaluated with MC simulations of  $MGD_{MC}$  performed considering the 1.45 mm thick skin envelope with a dedicated composition<sup>20</sup>, instead of 5 mm thick skin layer made by adipose tissue, as mentioned in the current protocols.<sup>5-7</sup>

To compare  $MGD_{MC}$  calculated by MC simulations and 2ABD evaluated by experimental measurements, both quantities must be normalized to the respective  $k_{a,i}$ , as fully described in Tucciariello et al.<sup>26,29</sup>:

$$\left[\frac{\text{MGD}}{k_{a,i}}\right]_{\text{MC}} = \left[\frac{2\text{ABD}}{k_{a,i}}\right]_{\text{meas}} \cdot f(G)$$
(5)

Eq. (5) has been used to obtain a simple reliable function f(G) using a reference breast model of 4.5 cm of thickness.

#### Results

Starting from MC simulations, the function f(G) was evaluated by Eq. (5) for a 4.5 compressed breast thickness, varying the glandularity G in the range 0.01-1, where 0.01 means a nearly full adipose and 1

means a full glandular breast. In Figure 2, the conversion function f(G) is shown. The conversion function can be expressed as a  $2^{nd}$  order polynomial function:

$$\mathbf{f}(\mathbf{G}) = \mathbf{A}_0 + \mathbf{A}_1 \cdot \mathbf{G} + \mathbf{A}_2 \cdot \mathbf{G}^2 \tag{6}$$

where  $A_0=1.389\pm0.001$ ,  $A_1=-0.555\pm0.004$  and  $A_2=0.115\pm0.004$  are the fitting parameters ( $R^2=0.99$ ).

f(G) can be used to convert 2ABD in MGD<sub>2ABD</sub> values for all the analyzed breast thicknesses. Figure 3 shows the mean glandular dose values obtained converting the 2ABD estimates through f(G). Typical kVp and mAs used in DBT modality are indicated for each breast thickness. The effect of the glandularity is also reported in the figure 2.

The measure-based MGD<sub>2ABD</sub> values have been compared with  $\text{MGD}_{\mbox{\tiny MC}}$  values obtained with MC simulations, which consider the exact breast thicknesses and glandularities, as defined in the previous paragraph and in line with the current state-of-the-art dosimetry in the literature.<sup>17,24,30</sup> The comparison allows estimating the reliability of this method and the approximation observed involving the f(G) function versus a MC-based dosimetry. Results are reported in Table 1 for breast thicknesses T ranging from 3 to 7 cm and normalized glandularities in the range 0.01-1 with increments of 0.1. The results show a good agreement for all the analyzed breast thicknesses and glandularities, where a maximum error of 12.4% is found for a 3 cm thick breast with the lower glandularity. The agreement can be considered fairly good for all T and G (except T=3 cm and G $\leq$ 0.2).

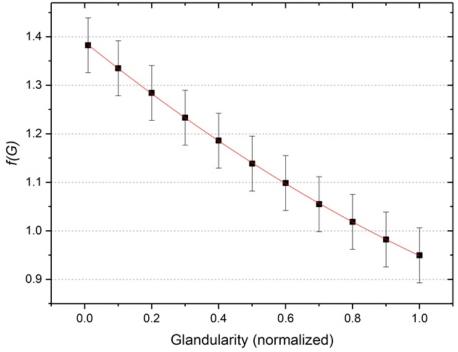


Figure 2. f(G) for normalized glandularities ranging from 0.01 to 1. Error bars are mainly affected by the uncertainties on the experimental-based 2ABD quantities (~20%), while uncertainties related to MC-based MGD<sub>MC</sub> quantities are negligible (less than 0.2%). Both MGD<sub>MC</sub> and 2ABD are normalized for their respective incident air kerma (Eq. (5)). Data refer to a 4.5 thick breast with a 30 kVp DBT investigation.

T (cm)	kilovoltage (kVp)	G (normalized)	$\left[\frac{MGD}{k_{a,i}}\right]_{MC}$	$\left[\frac{MGD_{2ABD}}{k_{a,i}}\right]_{meas}$	Discrepancies
	(, P)		0.459		<u>۹ ۵</u> ۵/
		0.01		$0.5 \pm 0.1$	8.9%
		0.10	0.445	$0.5 \pm 0.1$	12.4%
		0.20	0.430	$0.4 \pm 0.1$	-7.0%
		0.30	0.416	$0.4 \pm 0.1$	-3.8%
3	28	0.40	0.402	$0.41 \pm 0.09$	2.0%
		0.50	0.388	$0.40 \pm 0.09$	3.1%
		0.60	0.375	$0.38 \pm 0.09$	1.3%
		0.70	0.363	$0.37 \pm 0.08$	1.9%
		0.80	0.351	$0.35 \pm 0.08$	-0.3%
		0.90	0.340	$0.34 \pm 0.08$	0.0%
		1.00	0.330	$0.33 \pm 0.08$	0.0%
		0.01	0.396	$0.41 \pm 0.09$	3.5%
		0.10	0.382	$0.39 \pm 0.09$	2.1%
		0.20	0.368	$0.38 \pm 0.08$	3.3%
		0.30	0.354	$0.36 \pm 0.08$	1.7%
4	29	0.40	0.340	$0.35\pm0.08$	2.9%
	29	0.50	0.328	$0.34 \pm 0.07$	3.7%
		0.60	0.315	$0.32 \pm 0.07$	1.6%
		0.70	0.304	$0.31 \pm 0.07$	2.0%
		0.80	0.293	$0.30\pm0.07$	2.4%
		0.90	0.283	$0.29\pm0.06$	2.5%
		1.00	0.273	$0.28\pm0.06$	2.6%
		0.01	0.363	$0.36\pm0.08$	-0.8%
		0.10	0.350	$0.35 \pm 0.07$	0.0%
		0.20	0.336	$0.33\pm0.07$	-1.8%
		0.30	0.322	$0.32\pm0.07$	-0.6%
-	21	0.40	0.309	$0.31\pm0.06$	0.3%
5	31	0.50	0.297	$0.30\pm0.06$	1.0%
		0.60	0.286	$0.28\pm0.06$	-2.1%
		0.70	0.275	$0.27\pm0.06$	-1.8%
		0.80	0.265	$0.26\pm0.06$	-1.9%
		0.90	0.255	$0.26\pm0.05$	2.0%
		1.00	0.246	$0.25\pm0.05$	1.6%
		0.01	0.336	$0.32\pm0.07$	-4.8%
		0.10	0.324	$0.31 \pm 0.06$	-4.3%
		0.20	0.311	$0.30\pm0.06$	-3.5%
		0.30	0.298	$0.29\pm0.06$	-2.7%
		0.40	0.286	$0.28\pm0.06$	-2.1%
6	33	0.50	0.274	$0.27 \pm 0.05$	-1.5%
		0.60	0.264	$0.26 \pm 0.05$	-1.5%
		0.70	0.253	$0.25\pm0.05$	-1.2%
		0.80	0.244	$0.24 \pm 0.05$	-1.6%
		0.90	0.235	$0.23 \pm 0.05$	-2.1%
		1.00	0.227	$0.22 \pm 0.05$	-3.1%
		0.01	0.315	$0.22 \pm 0.05$ $0.30 \pm 0.06$	-4.8%
		0.10	0.303	$0.50 \pm 0.00$ $0.29 \pm 0.06$	-4.3%
		0.20	0.291	$0.29 \pm 0.00$ $0.28 \pm 0.06$	-3.8%
		0.20	0.279	$0.23 \pm 0.00$ $0.27 \pm 0.05$	-3.2%
		0.40	0.267	$0.27 \pm 0.05$ $0.26 \pm 0.05$	-2.6%
7	35	0.40	0.257	$0.26 \pm 0.03$ $0.25 \pm 0.05$	-2.7%
		0.60	0.246	$0.24 \pm 0.05$ 0.23 ± 0.05	-2.4%
		0.70	0.237	$0.23 \pm 0.05$	-3.0%
		0.80	0.228	$0.22 \pm 0.04$	-3.5%
		0.90	0.219	$0.21 \pm 0.04$	-4.1%
		1.00	0.212	$0.20 \pm 0.04$	-5.7%

**Table 1.** Comparison between the  $MGD_{2ABD}$  values obtained by converting the 2ABD estimates, and  $MGD_{MC}$  values obtained with dedicated Monte Carlo simulations using the formalism described in the text. Both MGD2ABD and  $MGD_{MC}$  are normalized for the respective incident air kerma. Data provided by MC calculations are in good agreement with Sarno et al.<sup>25</sup>

Moreover, in order to test the approach presented in this work, a comparison between  $MGD_{2ABD}$  and  $MGD_{Dance}$  values provided in the current dosimetry protocols based on the Dance's approach was

performed for normalized glandularities ranging from 0.2 to 1 with steps of 0.2.<sup>5-7</sup> It should be noted that c and g Dance's coefficients have been interpolated in order to match the exact glandularity to perform the comparison. The results are reported in

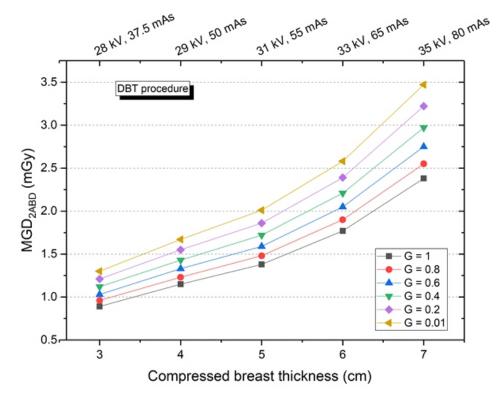


Figure 3. Mean glandular dose values obtained converting the 2ABD estimates. kV and mAs are typical parameters automatically selected by the DBT unit for the specified breast thickness.

G (normalized)	Phantom thickness (cm)	Tube load (mAs)	Tube voltage (kVp)	MGD <sub>2ABD</sub> (mGy)	MGD <sub>Dance</sub> (mGy)	Relative difference (%
	3	37.5	28	$0.9\pm0.2$	$1.0 \pm 0.2$	-10.0%
	4	50	29	$1.2 \pm 0.2$	$1.2 \pm 0.2$	0.0%
1	5	55	31	$1.4 \pm 0.3$	$1.5 \pm 0.3$	-6.7%
	6	65	33	$1.8 \pm 0.3$	$1.9 \pm 0.4$	-5.3%
	7	80	35	$2.4 \pm 0.4$	$2.6 \pm 0.3$	-7.7%
	3	37.5	28	$1.0 \pm 0.2$	$1.1 \pm 0.2$	-9.1%
	4	50	29	$1.3 \pm 0.2$	$1.3 \pm 0.3$	0.0%
0.80	5	55	31	$1.5 \pm 0.3$	$1.6 \pm 0.3$	-6.3%
	6	65	33	$2.0\pm0.3$	$2.0 \pm 0.4$	0.0%
	7	80	35	$2.6 \pm 0.5$	$2.8 \pm 0.6$	-7.1%
	3	37.5	28	$1.1 \pm 0.2$	$1.1 \pm 0.2$	0.0%
	4	50	29	$1.4 \pm 0.3$	$1.4 \pm 0.3$	0.0%
0.60	5	55	31	$1.7 \pm 0.3$	$1.7 \pm 0.3$	0.0%
	6	65	33	$2.1 \pm 0.4$	$2.2 \pm 0.4$	-4.5%
	7	80	35	$2.9 \pm 0.5$	$3.0 \pm 0.6$	-3.3%
	3	37.5	28	$1.2 \pm 0.2$	$1.2 \pm 0.2$	0.0%
	4	50	29	$1.5 \pm 0.3$	$1.5 \pm 0.3$	0.0%
0.40	5	55	31	$1.8 \pm 0.3$	$1.8 \pm 0.4$	0.0%
	6	65	33	$2.4 \pm 0.4$	$2.4 \pm 0.5$	0.0%
	7	80	35	$3.2 \pm 0.5$	$3.3 \pm 0.7$	-3.0%
	3	37.5	28	$1.3 \pm 0.3$	$1.3 \pm 0.3$	0.0%
	4	50	29	$1.7 \pm 0.3$	$1.6 \pm 0.3$	6.2%
0.20	5	55	31	$2.0\pm0.3$	$2.0\pm0.4$	0.0%
	6	65	33	$2.6 \pm 0.4$	$2.6 \pm 0.5$	0.0%
	7	80	35	$3.5 \pm 0.6$	$3.6 \pm 0.7$	-2.8%

**Table 2.** Comparison between  $MGD_{2ABD}$  and the  $MGD_{Dance}$  calculated using the Dance's approach for different breast thicknesses and glandularities.

Table 2.

There is a good agreement between the  $MGD_{Dance}$ and  $MGD_{2ABD}$  with a mean discrepancy among all data provided in Table 2 of -2.4% and a maximum relative percentage difference of -10.8% occurring with a 3 cm thick and 100% glandular breast (G=1). It should be stressed that in this work, the 1.45 mm thick skin model has been used, probably affecting the comparison mainly for low breast thicknesses with the MGD<sub>Dance</sub> coefficients, which are obtained using a 5 mm thick adipose skin. Indeed, in the 5 mm skin case, the breast tissue volume, i.e. the scoring volume for MC calculations, is thinner compared to the case of 1.45 mm thick skin layer, where the breast tissue is thicker; this implies different dose estimates because MC dose estimates are not performed in the skin tissue.

## Discussion

Currently two similar methods for the mean glandular dose evaluation in DM or DBT are employed, both based on MC calculations: the Dance<sup>8–12</sup> and the Wu and Boone methods.<sup>18–20</sup> The method introduced by Dance is the most widely used in the international dosimetry protocols.<sup>5-7</sup> It allows obtaining a reliable dosimetric index related to the ionizing radiation risk through the conversion of the incident air kerma k<sub>ai</sub> in a mean glandular dose MGD<sub>Dance</sub>. MGD<sub>Dance</sub> estimates are based on dedicated correction factors tabulated as a function of the beam quality, patient age, projections angle and breast thickness: interpolation is often required for an accurate evaluation of  $MGD_{Dance}$  and the dependence of glandularity on the age of the patient is sometimes questionable.

In this work, another method for evaluating the mean glandular dose absorbed by the patient's breast during the DBT examinations was presented. In previous works<sup>14,15</sup>, a simple approach based on experimental measurements has been introduced to individually evaluate the average absorbed dose in DM and DBT procedures. This method is based on the evaluation of a new dosimetric index, named 2ABD (Eq. 1), which quantifies the average dose absorbed in a homogenous phantom simulating a 100% glandular breast (G=1). The glandular dose is highly dependent on the glandularity of the breast, which results in wide variations among women, often not related to their age.

In order to extend this last method to any glandularity, in this work a new approach for relating the Average Absorbed Breast Dose 2ABD to a glandular dose  $MGD_{2ABD}$  was proposed. Using a validated GEANT4-based MC code, 2ABD was converted in a  $MGD_{2ABD}$  by a 2<sup>nd</sup> order polinomial function f(G) which depends only on different glandularities G. Following the geometrical assumptions of the breast model involved in international dosimetry protocols, a skin envelope was used to surround the sensitive volume (breast tissue) for the MC calculations. Based on the new results published in the literature obtained using bCT investigations, a skin layer of 1.45 mm of thickness and dedicated composition was used.<sup>17,20,24,25</sup>

f(G), obtained for a 4.5 thick breast (Figure 2), converted the mean dose  $_{2ABD}$  in a mean glandular dose MGD $_{2ABD}$  for different breast thicknesses and glandularities. A comparison between the measure-based MGD $_{2ABD}$  and the MC-based MGD $_{MC}$  values

showed a maximum discrepancy of 12.4% for a 3 cm thick breast with the 0.1 glandularity. The agreement between  $MGD_{2ABD}$  and  $MGD_{MC}$  can be considered fairly good for all T and G except T=3 cm and G≤0.2.

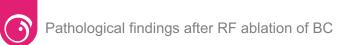
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Moreover, in order to compare the presented method to that currently involved in the dosimetry protocols,  $MGD_{2ABD}$  values were compared with  $MGD_{Dance}$  values. In this case, a maximum relative percentage difference of -10.0% was obtained for a 100% glandular (G=1) and 3 cm thick breast. In this work, MC simulations were performed using a 1.45 mm thick skin, probably affecting the comparison with  $MGD_{Dance}$  estimates (obtained considering a 5 mm thick adipose skin) especially for small compressed breast thicknesses.

The method presented in this paper allows the evaluation of the average glandular dose in a simple way. Few (and very easily accessible) parameters are required: tube voltage kVp, tube load mAs, breast thickness T, focus-to-surface distance FSD, tube yield Y<sub>th</sub> and finally the breast glandularity G. Some of these parameters (kVp, mAs and T) are characteristics of each DBT examination and can be easily found in the DICOM header of the images; FSD is typical of the specific employed mammographic device and Y<sub>th</sub> is calculated once and periodically verified. Therefore, if the glandularity of the breast is known, the evaluation of  $MGD_{2ABD}$ using the f(G) conversion function can be done for each patient. Thanks to its simplicity, the MGD<sub>2ABD</sub> based method could be easily implemented in any mammographic device. The proposed method is based on the above-presented coefficients  $\alpha$ ,  $\beta$ ,  $\gamma$ , a and b which are strictly related to the form of the Xray spectra. For this reason, while the method is general, the values of  $\alpha$ ,  $\beta$ ,  $\gamma$ , a and b presented in this paper can be used only for the mammographic devices whose anode-filter combination is W/Al.

Some limitations of the proposed approach should be noted. Specifically, there are some approximations in our 2ABD model. For example, a simple exponential decay relationship was employed to describe the dose distribution with depth, neglecting the polychromatic nature of the Xray beams.<sup>14,15</sup> Additionally, the absorbed dose distribution was considered homogeneous at each depth of the phantom. On the other hand, even the Monte Carlo simulations included some simplifications in the mammographic hardware modeling (e.g. modelisation of anode inclination, filtration thickness, compression paddle and breast support) which affect the final X-ray spectra and thus the absorbed dose and MGD estimation.

In conclusion,  $MGD_{2ABD}$  represents an easily evaluable index related to the risk induced by the exposure to the ionizing radiation, which can be included in the report of each DBT examination, in line with the indications of the 2013/59/Euratom Directive.



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## **Conflict of Interests**

The authors declare that they have no conflict of interests.

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## Tumor Control Probability (TCP) and Normal Tissue Complication Probability (NTCP) in Mono and Dual-isocentric Techniques of Breast Cancer Radiation Therapy

Kaveh Shirani Tak Abi<sup>a</sup>, Sediqeh Habibian<sup>b</sup>, Marzieh Salimi<sup>\*c.d</sup>, Ahmad Shakeri<sup>b</sup>, Mohammad Mahdi Mojahed<sup>b</sup>, Hussain Gharaati<sup>d</sup>

<sup>®</sup> Sina Radiation Oncology Department, Bu Ali hospital, Tehran, Iran

<sup>b</sup> Valiasr Radiation Oncology Center, Qom, Iran

<sup>°</sup> School of Physics and Astronomy, University of Exeter, Exeter, UK

<sup>d</sup> Department of Medical Physics and Biomedical Engineering, Tehran University of Medical Sciences, Tehran, Iran

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## ABSTRACT

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Key words: Tumor control probability, normal tissue complication probability, external beam, radiation therapy, breast cancer. **Background:** Nowadays, radiation therapy plays an important role in the treatment of breast cancer. The important point is the optimal control of the tumor along with the protection of organs at risk. This study aims to investigate and compare the radiobiological factors of the tumor and organs at risk in two different radiation therapy techniques of breast cancer.

**Methods:** Ten left-sided breast cancer patients with breast-conservative surgery were selected for this study. Three-dimensional treatment planning was performed using CT scan images of the patients using PCRT 3D software. Two different tangential external beam techniques were compared: first, dual-isocentric technique (DIT) with two isocentre, one on the breast tissue, and the other one on the supraclavicular lymph nodes and second, a mono-isocentric technique (MIT) with one isocentre at the intersection of the tangential and the supraclavicular field. The total prescribed dose was 5000 cGy per 25 fractions. Dose-volume histograms (DVHs), Tumor control probability (TCP), and normal tissue complication probability (NTCP) curves were used to compare the dosimetric and radiobiological parameters of the tissues in the prementioned techniques.

**Results:** The results showed that the maximum doses in planning target volume (PTV) with mean values of 109% and 110% in the SI and DIT were not significantly different in both techniques and that they were indeed at the optimum level based on the RTOG 1005 protocol. The dose homogeneity index in MMIT was more than that in DIT, while the conformity index and the mean TCP did not show a significant difference in the two techniques. Furthermore, minimum, mean, and maximum dose in the lung and the probability of pneumonitis decreased in MIT. On the other hand, the maximum dose, the dose of 33%, 66%, and 100% of the heart, and the probability of preicarditis in MIT were lower than the figure in DIT.

**Conclusion:** Due to the absence of hot spots at the intersection of tangential and supraclavicular fields and the reduction of mechanical movements of the coach and collimator in MIT, the superiority of this method was confirmed.

\* Address for correspondence: Marzieh Salimi, PhD Address: School of Physics and Astronomy, University of Exeter, Exeter, UK Tel: :+44-1392-725652 Email: <u>m.a.salimi@exeter.ac.uk</u>

## Introduction

Breast cancer is one of the most commonly diagnosed cancers among women worldwide with 1.4 million cases per year, accounting for almost one-third of malignancies in women.<sup>1,2</sup> Postoperative radiation therapy has a proven role in the treatment of



early stages of breast cancer to improve local control, survival, and reducing cancer recurrence.<sup>3-10</sup> On the other hand, breast radiation therapy can be accompanied by several complications such as cardiovascular and pulmonary damages that are due to irradiation of non-target tissues around the breast, chest wall, and regional lymph nodes.<sup>1, 4, 6, 11-19</sup> Two opposite-parallel tangent fields are used for breast tissue irradiation and one anterior field is applied for the supraclavicular irradiation. It is possible to have overdose or underdose at the intersection of two adjacent radiation fields that can be due to mismatch of the borders of the fields, which will ultimately have a direct impact on the treatment complications and tumor control.

Current guidelines for the locally advanced breast cancer normally recommend that elective nodal irradiation should be applied to the regional lymphatics as well as the whole breast.<sup>20</sup> Also, nodal irradiation is recommended strongly by the National Comprehensive Cancer Network (NCCN) guidelines for N1 breast cancer patients.<sup>21</sup> Furthermore, supraclavicular-axillary irradiation decreases the locoregional recurrence and mortality in the patients with lymph-node-positive breast cancer.<sup>22</sup> In the study of Kim et al., regional recurrence in the supraclavicular nodes occurred in only 1% of patients. This is a very low recurrence rate showing that supraclavicular-axillary irradiation should be performed in all patients. This study has also shown that supraclavicular-axillary irradiation can significantly decrease the risk of distant metastasis, as well as regional lymph-node recurrence.<sup>23</sup>

A study by Whelan et al. showed that metastasisfree survival increased in the patients who received supraclavicular-axillary irradiation compared to other patients who did not have regional nodal irradiation (78% vs. 75%, P=0.02). Also, breast cancer mortality was lower among patients in the nodal-irradiation group than control patients. Besides that, the rate of heart disease or deaths from heart disease did not increase among patients who received regional nodal irradiation at a follow up of 9.5 years.<sup>24</sup> Tai et al. evaluated the role of supraclavicular-axillary irradiation according to the nodal ratio (NR). The results revealed that for patients with >10 nodes examined, supraclavicular-axillary irradiation significantly increased the survival in the median and high NR patients but not in the low NR patients. In their study, the patients were considered in three NR groups: low (LNR, <25%), medium (MNR, 25% to 75%), and high (HNR >75%) nodal involvement.<sup>25</sup>

The effects of radiation therapy on cancer and healthy cells are characterized by two probabilities: first, tumor control probability (TCP), which indicates the probability of not having any cancer cells after radiation treatment, and second, the likelihood of expected complications called normal tissue complication probability (NTCP).<sup>26-28</sup> One of the most critical factors that have a significant effect on the treatment complications is the radiation therapy technique. Several techniques have been proposed for external breast cancer radiotherapies such as 2D, 3D, Intensity-modulated radiation therapy (IMRT), Image-guided radiation therapy (IGRT), and field-in-field technique with advanced technologies and special software. Moreover, several methods such as mono-isocenter, multiisocenter, half-beam, and full beam have been developed for application in the prementioned techniques.<sup>29,30</sup> In this study, two different techniques in 3D radiation therapy including mono-isocentric technique (MIT) and dual-isocentric technique (DIT) were selected for dosimetric and radiobiologic evaluation and comparison. The dosimetric comparisons between the two techniques have been performed in many studies<sup>4, 17, 19, 29, 31</sup>; so in this study, we evaluated the radiobiologic factors such as TCP and NTCP as well as the dosimetric factors and lung and cardiac exposure rate using different external breast radiation therapy techniques.

#### Methods

#### Patients' information

In this study, ten patients with early-stage invasive ductal carcinoma breast cancer and conservation surgery were selected at the Radiation

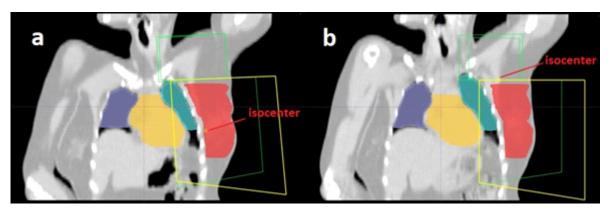


Figure 1. Isocenter positioning. a) dual-isocentric technique with isocenter applied to the breast tissue, b) monoisocentric technique with isocenter located at the intersection of the two tangent and supraclavicular fields.

and Oncology Center of Vali-e-Asr Hospital in Qom.

#### Treatment Planning

In the DIT which is the most common method of whole breast radiation therapy, two isocenters are defined; one on the breast tissue and the other on the upper area of the breast tissue to irradiate the supraclavicular lymph nodes.<sup>32</sup>It is worth mentioning that, in this technique, there is a gap between the two adjacent fields due to the radiation beam divergence (Figure 1a).

In the MIT, only one isocenter is defined at the intersection of the tangent and supraclavicular fields, so that the upper field is blocked for the radiation of the tangent field, and then the lower field is blocked for the radiation of the supraclavicular field (Figure 1b).<sup>33</sup> In this case, a non-divergence beam leads to the optimal matching of adjacent fields. Unlike the DIT, there is no collimator and couch rotation in the MIT.

Patients' CT scans were transferred to PCRT 3D (6.0.2.14) software for planning radiation fields. The lung, heart, and PTV tissues were contoured in all slices of the axial CT scans by a radiation oncologist. Due to breathing, patient position, breast swelling, and setup inaccuracies, usually 1 cm margin was considered around the clinical target volume (CTV) and Planning Target Volume (PTV).

#### Radiation dose and fractionation

A dose of 50 Gy in 25 fractions over 5 weeks was prescribed for 100% of the isodose, so that patients received 2Gy per fraction. Treatment planning was performed to cover the entire PTV by the isodose 95% and the maximum PTV dose less than 110%.<sup>34</sup>

## Beam characteristics

The SHINVA linear accelerator with 6 MV Xrays, 40 cm $\times$  40 cm asymmetrical jaws, and orthogonal mechanical wedges was capable of matching the 40 cm length of the field of view (FOV). The PCRT treatment planning software was also used for this study to access the target volume and organ at risk dose rate.

#### Limitations of lung and heart radiation

Dosimetric goals of study for lung and heart were defined according to the recommendations of the radiotherapy and oncology group, termed RTOG, as follows:<sup>35</sup>

 $V20 \le 20\%$ ,  $V10 \le 40\%$ ,  $V5 \le 55\%$  for the lung

D33%  $\leq$ 60Gy, D66%  $\leq$ 45Gy, D100%  $\leq$ 40Gy, and V10 $\leq$ 35% for heart

These values were extracted from DVHs for each organ.

## Dose homogeneity index and conformity index Dose homogeneity index (HI) and conformity index (CI) are two possibilities used to evaluate

breast conformal treatment plans.<sup>34,36,37</sup>

The following equation was used for HI calculation:

HI = D5 / D95 (1)

Where D5 is a minimum dose of 5% in PTV, indicating a maximum dose (Dmax) and D95 is a minimum dose of 95% PTV, representing a minimum dose (Dmin). The lower (close to one) the factor, the better is the dose homogeneity.

CI is also defined as the ratio of the volume surrounded by the reference isodose (which according to ICRU, is 95%) to the target volume planned by the physicist and its formula is as follows:

$$CI = VRI/TV$$
 (2)

Where VRI is the reference isodose volume and TV is the target volume.

TCP and NTCP assessment for lung and heart tissues

The TCP is the probability that no clonogenic cell can survive in the treated volume at the end of the treatment. It is described by a Poisson distribution with parameter  $\lambda$  which gives the final number of clonogenic cells. The model parameters are:

 $\begin{array}{l} \alpha m: \mbox{ mean linear radio sensitivity coefficient for the tumor (Gy-1)} \\ \beta m: \mbox{ mean quadratic radio sensitivity coefficient for the tumor (Gy-2)} \\ \sigma \alpha: \mbox{ standard deviation of } \alpha \\ \sigma \beta: \mbox{ standard deviation of } \beta \\ T \mbox{ dup: doubling time for the tumor (days)} \\ T \mbox{ time for accelerated proliferation (days)} \\ T: \mbox{ total time (natural days) of treatment (days)} \\ Q0: \mbox{ initial density of clonogenic tumor cells} \end{array}$ 

Initially, since all of the radiobiological parameters for standard fractionation (2 Gy per session) have been calculated, the dose-response curve for this fractionation is plotted. In the case of breast cancer, because of the uniformity of dose per fraction with standard fractionation, there is no need for uniformity and change in the dose-response curve. The mean values of radiation sensitivity and their related standard deviations are already available in the software. Finally, using the model, the final number of clonogenic cells is calculated with each radiation sensitivity.

$$N(i,j) = \sum_{h=1}^{\kappa} \rho_0 . v_h e^{\left\{-(\alpha i.Dh + \beta j.D_h.d) + \left(\frac{Ln2}{Tdup}\right).(T-Tk)\right\}}$$
(3)

$$TCP = \sum_{i=1}^{200} g_{\alpha i} \sum_{j=1}^{200} g_{\beta j} e^{-N(i,j)}$$
(4)

Where  $\alpha$ = 0.51 Gy<sup>-1</sup>,  $\beta$ = 0.061 Gy<sup>-2</sup>,  $T_k$  = 12,  $T_{pot}$  = 12, and Density of 1000 Cell/Cm<sup>3</sup> were considered for breast cancer.

NTCP calculation was performed using Lyman-Kutcher and Burman model. This model also known as a normal or empirical model which calculates the complications probability of normal tissues in a nonuniform irradiation using dose-response histograms. Moreover, this model can estimate the probability of complications for uniformly irradiated organs. In this regard, we applied the method of effective volume in which a non-uniform dose-volume histogram was mapped onto a uniform dose-volume histogram with a volume equal to the effective volume and a dose equal to the organ maximum dose. This effective volume was calculated by the following equation:

$$V_{eff} = \sum_{i=1}^{k} v_i \left(\frac{Di}{D_{max}}\right)^{\frac{1}{n}}$$
(5)

Where (vi , Di) are the histogram pairs, vi is normalized to 1,  $D_{max}$  is the organ maximum dose and k is the number of histogram pairs. First, the histogram must be transformed to the standard fractionation schedule (2 Gy/fraction). Subsequently, the following equation is used for NTCP:

$$NTCP = \frac{1}{(2\pi)^{\frac{1}{2}}} \int_{-\infty}^{t} e^{\frac{-x^2}{2}} dx$$
(6)

$$t = \frac{(D_{max}.V_{eff}^{n})}{m.D_{5\ 0}}$$
(7)

Where n and m are obtained empirically by fitting the expression for NTCP to the tolerance doses for each organ totally and partially irradiated with a uniform dose; D50 is the dose that causes complications with 50% probability when the tissue is homogeneously irradiated. For NTCP calculation, the parameters were defined in Table 1.

**Table 1.** The parameters used for NTCP calculation

 in left lung and heart

Parameters	Left lung	Heart
$\alpha$ (Gy <sup>-1</sup> )	0.035	0.058
$\beta$ (Gy <sup>-2</sup> )	0.008	0.029
n	0.87	0.35
m	0.18	0.1
$D_{50}(Gy)$	24.5	48

#### Results

PTV

The results of dosimetric analysis in PTV are summarized in Table 2 and Figures 2 and 3.

The minimum dose in MIT (1044 cGy) was significantly lower than that in DIT (1641cGy) (p-value = 0.03). Also, the mean dose in the MIT (4810 cGy) was lower than that in DIT ( 4928 cGy) (p-value = 0.00). On the other hand, the maximum dose in the MIT (5463 cGy) and DIT (5510 cGy) did not show a significant difference (p-value = 0.19). The maximum dose percentage of PTV was 109% and 110% in the MIT and DIT, respectively (Table 2). This value was lower than the ideal threshold recommended by RTOG 1005 (115%), indicating that there was no hot spot in both techniques (Figure 2).

**Table 2.** Comparison of calculated values in two mono-isocentric and dual-isocentrictechniques for PTV. The values include  $D_{min}$  (minimum dose),  $D_{mean}$  (mean dose),  $D_{max}$  (maximum dose), HI (Homogeneity index), and CI (Conformity index)

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parameter	MIT*	DIT*	P-value	R	TOG*
				Ideal	Acceptable
D <sub>min</sub>	1044.1	1641.5	0.03		
$\mathbf{D}_{\text{mean}}$	4810.4	4928.6	0		
$\mathbf{D}_{\max}$	5463.4	5510.1	0.19		
D <sub>max</sub>	109.29	110.21	0.2	<115%	<120%
HI*	1.15	1.12	0		
CI*	1.52	1.51	0.96		

\*Abbreviations: DIT = Dual-isocentric Technique, MIT= Mono-Isocenteric Technique, HI= Hemogeneity Index, CI= Conformity Index, RTOG= Radiation Therapy Oncology Group

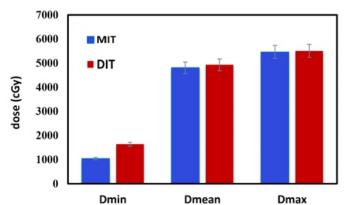


Figure 2. The mean values of the minimum, mean and maximum dose of PTV in both mono-isocenteric and dual-isocentric techniques.

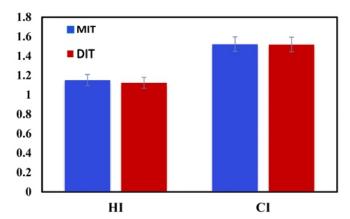


Figure 3. Homogeneity index and conformity index in the mono-isocenteric and dual-isocentric techniques

The homogeneity index of the beam in the MIT (1.15) was higher than that in the DIT (1.12). Given the fact that the amount of this factor was close to 1 in both techniques, the results showed that homogeneity was acceptable in these techniques (Figure 3). Furthermore, the conformity index with mean values of 1.52 in the MIT and 1.51 in the DIT did not show a significant difference (p-value = 0.96). As Figure 4 shows, the isodose curves in the DIT and MIT are similar and there is no significant difference between the two techniques.

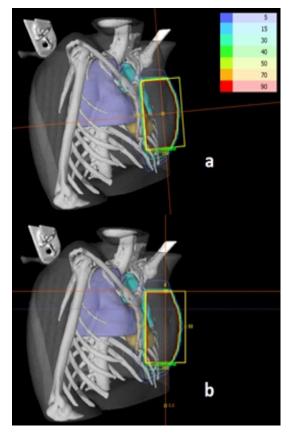
#### Left lung

As shown in Table 3, all parameters calculated for the left lung decreased in MIT (p-value < 0.05), except V20 (Figure 5a).

V20 was not significantly different in the two techniques (p-value = 0.1); the values of this parameter in both MIT and DIT (23% and 26%, respectively) were higher than the acceptable threshold by RTOG 1005 (20%). Additionally, V5 and V10 were ideal and lower than the threshold <sup>38</sup> (Figure 5b).

#### Heart

Based on the information given in Table 4, the mean value of  $D_{min}$  was 127.9 and 173.3 for MIT and DIT, respectively. Also, the mean value of  $D_{max}$  was 4843 and 4960 in SI and DIT, respectively (Figure 6a). The P-values calculated for these parameters indicated a reduction in the MIT, while the  $D_{mean}$  with a mean value of 831 for SI and 883 for DIT did not show a significant difference between the two techniques. D33%, D66%,



**Figure 4.** Isodose curves in the medial tangent beam. a) the dual-isocentric technique with collimator and couch rotation and b) the mono-isocentric technique without those rotations.

Table 3. Comparison of the calculated values in two mono-isocentric	c and dual-
isocenteric techniques for the left lung	

parameter	MIT*	DIT*	P-value	R	TOG
				Ideal	acceptable
$D_{min}$ (cGy)	125.6	149.8	0.01		
D <sub>mean</sub> (cGy)	1219.8	1374.2	0.04		
$D_{max}(cGy)$	4914.3	5058	0.00		
V20* (%)	23.33	25.98	0.1	<15%	<20%
V10* (%)	25.89	30.24	0.02	<35%	<40%
V5* (%)	29.99	37.24	0.00	<50%	<55%

\*Abbreviations: DIT = Dual-Isocentric Technique, MIT= Mono- Isocenteric Technique, V5, V10 and V20 = the volume of lung receiving 5,10 and 20 Gy, respectively.

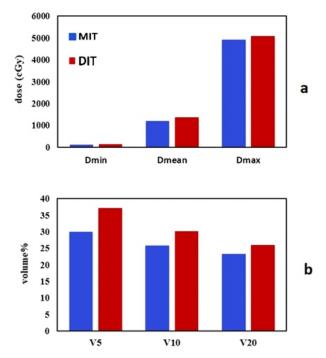


Figure 5. Parameters were calculated for the lung in two mono-isocentric and dual-isocentric techniques. a) the minimum, mean and maximum dose of the lung, b) the volume of the lung (V5, V10, and V20) receiving 5, 10, and 20 Gy, respectively.

and D100% were lower in MIT; however, the values for both techniques were acceptable according to RTOG. V10 was lower than the RTOG threshold with no significant difference between the two techniques (Figure 6b).

#### TCP and NTCP

TCP and NTCP calculations were performed using the radiobiological part in the PCRT 3D software. The corresponding curves were drawn and the TCP and NTCP values were extracted for the 50Gy prescribed

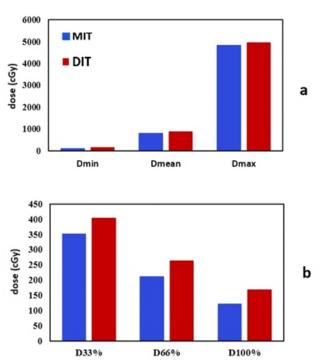


Figure 6. Parameters calculated for the heart in MIT and DIT. a) the minimum, mean, and maximum heart dose, b) the dose of 33%, 66% and 100% volume of the heart.

dose. As shown in Table 5, TCP in the MIT with a mean value of 82.8% (the range of 69-89%) and a mean value of 84.8% in the DIT (the range of 73-95%) did not reveal a significant difference (P-value = 0.08). On the other hand, the mean NTCP for the left lung in MIT was 6% (the range of 2.9-10.6%), less than that in the DIT (mean value of 7.5% and the range of 4.1-12.4%). The heart NTCP was 0.77% (the range of 0.19-1.8%) and 0.97% (the range of 0.24-1.9%) in the MIT and DIT, respectively (Figure 7).

**Table 4.** Comparison of calculated values in two mono-isocenteric and dual-isocentric techniques for the heart.

parameter	MIT	DIT	P-value	R	TOG
				Ideal	Acceptable
$D_{min}(cGy)$	127.9	173.3	0.00		
$D_{mean}(cGy)$	831.4	883.8	0.24		
$D_{max}(cGy)$	4843.1	4960.8			
D <sub>33%*</sub> (cGy)	353.2	404.2	0.00	≤60Gy	
D <sub>66%*</sub> (cGy)	212.9	265.1	0.00	≤45Gy	
D <sub>100%*</sub> (cGy)	122.2	170.3	0.00	≤40Gy	
V <sub>10</sub> (%)	16.47	17.71	0.26	<30%	<35%

 $D_{33\%}$ ,  $D_{66\%}$ , and  $D_{100\%}$  = dose of 33%, 66% and 100% of heart volume.

Table 5. Values of target volume TCP and NTCP of organs at risk
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parameter	MIT	DIT	P-value
TCP of PTV (%)	82.8±2.2	84.8±2	0.08
NTCP of Lung (%)	$6.16 \pm 0.8$	7.57±1	0.02
NTCP of Heart (%)	$0.77 \pm 0.2$	$0.97 \pm 0.17$	0.04

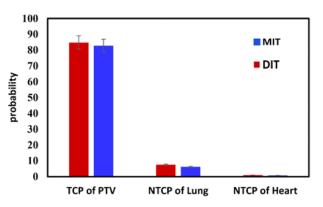


Figure 7. TCP and NTCP calculated for dual and mono-isocentric technique.

#### Discussion

In general, the outcomes of successful radiation treatment planning are tumor control and the incidence of complications. However, the simultaneous control of these outcomes is not only possible by dosimetric factors; the radiobiologic factors seem to be also essential to evaluate and optimize treatment planning. In this study, two external radiation therapy techniques for breast cancer including MIT and DIT were investigated using radiobiological factors such as TCP and NTCP as well as dosimetric parameters.

Despite dual or mono-isocentric (conventional) techniques, advanced radiotherapy techniques such as VMAT, IMRT-IGRT, tomotherapy and protontherapy are not widely available for breast cancer radiotherapy and are restricted to highly selected patients.<sup>39</sup> Although advanced techniques such as intensity-modulated radiotherapy (IMRT) and volumetric imageguided radiotherapy (V-IGRT) improve the tumor control and normal tissue complication, conventional radiotherapy in breast cancer have remained the standard treatment techniques. The interaction between tomotherapy and nodal irradiation improve the outcome, although it did not reach significance.<sup>40</sup>

Taylor et al. published a review article on heart dose in breast cancer, summarizing 149 articles and 398 regimens, showing that the mean heart dose was 5.4Gy (range between 1.6-8) in the 3D breast cancer radiotherapy without intramammary lymph nodes. The values obtained in our study were 8.3Gy for MIT and 8.8Gy for DIT that was at the upper limit of Taylor's article.<sup>4</sup> The values obtained in the study by Adam et al. for the mean and maximum heart dose in the 3D technique were 13 Gy and 51Gy, respectively<sup>37</sup>, which were more than the values in our study for both DIT and MIT (Table 6).

According to the protocol published by the RTOG 0972<sup>41</sup>, cardiac exposure limitations for 33%, 66%, and 100% of the heart are 60, 45, and 40Gy, respectively. The values of heart tissue tolerance in the study by Emami et al. were similar to those of RTOG 0972, although the extracted values in our

study were significantly lower than those in their study. According to the RTOG 1005 protocol, the ideal volume limit for the heart tissue receiving 10Gy and more is 30% and in our study, these values were 16% and 17% for MIT and DIT, respectively.<sup>41</sup> In contrast, Chan reported a V10 of  $3.4 \pm 5.5\%$  for heart in left breast 3D radiotherapy<sup>17</sup>, which is lower than those in our study.

Ohashi et al. claimed that cardiac complications would be minimized if the heart receives less than 30Gy.<sup>42</sup> Emami et al. also stated that if the mean heart dose is less than 26Gy, the pericardial inflammation would be less than 15%.43 Radiationinduced heart-related injuries include acute and chronic damage. Pericardial inflammation is an acute injury that is often transient but can be chronic. The probability of cardiac complications calculated in our study for pericardial inflammation with a mean of 0.77 in SI and 0.97 in DIT was consistent with the study by Astudillo et al. who reported 0-1 for heart NTCP. In the current study, the heart dosimetric parameters in MIT were lower than that in DIT, and in fact, heart exposure was reduced in MIT. Furthermore, NTCP results indicated that the inflammation risk of the pericardium decreased due to the heart dose reduction.<sup>44</sup>

Chan et al. reported 52.3 and 10.7 Gy for the maximum and mean doses of the lung, respectively.<sup>17</sup> The maximum dose of the lung in the current study with a mean value of 49 Gy in MIT and 50 Gy in DIT was lower than that in the study by Chan et al; while the mean dose calculated for MIT with 12 Gy and DIT with 13.7 Gy was higher than that in their study. In Chan et al.'s study, V5, V10, and V20 values for the lung were 35.9, 28.5, and 21.8%, respectively, and V5 and V10 in their research in MIT were more than that in our study, while these values in DIT were less than those in our study.

On the other hand, V20 in our study was higher than reported values in both techniques in the previous research and the RTOG 1005 threshold.<sup>38</sup> Although in the previous RTOG recommendation (version 0972<sup>30</sup>), the threshold for V20 was 35% which was consistent with our study. In Adam's

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study, V20 for lung was 24% and higher than that in our study. The mean dose of the left lung was reported to be 12 Gy which was identical to the values of the MIT but less than the DIT values in our study. Emami et al. claimed that pulmonary inflammation due to irradiation was the most common complication among patients with breast radiation therapy and that the risk of this complication often limited the prescription dose in the treatment. They estimated the risk of pulmonary inflammation at 10% when V20 was less than 31%, arguing that the likelihood of inflammation was 5% for V5 less than 42%. These values resemble the values obtained in the current study and consequently, it was expected that the NTCP for the pulmonary inflammation would be the same as the value reported by Emami et al.43 In our study, the NTCP was 7.57 and 6.16 for the DIT and MIT, respectively while the mean value of the left lung NTCP in the study conducted by Astudillo ranged from 6 to 53%. Hurkman also reported that pulmonary inflammation was not observed in the patients receiving 8 Gy mean lung dose.<sup>14</sup>

The normalization point of the tangential field was defined in the same region with a slight difference in both techniques. The maximum dose was received in the DIT and it was in the acceptable limit according to RTOG 1005; no hot point was observed. Reducing the hot spots in the isodose curves has a direct effect on the treatment outcomes and decreases the superficial breast skin burns. The average value of TCP, which represented the result of all dosimetric calculations, did not show a significant difference in the two techniques, and in fact, both techniques provided similar tumor control. Kara et al. pointed to the superiority of the MIT, with a reduction of more than 50% in hot spots, and the values of the minimum and mean dose in the MIT were close to the values of the prescription dose.<sup>45</sup> In our study, all values for the left lung, except V20, were significantly different in the two methods, and in the MIT, the dose and volume of the exposed lung decreased. A significant decrease in the parameters for the heart was observed in the MIT technique in the DIT in our study. Rosenow et al. also reported lung dose reduction in the MIT technique.<sup>11</sup>

However, the dose-volume curves of organs at risk of the lung and heart showed a significant difference in all patients with a history of breast conservative surgery and mastectomy.<sup>11</sup>

Kagkiouzis published a review of three-field techniques in breast cancer radiation therapy, which introduced tangent and supraclavicular fields matching as the most complex clinical problem that could be due to breast disordered morphology (Such as breast shape and chest slope) and divergence of radiotherapy fields.<sup>46</sup> Also, in the clinic, these parameters such as patient setup and fixing the collimators have more effect on the adjacent radiation fields matching than the type of treatment technique. Several papers pointed to the reduction of hot spots in MIT. The MIT technique reduces the overall time of the treatment and also decreases the errors caused by the patient's movements but the disadvantage is that treatment planning takes longer. It has been argued that a slight difference in breast and supraclavicular field matching leads to high dosimetry changes in the target volume, lung, and bilateral breast, and there is a need for high precision and jaw control in the treatment.<sup>46</sup>

The presence of one isocenter as well as the absence of collimator and couch rotation in the MIT increases the speed of patient setup and reduces the treatment time. On the other hand, the need for displacing the isocenter point for two different fields increases precision and repeatability. The main point is the absence of hot spots, cold spots, overlaps of the tangent and supraclavicular fields, which reduces the risk of treatment complications and cancer recurrence.11,47,48 It is worth mentioning that the collimators cover a maximum length of 40 cm. Using asymmetric jaws and the MIT allows the opening of therapeutic fields up to 20 cm, which may not be enough for some patients with a large breast (length of more than 20 cm); so in these cases, the dual-isocentric technique should be used. Another issue that we encountered in the clinic is the effect of the isocenter location on the treatment accuracy. In the MIT, the isocenter is located close to the axillary region and the repeatability of the treatment is reduced in patients with obesity or those having tissue irregularities and flexure due to breast surgery. Consequently, the treatment does not have adequate accuracy and, thus, it is recommended to use a dualisocentric technique with two separate isocenters.

To compare different conventional breast radiotherapy techniques, the mean absolute dose deviation (MADD) has been developed; this parameter measures how widely the dose delivered to an organ deviates from a reference dose prescribed for that organ and integrates the balance between tumor control and normal tissue complication. Wang et al. evaluated the dosimetric advantage of prone setup compared to supine for left-breast radiotherapy. In their study, radiation doses to heart, lungs, breasts, and tumor bed were assessed using MADD. Subsequently, as a weighted sum of the MADDs was normalized to the breast prescribed dose, a penalty score was computed for each treatment plan.<sup>49</sup>

Several limitations to this study need to be acknowledged. First, the sample size was small due to the inclusion/exclusion criteria in this study and the time limitation that the researchers encountered during this project. With a larger sample size, more significant results could have been extracted from the data. Second, the present study was subject to some potential methodological weaknesses; for example, a) TCP and NTCP are multi-parametric non-linear

		Curre	nt Study	Taylor	Adam	Emami	RTOG	RTOG	Chan	Astudilo
		DIT	MIT	·			0972	1005		
Heart	Mean dose (Gy)	8.8	8.3	5.4	13					
	Max dose (Gy)	49	48		51					
	D33% (Gy)	4	3.5			60	60			
	D66% (Gy)	2.6	2.1			45	45			
	D100% (Gy)	1.7	1.2			40	40			
	V10 (%)	17	16					30	3.4	
	NTCP (%)	1	0.8							
										0-1
Lung	Max dose (Gy)	50	49						52.3	
-	Mean dose (Gy)	14	12		12				10.7	
	V5 (%)	37	30					55%	35.9	
	V10 (%)	30	25					40%	28.5	
	V20 (%)	25	23		24		35%	20%	21.8	
	NTCP (%)	7.6	6.2		10	% for V20<31%	)			6-53%

Table 6. The results of our study and those of the other s	studies cited in this study.
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models that have not received formal validation. The more parameters, the greater the likelihood of a model being wrong, b) TCP and NTCP were evaluated differently, one set of equations for targets, another set for organs, intrinsically disparate metrics, c) Using TCP and NTCP can only be hypothesisgenerating.

Regarding the findings of this study and the review of other studies, in both MIT and DIT the dose coverage of PTV and tumor control probability was similar, while the dose of organs at risks such as heart and lung was reduced in the MIT. All in all, it can be stated that the MIT provided an improved treatment plan compared to the DIT.

#### **Conflict of Interest**

None.

#### **Ethical Consideration**

This study have obtained research ethics committee approval from Tehran University of Medical Sciences.

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# Growth Inhibitory Efficacy of Tabebuia Avellanedae in a Model for Triple Negative Breast Cancer

Nitin T. Telang\*<sup>a</sup>, Hareesh B. Nair<sup>b</sup>, George YC Wong<sup>c,d</sup>

<sup>a</sup> Cancer Prevention Research Program, Palindrome Liaisons Consultants, Montvale, New Jersey, USA

<sup>b</sup> University of Texas Health Science Center, San Antonio, Texas, USA

<sup>°</sup> American Foundation for Chinese Medicine, New York, USA

<sup>d</sup> Breast Center, Maimonides Medical Center, Brooklyn, USA

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#### ABSTRACT

Received: 02 March 2021 Revised: 28 March 2021 Accepted: 02 April 2021 **Background:** Tabebuia avellanedae (TA) is a tree that is indigenous to the Amazon rainforest. The experiments in the present study were designed to examine the inhibitory effects of TA, and to identify mechanistic targets for its efficacy in the estrogen- $\alpha$  receptor (ER- $\alpha$ ), progesterone receptor (PR) and human epidermal growth factor receptor-2 (HER-2) negative MDA-MB-231 model for triple negative breast cancer (TNBC).

**Methods:** Non-fractionated aqueous extract from the inner bark of TA was used in the experiments. Anchorage dependent growth, anchorage independent (AI) colony formation, cell cycle progression, and expressions of relevant regulatory proteins represented quantitative end points.

**Results:** Long-term treatment for 21 days with the maximum cytostatic concentration of 2.5% TA resulted in a 90% inhibition (P=0.014) in AI colony number. Short-term treatment for 2 days with 1.0% TA (IC<sub>50</sub>) resulted in about a 1.3 fold increase (P=0.014) in G<sub>1</sub>: S+G<sub>2</sub>/M ratio, about a 1.48 fold increase (P=0.010) in the sub G<sub>0</sub> (apoptotic) cells and about a 3.2 fold increase (P=0.014) in the pro-apoptotic caspase 3/7 activity. Mechanistically, the short-term treatment with 2.5% TA decreased Cyclin D1 expression by about 83.3%, and pRB expression by about 73.3%.

#### Key words:

Triple negative breast cancer, *Tabebuia avellanedae*, cell cycle, RB signaling, cellular apoptosis, caspase 3/7 activity **Conclusion:** TNBC represents an aggressive cancer notable for its resistance to conventional and targeted therapy. Non-toxic natural substances may represent testable alternatives. This study identifies potential mechanistic leads for TA as a novel naturally occurring testable alternative for secondary prevention/therapy of TNBC, and validates a novel mechanistic approach to evaluate efficacious non-toxic phytochemicals and herbs as testable alternatives against therapy resistant breast cancer.

#### Introduction

Therapy resistant invasive breast cancer accelerates disease progression and represents a predominant cause of mortality in the US. The American Cancer Society projections for the breast cancer incidence and mortality estimate 246,660

\* Address for correspondence: Nitin T. Telang, Ph.D. Address: Palindrome Liaisons Consultants, 10 Rolling Ridge Road, Suite B, Montvale, NJ 07645-1559, USA Email: <u>ntelang3@gmail.com</u> newly diagnosed breast cancer cases and 40,450 breast cancer related deaths in women in 2020.<sup>1</sup> The mortality rate is due to the incidence of advanced metastatic cancer. Clinical triple negative breast cancer (TNBC) represents an aggressive subtype that lacks the expression of ER- $\alpha$ , PR and HER-2. TNBC is notable for its resistance to conventional endocrine or HER-2 targeted therapy.<sup>2-4</sup> Current treatment options for the TNBC subtype are mostly restricted to anthracyclin, taxol and platinum based conventional chemotherapy. In addition, selective inhibition of



poly (ADP-ribose) polymerase (PARP), phosphoinosine-3-kinase (PI3K) or molecular target of rapamycin (m-TOR) pathways via small molecule based targeted therapy has documented clinical efficacy.<sup>5</sup> These conventional and targeted treatment options are frequently associated with long-term systemic toxicity, de novo or acquired tumor resistance and emergence of therapy resistant cancer stem cell population.<sup>6</sup> These limitations emphasize a need to identify novel, less toxic options as testable alternatives to existing treatment strategies.

Complementary and alternative approaches utilizing herbal medicines are being used for general health issues and for palliative care of breast cancer in women.<sup>7,8</sup> Efficacious non-toxic natural phytochemicals and nutritional herbs may offer testable alternatives as a novel approach to eliminate therapy associated systemic toxicity and/or to reduce the emergence of therapy resistant tumor cell phenotype. Non-fractionated aqueous extracts from several nutritional herbs have documented growth inhibitory efficacy via distinct mechanisms in a human breast carcinoma derived cellular model for ER- $\alpha^+$ /PR<sup>+</sup>/HER-2- Luminal A subtype<sup>9-11</sup>, and in a model for ER- $\alpha^-$ , PR<sup>-</sup> and HER-2<sup>-</sup> triple negative subtype.<sup>12,13</sup>

TA is a tree that is indigenous to the Amazon rainforest. A tea made from the inner bark of TA, also known as Taheebo or Pau d'Arco, has been traditionally used by the people in that region to treat a variety of ailments including bacterial, fungal and viral infections.<sup>14</sup> Non-fractionated aqueous extract from TA represents a source for furano-naphthoquinines, quinines, naphthoquinones and flavonoids, and has documented efficacy in preclinical model for colon cancer CT-26.<sup>15, 16</sup> Since the relevant part of TA for medical usage is the inner bark, the abbreviation TA in this manuscript refers to the inner bark of this tree.

Global gene expression profiling to monitor differential gene expressions for growth inhibitory has demonstrated that non-fractionated aqueous extract of TA decreases the expression of positive growth regulatory cyclins, while increasing the expression of genes relevant to cellular apoptosis in the MCF-7 model for the Luminal A molecular subtype of breast cancer<sup>9</sup>, and decreases the aromatase activity in the aromatase expressing MCF7-AROM model for post-menopausal breast cancer.<sup>11</sup> Growth inhibitory efficacy of aqueous extracts of Chinese nutritional herbs Dipsacus asperoides and Cornus officinalis via the RB signaling pathway have been demonstrated in the MDA-MB-231 model for TNBC molecular subtype of breast cancer.<sup>12,13</sup> These data on the Luminal A and TNBC models identify potential mechanistic leads. However, growth inhibition and the molecular mechanisms for efficacy of TA in the TNBC model are not identified.

The TNBC subtype frequently leads to progression of therapy resistant metastatic disease.<sup>3,5</sup> The experiments in the present study are designed to evaluate growth inhibitory effects of TA, and to identify susceptible mechanistic pathways and molecular targets for its efficacy in a cellular model for TNBC.

#### Methods

#### Experimental model

The TNBC cell line MDA-MB-231 is ER- $\alpha$  negative, PR negative and HER-2 non-amplified.<sup>17,18</sup> This cell line was obtained from American Type Culture Collection (ATCC, Manassas, VA, USA), and was maintained in RPMI medium with L-glutamine and 5% fetal bovine serum (Life Technologies, Grand Island, NY) following the protocol recommended by the vendor.

#### Tabebuia avellanedae (TA)

The test agent is provided by Taheebo Japan Co., Ltd., Osaka, Japan in the form of water soluble powder prepared from the inner bark of the TA tree. This product is commercially available under the name of Taheebo NFD Essence. The aqueous extract of TA typically contains about 100  $\mu$ g of naphthofuran dione (NFD, 3.9  $\mu$ M, Molecular Mass: 258) and about 0.4 $\mu$ g of  $\beta$ -lapachone ( $\beta$ -LAP, 1.6 nM, Molecular Mass: 242.27). These compounds represent known bioactive agents of TA (Personal Communication: Prof. Fukuda, Taheebo Japan Co. Ltd. Osaka, Japan).<sup>15-17</sup>

The Stock solution of TA was prepared by dissolving 500 mg of the powder in 100 ml of double distilled water using the boiling extraction protocol.<sup>9,11</sup> The stock solution was serially diluted using the culture medium to obtain the concentrations of 2.5%, 2.0%, 1.5%, 1.0% and 0.5% TA for the dose response experiments.

#### Anchorage Independent (AI) Growth Assay

This assay was performed following the optimized protocol.<sup>11-13</sup> TA treated and untreated control cells were suspended in RPMI medium containing 0.33% agar and were overlaid on the basement layer of 0.6% agar. The cultures were incubated at 37°C in a CO<sub>2</sub> incubator for 21 days. The AI colonies were stained with 0.005% crystal violet and colony counts were determined at 10X magnification. The data were expressed as AI colony number.

#### Cell Cycle Progression

Cells were monitored for cell cycle progression following published protocol.<sup>11-13</sup> Cellular DNA content was analyzed using a Becton Dickinson FACSCAN Flow Cytometer (BD Biosciences, Research Triangle Park, NC, USA) and analyzed with FACS Express software (De Novo Software, Glendale, CA, USA). The cell cycle progression was presented as % of cells in  $G_1$ , S and  $G_2/M$  phases of the cell cycle, and as  $G_1$ : S+ $G_2/M$  ratio.

#### Western Blot Analysis

Cellular proteins were separated by 10% SDS-PAGE (Mini-PROTEAN TGX, Bio-Rad Laboratories), transferred onto a nitrocellulose membrane (Bio-Rad Laboratori3es), and incubated with primary and secondary antibodies according to the published protocol.<sup>13</sup> Antibodies against the following proteins were used:  $\beta$ -actin, cyclin D1 and RB (Santa Cruz Biotechnologies, Santa Cruz, CA, USA). CDK4 and phospho-Rb Ser 780 (Cell Signaling Technology, Inc. Danvers, CA, USA). The chemo-luminescent signal was developed with ECLplus reagent (Bio-Rad, Hercules, CA, USA), and detected by autoradiography. The data were quantified by arbitrary scanning units (ASU).

#### Caspase Assay

Caspase-3/7 activity in the MDA-MB-231 cells was measured using Caspase-Glo assay kit (Promega, Madison, WI, USA) following the protocol provided by the vendor. The luminescence was measured using Luminometer (Thermo Scientific Co, Waltham, MA, USA). The data were expressed as relative fluorescence units (RLU).

#### Statistical Analysis

The experiments for dose response, AI growth, cell cycle progression, and Caspase 3/7 activity were conducted in triplicate. The data were expressed as mean  $\pm$  SD. Comparison of statistically significant differences between the common control and multiple treatment groups was analyzed using analysis of variance and Dunnett's multiple comparison test as a

post-hoc with a threshold of  $\alpha$ =0.05. The data were analyzed using the Microsoft Excel 2013 XLSTAT-Base software.

#### Results

Growth Inhibitory Effects of TA

The data on the dose response of TA on MDA-MB-231 cells are presented in Table 1. Treatment with TA resulted in a dose dependent cytostatic growth arrest of MDA-MB-231 cells, and identified IC50 as 1.0%, and IC90 as 2.5%, respectively. Statistical analysis revealed that 1% TA induced a 52.0% inhibition (P=0.037) and 2.5% TA induced a 90.0% inhibition (P=0.014) in the viable cell number, relative to the control. Treatment with TA at concentration higher than 2.5% resulted in a viable cell number that was lower than the initial seeding density of 1.0X105. Therefore, this concentration was considered toxic (data not shown).

The data on the effect of TA on AI colony formation are presented in Table 2. Treatment with TA within the cytostatic range of 1.0% and 2.5% induced a 50.9% reduction (P=0.037) and a 90.2% reduction (P=0.014) in the number of AI colonies, relative to the control.

#### Effects of TA on Cell Cycle Progression

The data presented in Table 3 examined the effect of TA on the cell cycle progression of MDA-MB-231 cells. In response to the treatment with 1.0 % TA, the cells exhibited a 1.3 fold increase (P=0.014) in the  $G_1$ : S+G<sub>2</sub>/M ratio, relative to the control. This increase was due to an inhibition of  $G_1$  to S phase transition and resultant  $G_1$  arrest.

The data presented in Figure 1 examined the effect of TA on the status of select cell cycle regulatory proteins. In response to treatment with 1% and

 Table 1. Growth inhibitory effect of TA on MDA-MB-231 cells

Treatment	Concentration (%)	Viable cell number $(10x^5)^{a}$	Inhibition (% control)
Control TA	0.5 1.0 1.5 2.0 2.5	$25.0\pm2.5 \\18.0\pm3.2 \\12.0\pm1.4* \\11.0\pm1.4 \\7.0\pm0.7 \\2.5\pm0.3**$	28 52 56 72 90

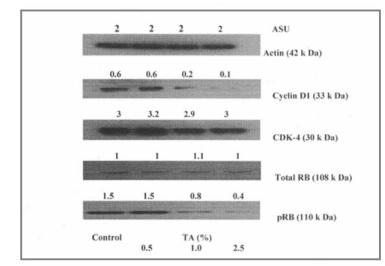
<sup>a</sup> determined at day 7 seeding of  $1.0 \times 10^5$  cells by trypan blue dye exclusion test. Mean  $\pm$ SD, n= 3 per treatment group. \* P= 0.037, \*\* P= 0.014. Data analyzed using ANOVA with Dunnett's post-hoc multiple comparison test ( $\alpha$ =0.05). TA,

*Tabebuia avellandae*; ANOVA, analysis of variance.

Table 2. Effect of TA on anchorage independent growth in MDA-MB-231 cells

Treatment	Concentration (%)	AI colony number <sup>a</sup>	Inhibition (% control)
Control TA	1.0 2.5	255±76 125±37* 25±5**	50.9 90.2

<sup>a</sup> determined at day 21 post-seeding of  $5.0 \times 10^5$  cells. Mean ±SD, n=3 per treatment group. \* P=0.037, \*\* P= 0.014. Data analyzed using ANOVA with Dunnett's post-hoc multiple comparison test ( $\alpha$ =0.05). AI, anchorage independent; TA, Tabebuia avellandae; ANOVA, analysis of variance.



**Figure 1.** Western blot analysis of select proteins of the RB pathway. The expressions of Cyclin D1 and p-RB exhibit a dose dependent inhibition in response to treatment with TA. ASU, arbitrary scanning unit; CDK4, cyclin dependent kinase-4; RB, retinoblastoma; pRB, phosphorylated RB; TA, *Tabebuia avllanedae*.

2.5%TA, expression of cyclin D1 was reduced in a dose dependent manner by about 66.7% and 83.3%, respectively, relative to the control. Similarly, the expression of pRB was reduced by about 46.7% and 73.3% respectively, relative to the control.

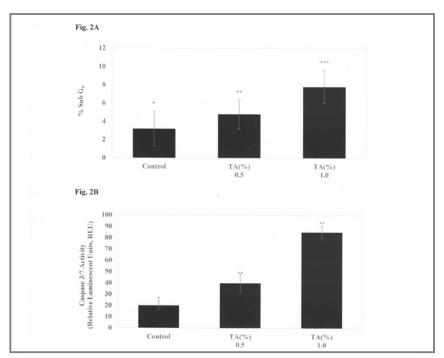
#### Effects of TA on Cellular Apoptosis

This experiment examined the effect of TA on cellular apoptosis in MDA-MB-231 cells. Treatment with 0.5% and 1.0% TA resulted in about a 50.0% increase (P=0.037), and about a 1.43 fold increase (P=0.010) in apoptotic cells that were represented as the sub  $G_0$  phase of the cell cycle (Figure 2A).

Additionally, treatment with 1.0% TA resulted in about a 3.2 fold increase (P=0.014) in caspase 3/7 activity (Figure 2B).

#### Discussion

Human tissue derived cell culture models offer valuable mechanistic approaches to identify clinically translatable leads. Published comparative studies on non-tumorigenic triple negative 184-B5 cells, and on the carcinoma derived MDA-MB-231 cells have revealed that in contrast to 184-B5 cells, MDA-MB-231 cells exhibit aberrant hyperproliferation, accelerated cell cycle progression,



**Figure 2.** Induction of cellular apoptosis. A. Incidence of cells in subG<sub>0</sub> (apoptotic phase) exhibits an increase in response to treatment with TA. \*\_\*\* P=0.037, \*\_\*\*\* P=0.010. Data analyzed by ANOVA with Dunnett's multiple comparison post-hoc test. B. Increase in caspase 3/7 activity in response to treatment with TA. \*\_\*\* P=0.040, \*\_\*\*\*P=0.014. Data analyzed by ANOVA with Dunnett's multiple comparison post-hoc test. TA, Tabebuia avellanedae; ANOVA, analysis of variance.

Treatment <sup>a</sup>	Concentration	Cell cycle phase <sup>b</sup>				
	(%)	$% G_1$	% S	% G <sub>2</sub> /M	$G_1: S+G_2/M$	
Control		29.9±5.0	11.9±0.6	41.7±2.9	0.6±0.3	
TA	0.5	39.4±2.0	12.2±0.6	36.2±1.8	$0.8 \pm 0.4$	
	1.0	49.2±3.5*	22.2±1.1	12.8±0.6	1.4±0.3**	

 Table 3. Effect of TA on cell cycle progression in MDA-MB-231 cells

<sup>a</sup> TA treatment for 48 hr.<sup>b</sup> determined by flow cytometry based FACS assay. Mean  $\pm$  SD, n=3 per treatment group. \*P=0.02. \*\*P=0.014. Data analyzed using ANOVA with Dunnell's post-hoc multiple comparison test ( $\alpha$ =0.05). TA, *Tabebuia avellandae*; FACS, fluorescence assisted cell sorting.

down-regulated cellular apoptosis and AI growth in vitro.<sup>14</sup> These data suggest loss of homeostatic growth control and retention of cancer risk in MDA-MB-231 cells. This aspect is also documented in the ER- $\alpha$ +/PR+/HER-2-Luminal A model.<sup>9,10,19</sup> Furthermore, it is notable that AI colonies are observed in carcinoma derived MDA-MB-231 cells, but not in non-tumorigenic 184-B5 cells. Thus, AI growth represents an important in vitro surrogate end point marker for in vivo tumor development, and AI growth provides a quantifiable marker for cancer risk.

Treatment options for TNBC are associated with long-term systemic toxicity, acquired tumor resistance and emergence of drug resistant stem cells.<sup>2,5,6</sup> These limitations emphasize a need to identify effective, non-toxic alternatives as testable therapeutic options. Nutritional herbs Dipsacus asperoides and Cornus officinalis have documented growth inhibitory efficacy in the present model for TNBC.<sup>12,13</sup> It is noteworthy that there are several cellular models for clinical TNBC, and that the MDA-MB-213 model represents one such model. The present study outcome on the MDA-MB-231 model provides a mechanistic proof of concept for investigations on other TNBC models to examine the efficacy of naturally occurring phytochemicals and herbal extracts as testable alternatives for prevention/therapy of TNBC.

Little published evidence is available for clinical efficacy of TA on cancer patients. Anecdotal evidence on a limited number of patients demonstrates the effect of TA on the status of quality of life in advanced metastatic multiple organ site cancers.<sup>20</sup> In addition, the effects of NFD have been documented in three head and neck cancer patients and in one patient with lung metastasis from rectal cancer.<sup>20,21</sup>

In the studies discussed above, TA and NFD were administered as aqueous solutions. In traditional Chinese medicine, herbal formulations are commonly administered to patients in the form of herbal tea that is prepared by boiling the herbs in water. Thus, non-fractionated aqueous extracts of herbal formulations represent a commonly used method in patients. To simulate patient consumption of Taheebo tea, non-fractionated aqueous extract from TA was used in the present study.

At the mechanistic level, NFD, a major bio-active component of TA, has documented efficacy as a small molecule inhibitor of recombinant dual-specificity phosphatase Cdc-25 that regulates cell cycle transition. In addition, NFD induced  $G_1/S$  and  $G_2/M$  arrest and inhibited the proliferation of PC3 prostate carcinoma derived cells and MDA-MB-435 breast carcinoma derived cells at  $\mu$ M concentrations.<sup>22</sup>

PARP inhibitors represent a clinical option for targeted therapy of TNBC. Monotherapy with PARP inhibitors induces systemic toxicity and therapy resistance.<sup>5</sup> Combined treatment with PARP inhibitors and a naturally occurring quinone  $\beta$ -LAP has demonstrated synergistic interactions resulting in enhanced therapeutic efficacy of PARP inhibitors in pre-clinical xenograft models of lung, pancreatic and breast cancer.<sup>23, 24</sup> In response to treatment with TA within its cytostatic range, MDA-MB-231 cells exhibited a dose dependent growth inhibition and reduction in the number of AI colonies. The effective half-maximum concentration (IC<sub>50</sub>) of TA was determined at 1.0%. This concentration was estimated to contain 0.039 µM of NFD. The maximum cytostatic concentration (IC<sub>90</sub>) of TA was determined at 2.5%. This concentration is estimated to contain about 0.097 µM of NFD. Thus, the data on cytostatic growth arrest and reduction in the number of AI colonies suggest that TA may have effectively reversed the loss of homeostatic growth control and inhibited cancer risk in part, due to the presence of NFD in the present model for TNBC. In addition to NFD,  $\beta$ -LAP represents a minor constituent of TA.<sup>15,16</sup> The  $\beta$ -LAP content of 1.0% and 2.5% TA was estimated to be 0.016 nM and 0.040 nM, respectively. Thus, these concentrations represent non-effective low concentrations of  $\beta$ -LAP. Additionally, preclinical in vivo studies on the effects of TA in mice transplanted with Ehrlich's ascites carcinoma cells have shown that the levels of  $\beta$ -LAP are nondetectable as measured by thin layer chromatography based, and high pressure liquid chromatography based assays.<sup>16</sup> High  $\mu M$  concentrations of  $\beta$ -LAP inhibit growth and induce BCL-2 and caspasedependent apoptosis in the T24 model of bladder cancer.<sup>25</sup> However, despite the documented efficacy of  $\beta$ -LAP at the higher pharmacological concentrations, these effective high  $\mu$ M concentrations of  $\beta$ -LAP are of limited relevance in the present study where concentrations of TA are low, and consequently, those of  $\beta$ -LAP.

Non-fractionated aqueous extract of TA also has documented growth inhibitory efficacy in ER- $\alpha^+/PR^+/HER$ -2- MCF-7 cells that represent a model for endocrine therapy responsive Luminal A



molecular subtype of clinical breast cancer. In this model, TA within the cytostatic dose range inhibited cell cycle progression and induced cellular apoptosis. At the mechanistic level, the biological effects of TA were associated with up-regulated expression of select proliferation specific genes, modulated expression of apoptosis specific genes and enhanced expression of genes specific for xenobiotic metabolism.<sup>9</sup> In the present study, the effect of TA on cell cycle progression revealed a dose dependent  $G_1$  phase blockade and inhibition of S+ $G_2/M$  phases of the cell cycle. These alterations in the cell cycle resulted in a dose dependent increase in the  $G_1$ : S+ $G_2/M$  ratio.

The cell cycle regulatory function of RB via the cyclin D1-CDK4/6-p-RB axis is compromised in therapy resistant basal-like and triple negative subtypes of clinical breast cancer.<sup>26, 27</sup> The data from the experiment designed to examine the effect of TA on the RB pathway clearly demonstrated that TA inhibited the expression of cyclin D1 and p-RB in a dose dependent fashion, while, intriguingly, the expression of CDK4 remained essentially unaltered. Kinase-dependent site specific phosphorylation of RB represents a critical post-translational modification for the tumor suppressive function via inactivation of cell cycle progression and promotion of cellular apoptosis.<sup>28</sup> Thus, the present data on inhibition of cyclin D1 and p-RB provide mechanistic lead to suggest that the RB pathway might represent a molecular target for the efficacy of TA in the present model system.<sup>29,30</sup>

Mitochondria-mediated intrinsic apoptotic pathway involves altered membrane permeability, cytochrome-c release, and apoptosome-mediated activation of Caspase 9 and subsequently of Caspase 3/7.<sup>31, 32</sup> The experiment designed to examine the effect of TA on cellular apoptosis demonstrated that TA produced a dose dependent progressive increase in the Sub G<sub>0</sub> (apoptotic) phase of the cell cycle, and an increase in the pro-apoptotic Caspase 3/7 activity. Collectively, these data on cellular apoptosis provide mechanistic leads to support the evidence that induction of cellular apoptosis by TA may function via caspase dependent mechanism in the present model system.

Differential gene expression profiling in the MCF-7 model revealed that TA resulted in downregulation of cell cycle regulatory genes, cyclins B1 and E and cyclin dependent kinases CDK2, CDK4 and CDK6 and in upregulation of apoptosis related genes, caspase 4, caspase 7 and caspase 8.° Thus, TA appears to be effective via mechanistically distinct pathways in cell culture models for Luminal A and TNBC subtypes of clinical breast cancer, thereby supporting the concept that growth inhibitory effects of TA may be independent of expressions of hormone receptors. In this context, it is notable that mechanistically distinct nutritional

herbs demonstrate differential growth inhibitory efficacy in a model where isogenic phenotypes exhibit modulated ER function.<sup>33</sup>

In conclusion, the results of the present study exhibiting anti-proliferative and pro-apoptotic effects of TA, together with the published evidence for the efficacy of extracts from several nutritional herbs on the ER- $\alpha$  positive, PR positive and HER-2 non-amplified (Luminal A) model<sup>9-11</sup>, and on the ER- $\alpha$  negative, PR negative, HER-2 non-amplified negative (TNBC) model<sup>12, 13</sup> may provide a mechanistic rationale for future preclinical and clinical investigations.

#### **Conflict of Interest**

The authors declare that there is no conflict of interests. This study was supported by philanthropic contributions to the American Foundation for Chinese Medicine from the Randall and Barbara Smith Foundation, the Saint Agatha Foundation and the Sophie Stenbeck Family Foundation.

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### DOI: 10.32768/abc.202183210-215 Breast Cancer Awareness in Myanmar: Results of a Hospital-based Study in Mandalay

Myo Khin\*<sup>a</sup>, San Shwe<sup>a</sup>, Khin May Oo<sup>a</sup>, Le-Le Win<sup>a</sup>

<sup>a</sup> Ministry of Health and Sports, Myanmar

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#### ABSTRACT

**Background:** Although breast cancer is an important health problem in Myanmar, awareness of breast health has not been widely described.

**Methods:** A cross-sectional descriptive study was carried out to explore awareness of early signs and risk factors of breast cancer among outpatient attendees at the Mandalay Central Women Hospital.

**Results:** A total of 402 respondents with mean age of  $31.4\pm9.0$  years participated in the study. More than half of the respondents (65.9%) were aware of breast lumps as a breast cancer sign. Nearly half (42% to 48.7%) of the respondents were aware of the various risk factors of breast cancer. Although 54.1% had heard of breast self-examination, only 25.3% had knowledge of mammograms. Older women (age 49 years or more) had significantly better awareness of two early breast cancer signs; change of breast shape (54.1% vs 39.9%) and discharge from nipple (49.2% vs 33.4%). Those with less than high school education had significantly lower awareness of the following signs of breast cancer; thickening of the breast skin (39.3 % vs 20.45%), dimpling of the breast skin (43.2% vs 24.6%), change of breast shape (52.5% vs 35.2%), and discharge from nipple (44.9% vs 31.9%). They also displayed significantly lower awareness of risk factors of breast cancer.

Key words: Breast cancer, risk factors, female outpatient attendees, Myanmar

**Conclusion:** Among the study respondents, only one third had heard of selfbreast examination and only a few (10%) examined both breasts regularly. Greater awareness of breast health and breast cancer screening should be imparted to promote breast health among women in Myanmar.

#### Introduction

Cancer is a leading cause of disease worldwide. The International Agency for Research on Cancer (IARC) has estimated that 19.3 million cancer cases and 9.96 million cancer deaths occurred globally in the year 2020.<sup>1</sup> According to the World Health Organization (WHO), breast cancer is the most common cancer among women worldwide, claiming the lives of hundreds of thousands women each year and affecting countries at all levels of modernization.<sup>2</sup> It has also been estimated that over 2.1 million new cases of breast cancer occurred during 2018 with over 62 thousand deaths.<sup>3</sup> Among the Asian countries, a higher mortality-to-incidence ratio, more advanced stage at diagnosis, and reduced survival was observed in less developed countries as compared developed countries. The crude rate of breast cancer incidence in Myanmar is also estimated at 22.9 per 100,000 women.<sup>4</sup>

Although breast cancer in Myanmar has been studied since the late 70's<sup>5</sup>, little epidemiological data exists in Myanmar. Also, few studies have been conducted to address breast cancer awareness in Myanmar. A study on the awareness, knowledge and perceptions regarding common female cancers revealed that information regarding prevention and

<sup>\*</sup> Address for correspondence: Myo Khin, MBBS, MD(NSW), DCH, FRCP Chair, Institutional Review Board No.1 Ministry of Health and Sports, Myanmar Email: <u>khinmmaja@gmail.com</u>

treatment of cervical and breast cancers needs to be promoted.<sup>6</sup> A recent study showed that women living in rural Myanmar had inadequate information about breast cancer.<sup>7</sup>

For control of diseases, effective health education programs need to be established. In the development of health education programs for control of breast cancer, it is very important to investigate the awareness of breast cancer among the indigenous female population of the country. We carried out the present study to examine the awareness of breast health and breast cancer among women in Myanmar attending the Central Women Hospital, Mandalay. We expect that the findings will contribute to the objectives of developing an appropriate program to promote awareness of breast cancer and increasing women's participation in breast cancer screening activities in Myanmar. The ultimate goal of the study is to reduce morbidity and mortality due to breast cancer among women in Myanmar.

#### Methods

#### Study Design

A hospital based cross-sectional descriptive study was carried out to assess the awareness of women regarding early signs of breast cancer and the risk factors.

#### Study area and population

The study was carried out during 2018 in the Central Women Hospital in Mandalay. Mandalay is situated in central Myanmar and is the secondlargest city after Yangon. It is the cultural capital of Myanmar, with a population of 1.2 million residents. The Central Women Hospital is the tertiary specialist hospital located in the center of Mandalay. The hospital is attended by women for antenatal care and for management of gynecological problems. Daily out-patient attendance is between 300 to 500.

#### Sampling

Consecutive non-probability sampling method was used. Study respondents were those attending the hospital for different purposes related to women health and conditions (antenatal care, gynecological problems, etc.) Prior to the interview, the study objective was explained to the participants who were assured that all information would remain confidential and gave their informed consent. Recruitment was made until the required sample size was achieved.

#### Sample size

Considering the proportion of women having awareness of breast cancer at  $38\%^6$ , margin of error 0.05, and non-response rate 10%, the required sample size was 398.

#### Questionnaires and data analysis

Self-administered questionnaires were used. One of the authors (SS) developed the questionnaire based on a valid and reliable questionnaire used in a previous epidemiological study carried out in Myanmar, in which structured questions regarding socio-demographic characteristics and awareness of common female cancers (breast and cervical cancers) were presented to 400 women.<sup>6</sup> The questionnaires were arranged in two parts. Part one contained questions on demographic characteristics (age, marital status, education, and occupation). The second part comprised knowledge on symptoms of breast cancer, risk factors and screening tests. Data analyses were conducted with SPSS software version 16.0. Descriptive statistics (mean, SD for intervals, frequency with percentages for categorical variables) were calculated. Age and education levels were grouped for categorical analysis. To determine associations between categorical dependent variables, Chi-square tests were carried out. Data were shown as frequency/percentage for categorical variables and mean (standard deviation-SD) for continuous variables.

#### Ethical Consideration

Ethical approval was obtained from the Ethics Review Committee, Department of Medical Research, Ministry of Health and Sports. Written informed consent was obtained from all respondents after thorough explanation about the study.

#### Results

#### Demographic characteristics

A total of 402 respondents, aged 18 years to 67 years participated in the study. The mean age (SD) of the participants was  $31.4\pm9.0$  years. Forty-two respondents (10.5%) were single (never married), the remaining majority (90%) were married (married, divorced or widowed). Concerning education, a few (6.5%) of them had never attended school. All other participants had attended school and more than 118 (29.6%) had studied at high school. Nearly half of them (48.5%) were dependents (Table 1).

# Awareness of early signs of breast cancer and screening for breast cancer

The majority of the respondents (65.9%) were aware of lump as a breast cancer sign. Also, most of them recognized other early signs of breast cancer such as change of breast shape (40.4%), discharge from nipple (35.8%), dimpling of the breast skin (30.1%), thickening of breast skin (25.9%) and swelling of the breast (24.6%). Very few were aware of soreness of breast (20.3%) and redness of breast skin (19.3%) as breast cancer signs. Also, the majority agreed to the fact that nulliparity (48.7%), no breast feeding (48.7%), lumps in breast (47.9%),

Characteristics of the respondents	Num	ber
	Ν	%
Age		
18 to 20 years	36	9.0
21 to 30 years	184	45.8
31 to 40 years	121	30.1
41 to 50 years	50	12.4
51 years and above	11	2.7
Marital status		
Single	42	10.5
Married	333	83
Divorced	12	3
Widowed	14	3.5
Education Level		
Illiterate, Read & Write	65	16.3
Primary	108	26.8
Middle	109	27.3
High	65	16.3
University/ Graduate	53	13.3
Occupation		
Dependent/unemployed	195	48.5
Manual labors	55	13.7
Private employee	19	4.7
Government employee	37	9.2
Small own business	96	23.5

Table 1. Demographic	characteristics	of the res	pondents
	1. (	NT 1	

breast cancer history in family (46%), and oral contraceptives (42.0%) increase the risk of breast cancer. Nearly two-thirds of the respondents (64%) had heard of breast cancer screening. Although half of them (54.1%) had heard of breast self-examination, only 25.3% had knowledge on mammograms.

#### Marital status

The study population included 341 married women and 41 never married women. We found no significant differences between the two groups on breast cancer awareness, knowledge of risk factors and on breast cancer screening.

#### Age of the respondents

The study population consisted of 41 respondents aged 40 years and above and 341 respondents younger than 40 years of age. Older women had

significantly better knowledge of two early breast cancer signs; change of breast shape (54.1% vs 37.9%, p=0.023), and discharge from nipple (49.2% vs 33.4%, p=0.021). Older women also knew better than the younger age group that breast lumps increased the risk of breast cancer (59.0% vs 46.3%, p=0.046). There were no differences on other breast cancer signs, knowledge of risk factors and screening procedures between the two groups. Apart from knowledge of a few signs and on mammography, older women had more correct answers about the awareness of breast cancer (Table 2).

#### Education status

The education of the respondents played an important role in their awareness of breast cancer. In the present study, 282 respondents had less than high school education. They had significantly lower awareness of the following signs of breast cancer; thickening of the breast skin, dimpling of the breast skin, change of breast shape, and discharge from nipple. Those who had more than high school level of education (118 respondents) had significantly higher knowledge of risk factors of breast cancer. (Table 3)

#### Discussion

Breast cancer is the commonest cancer in women both in the developed and the developing world. In most countries of the Association of South East Asia Nations (ASEAN), the burden of cancer is increasing due to population aging and growth, and the adoption of cancer-associated lifestyle behaviors.<sup>8</sup> Identification of breast cancer at an early stage is important in improving breast cancer outcome. A community-based cross-sectional study carried out in Yangon found that 40% of the respondents agreed that breast lump(s) are a condition that could further develop into breast cancer. Also, the majority (93%) agreed that breast self-examination should be carried out to detect breast cancer. More than half (56%) of

Table 2. Awareness of signs of b	reast cancer, risk factors and	breast cancer screening by the study
narticinant age groups		

Variables	Age 18-39	Age > 40	Chi-square
	years (N=341)	years (N=61)	P value
Signs of breast cancer			
Lump in breast	222(65.1)	43(70.5)	NS
Thickening of the skin of breast	85(25.4)	18(29.5)	NS
Swelling of the breast	86(25.4)	12(19.7)	NS
Dimpling of the skin of the breast	98(29.3)	21(34.4)	NS
Redness of the skin of the breast	66(19.6)	17(18.0)	NS
Soreness of the breast	67(19.8)	14(23.3)	NS
Change of breast shape	129(39.9)	33(54.1)	*0.033
Discharge from nipple	113(33.4)	30(49.2)	*0.021
Risk Factors		× ,	01021
Breast lumps increase risk	157(46.3)	36(59.6)	*0.046
Breast cancer history in family increases risk	178(52.4)	37(61.7)	NS
Oral contraceptives increases risk	41(12.1)	7(11.5)	NS
No breast feeding increases risk	142(46.8)	23(44.3)	NS
Nulliparity increase risk	162(47.3)	34(55.9)	NS
Screening	. /	· /	110
Heard of Breast Cancer Screening	226(66.9)	32(53.5)	NS
Heard of Breast Self-Examination	184(55.4)	34(55.7)	NS
Heard of Mammography	92(27.1)	10(16.7)	NS

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Variables	Low (less than	High (high school	Chi-square
	high school) (N=282)	and higher) (N=118)	P value
Signs of breast cancer			
Lump in breast	179(63.5)	85(72.0)	NS
Thickening of the skin of breast	57(20.4)	46(39.3)	*0.000
Swelling of the breast	69(24.5)	38(32.2)	NS
Dimpling of the skin of the breast	68(24.6)	51(43.2)	*0.000
Redness of the skin of the breast	47(16.8)	30(25.6)	NS
Soreness of the breast	53(19.0)	28(23.7)	NS
Change of breast shape	99(35.2)	62(52.5)	*0.001
Discharge from nipple	89(31.9)	53(44.9)	*0.013
Risk Factors			01012
Breast lumps increase risk	123(43.9)	70(59.3)	*0.005
Breast cancer history in family increases risl	x 136 (48.6)	77(65.3)	*0.002
Oral contraceptives increases risk	27(9.6)	21(17.8)	*0.023
No breast feeding increases risk	99(35.4)	69(58.5)	*0.000
Nulliparity increase risk	120(42.7)	76 (64.4)	*0.000
Screening			
Heard of Breast Cancer Screening	153(54.8)	105 (89.0)	*0.000
Heard of Breast Self-Examination	132(47.8)	86 (73.5)	*0.000
Heard of Mammography	167(59.2)	45(38.1)	*0.000

**Table 3.** Awareness of signs of breast cancer, risk factors and breast cancer screening by level of education

the respondents thought that breast cancer is preventable. The findings highlighted the fact that prevention of breast cancer is recognized well by the participants.<sup>6</sup> In the present study, a high proportion of the respondents (65.9%) also acknowledged that breast lumps are an important sign of breast cancer. Moreover, a considerable proportion of the respondents (40.4%) agreed that change in the shape of the breast is also an important sign of breast cancer.

The finding of low awareness levels of breast health, risk factors and breast cancer screening is in agreement with a study from Egypt where the authors reported that lowest levels of awareness were related to age, education and culture.<sup>9</sup> Awareness of breast cancer is important because it affects the likelihood that a woman will engage in appropriate preventive measures. The present study demonstrated that older women had significantly better awareness of two early breast cancer signs (change of breast shape and discharge from nipples) than their younger counterparts. They also knew the high risk of breast lumps better than their younger counterparts (59.0% vs 46.3%). The higher degree of awareness among older women is an encouraging finding as older women above 40 years of age are at increased risk for breast cancer.

It is agreed upon that knowledge of breast cancer and the benefits of breast cancer screening are important in facilitating breast cancer screening behavior.<sup>10</sup> Although breast cancer screening guidelines for women by different age-groups are well developed in developed countries, it is difficult for women in Myanmar to appreciate the risk and recognize the benefits of breast cancer screening. The main reason may be the often-asymptomatic behavior of the breast cancer in its early stages. In the present study, less than two-thirds of the participants (64%) had heard of breast cancer screening. This is much lower than the study from Qatar where 90% of the subjects had heard of breast cancer screening.<sup>11</sup> More importantly, a recent study on 248 nursing students in Myanmar revealed that only 60% of them had positive attitude towards breast cancer screening. Among them only 47% had the correct knowledge concerning breast self-examination and few practiced it regularly.<sup>12</sup> The need for providing correct breast health and breast cancer screening techniques to the health care providers should be seriously considered for efficient transfer of information to the patients. A recent study also highlighted the need to provide proper health education on breast cancer and breast self-examination to women residing in rural areas for early detection of breast cancer.<sup>7</sup>

More information on the breast health and breast cancer screening among the basic health care personnel should be further explored for providing effective health education to the community. Improved awareness of breast health and breast selfexaminations could improve the breast selfexamination behavior and reduce the number of deaths from breast cancer.<sup>13</sup> We found that nearly two-thirds of the participants reported awareness of lumps in the breast as a sign of breast cancer. A similar low level of awareness (38.8%) was reported in a previous study carried out in Myanmar.<sup>6</sup> Both studies concluded that awareness regarding lumps in breast should be increased. Globally, most commonly used breast cancer screening programs are Breast Self-Examination (BSE), Breast Clinical Examination (BCE), and mammography. Mammography is most effective but costly and accessibility is not easy for the general population. BSE could be the community-based screening method in Myanmar for the early detection of breast cancer. BCE could be introduced in secondary hospitals where specialist medical doctors are available. However, even in more developed countries, it has been suggested that identification of high-risk groups should be done incorporating breast cancer risk factors, breast cancer-related awareness and BSE practices, before referral to such centers.<sup>1</sup> It has been suggested that cultural-appropriate strategies should be designed to promote breast cancer screening in the regions where ethnic groups with different characteristics reside.<sup>15</sup> This could be a big challenge for a country like Myanmar where over 100 ethnic groups are widely dispersed throughout the country, especially in the hilly regions where health care infrastructure is weak. Mammograms are only recently available in two public tertiary hospitals for women and a few private hospitals in Myanmar, and its utilization is very limited. Almost 40% of women who came for mammogram to the Central Women Hospital in Yangon thought that it was very costly to have breast cancer screening.<sup>6</sup> Thus, it will be difficult to use mammography as a screening tool for breast cancer in Myanmar.

Our study had some limitations. Although the questions were based on a previous study, some of the items were not specific enough to show correct awareness which underlines appropriate preventive and screening practice. For instance, 54.1% of respondents had heard of breast self-examination, but we do not know what the source of information had been and if the information was provided correctly.

As the study was hospital-based, the respondents were women seeking medical care for antenatal care or gynecological problems. Thus, their awareness about screening and signs of breast cancer might be potentially different from the general population. However, the study supported the fact that promotion of awareness of breast cancer through education is needed in Myanmar. Considering the findings from the present study, emphasis should be given to those with young age and to those with lower education as there was a marked contrast in the awareness of breast cancer and breast health among them as compared to those with older age and those with higher education. Awareness of lumps in breast should be increased. Awareness raising materials specifically targeted at them should be widely distributed in the community. Breast cancer screening guidelines should be developed and tested for application in the field situation. Recently, a "Breast Health Club" has been introduced in Myanmar with the aim of promoting awareness and action on breast health.  $^{16}$  Similar free-of-charge membership-based health clubs should be encouraged to promote community-based breast cancer educational programs in the country.

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#### **Conflict of Interest**

The authors declared no potential conflict of interests with respect to the research, authorship, and/or publication of this article. Also, the authors received no financial support for the research, authorship, and/or publication of this article.

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### Assessment of Tumor Cell Death After Percutaneous Ultrasound– Guided Radiofrequency Ablation of Breast Carcinoma: A Prospective Study

Anna Gumà\*<sup>a</sup>, Teresa Soler<sup>b</sup>, César G. Chappuis<sup>c</sup>, Alazne Valdivielso<sup>a</sup>, Anna Petit<sup>b</sup>, Maria J. Pla<sup>d</sup>, Catalina Falo<sup>e</sup>, Sònia Pernas<sup>e</sup>, Raúl Ortega<sup>e</sup>, Laia Pérez<sup>a</sup>, María Vicente<sup>a</sup>, Eulalia Fernández-Montoli<sup>d</sup>, Miriam Campos<sup>d</sup>, Fernando Burdio<sup>f</sup>, Jordi Ponce<sup>d</sup>, Nahum Calvo<sup>a</sup>, Amparo García-Tejedor<sup>d</sup>

<sup>a</sup> Department of Radiology, Hospital Universitari de Bellvitge, Hospitalet de Llobregat, Barcelona, Spain

<sup>b</sup> Department of Pathology, Hospital Universitari de Bellvitge, Hospitalet de Llobregat, Barcelona, Spain

<sup>°</sup> Department of Pathology, Consorci del Laboratori Intercomarcal de l'Alt Penedès, l'Anoia i el Garraf. Sant Pere de Ribes, Barcelona, Spain

<sup>d</sup> Department of Gynaecology, Hospital Universitari de Bellvitge, Hospitalet de Llobregat, Barcelona, Spain

<sup>e</sup> Department of Oncology, Institut Català d'Oncologia, Hospitalet de Llobregat, Barcelona, Spain

<sup>t</sup> Department of Surgery, Parc de Salut-Hospital del Mar, Barcelona, Spain

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#### ABSTRACT

**Background:** Current trends in breast cancer treatment include the use of less aggressive surgeries to reduce morbidity, shorten hospital stays and improve cosmetic results. The aim of the study is to assess tumor cell viability after percutaneous ultrasound (US)-guided radiofrequency ablation (RFA) for small breast cancer by a combination of staining techniques.

**Methods:** A prospective study was conducted at a single institution from 2013 to 2017. Twenty women with invasive ductal carcinoma of the breast measuring  $\leq$  20 mm were treated with US-guided RFA followed immediately by surgical resection. Tumor viability pre- and post-RFA was assessed with Hematoxylin and Eosin (H&E), Nicotinamide adenine dinucleotide (NADH), Succinate dehydrogenase (SDH), Cytochrome c oxidase (COX), Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) and Cytokeratin 18 and 19 (CK18/CK19) staining techniques. Outcomes and correlation with the different techniques were evaluated with principal component analysis Cronbach's alpha.

**Results:** Oxidative enzymes in frozen sections showed loss of SDH and NADH in 13 of the 16 tumors (81%) and COX in 11 of the 13 tumors (84%). In paraffinembedded tissues, CK18 was negative or markedly reduced in 98% and CK19 in 100% of the cases. Lack of evidence of cell death was seen in 3 cases where the maximum temperature achieved at the center of the tumor was  $\leq$  70°C. The reliability and internal consistency between the different staining techniques was high (Cronbach's alpha, 0.8), with concordance between the staining results of the oxidative enzymes and of CK18/CK19.

**Conclusion:** Loss of tumor viability in small breast tumors after US-guided percutaneous RFA could be assessed in our series with different staining methods. CK18 and CK19 could be used in paraffin-embedded tissues as surrogate markers of tumor cell viability after immediate RFA.

\*Address for correspondence: Anna Gumà, MD

Address: Department of Radiology, Hospital Universitari de Bellvitge, Hospitalet de Llobregat, Carrer de la Feixa Llarga, s/n 08907 Hospitalet de Llobregat, Barcelona, Spain. Email: <u>anna.guma@bellvitgehospital.cat</u>

#### Introduction

Current trends in breast cancer treatment include the use of less aggressive surgeries to reduce morbidity, shorten hospital stays and improve cosmetic results. Several percutaneous ablation techniques have been studied, such as cryoablation, laser ablation, microwave irradiation, high-intensity focused ultrasound, electroporation and radiofrequency ablation (RFA).<sup>1,2</sup> RFA is one of the most promising techniques for the treatment of small breast cancers, with a high but variable success rate of complete ablation ranging from 77 to 100%.<sup>1.5</sup>

During an RFA, a needle is inserted into the middle of the tumor to induce tissue electrocoagulation, creating an ellipsoid region of necrotic tissue that aligns with the tip of the needle electrode.<sup>5,6</sup> Since Jeffrey et al. first introduced RFA for breast cancer treatment in 1999<sup>7</sup>, several electrodes have been used. Analysis of the tumor specimen after an RFA can be used to determine the diameter of the ablated lesion including the tumor with adequate margins.

A previous study reported that complete ablation with RFA was reached in 87% of the cases reviewed<sup>2</sup>, although it was unclear whether complete tumor necrosis was achieved as it is not easy to determine cell death after treatment.<sup>2,4,5</sup> In fact, one of the major challenges described in the literature involves the determination of tumor cell death after RFAs, especially in the periphery of the ablated tumor area.<sup>8,9</sup>

In general, cell damage is evaluated by a combination of Hematoxylin and Eosin (H&E) staining and Nicotinamide adenine dinucleotide (NADH) staining, which requires frozen material and poses technical difficulties. Although several studies have shown that evaluation of the therapeutic effects using NADH-diaphorase staining is useful, evaluation of therapeutic effects by RFA would become more reproducible and accurate if evaluations were effectively done using HE-stained sections of formalin-fixed, paraffin-embedded tissues.<sup>10-14</sup> Alternatively, Cytokeratins 8 and 18 (CK8/CK18) immunostaining can be used in paraffin-embedded sections, which are probably easier to standardize.

Some studies have shown that the CK8<sup>4,18</sup> and CK18<sup>15,16</sup> immunostaining of paraffin-embedded sections is comparable to the NADH-diaphorase staining of frozen sections in assessing cell viability, with fewer technical and cost requirements. Thus, CK8/18 could be surrogate markers of tumor cell viability in breast carcinomas subjected to RFAs since they are cleaved at an early step in cell death. Additionally, Cytokeratin 19 (CK19) is a type I keratin that has been described to be cleaved in cell death.

The aim of this study was to evaluate different staining techniques assessing cell death after ultrasound (US)-guided percutaneous radiofrequency ablation (RFA) in the treatment of invasive breast carcinoma.

#### Methods

An ex vivo study was first performed to determine

the optimal methodology to acquire an adequate lesion diameter and avoid adverse effects. This was followed by an *in vivo* experimental study.

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The study was approved by the Institutional Review Board (ethical committee) of our hospital (reference AC133/12), and the procedures were performed according to the ethical standards of the World Medical Association (Declaration of Helsinki). A prospective trial was conducted at a Health Insurance Portability and Accountability Act compliant single site. Institutional Review Board approval was obtained, and all participants signed informed consent. The trial was registered at ClinicalTrials.gov with the identifier number NCT02281812.

#### Ex vivo study

A preliminary RFA was performed on two mastectomy specimens to test the electrode, practice the US technique and evaluate the macroscopic and microscopic effects of the RFA. Under US guidance, electrodes were inserted through the tumour and RF was applied for less than 5 minutes.

#### In vivo study

The prospective study was conducted at the Multidisciplinary Breast Cancer Unit of Hospital Bellvitge-Institut Catala d'Oncologia. Twenty patients with T1 invasive ductal carcinoma of the breast were treated with RFA *in vivo* followed by immediate surgery from September 2013 to February 2017. These patients had been previously reported<sup>19</sup>, publishing the safety and efficacy of the US-guided percutaneous RFA as a local treatment for breast cancer with a reduction in intraoperative margin involvement compared with standard surgical treatment. The present study describes the technical aspects of US-guided percutaneous RFA and evaluates different staining techniques to assess cell death after RFA.

The inclusion criteria were: (1) women, (2) aged> 40 years, (3) invasive ductal carcinoma (not otherwise specified (NOS)) of the breast diagnosed by core needle biopsy, (4) intraductal component less than 20% of the tumor, (5) tumor size  $\leq 20$  mm, (6) tumor clearly visible by ultrasound and (7) tumor located at least 10 mm from the skin surface and chest wall. The exclusion criteria were: (1) men, (2) aged < 40 years, (3) pregnancy or breast-feeding women, (4) radiological suspicion of multifocal breast cancer, (5) extensive intraductal carcinoma, (6) lobular carcinoma, (7) neoadjuvant therapy and (8) prior surgery or radiotherapy of the ipsilateral breast. Subjects that met all the inclusion criteria and none of the exclusion criteria were included in the study.

Tumour size was measured by US and categorized into two groups:  $< \text{or} \ge 15 \text{ mm}$ .

A Cool-tip<sup>™</sup> RF ablation cluster system (Covidien, Tyco Healthcare Group LP, Boulder, CO 80301-3299 USA) was used. This system consists of three 17-gauge straight electrodes, with internally circulating chilled water designed to cool the tissue adjacent to the electrode, limiting tissue charring and maximizing energy deposition.

The *in vivo* procedure was performed in the operating room, under general anaesthesia and sterile conditions. Real-time US was performed using the Flex Focus 400 Ultrasound Machine (BK Ultrasound, BK Medical UK Ltd.). The transducer was covered with a sterile sheath.

Prior to the RFA, at least three additional fresh core needle biopsy specimens from the tumor were obtained for future pathological examination.

All Us-guided RFA procedures were performed by two radiologists with 23 and 11 years of experience in breast cancer diagnosis and interventional radiology (AG, AV). The three needle electrodes were guided by US, as parallel to the chest wall as possible. Proper positioning of the 3 electrodes was confirmed in three dimensions to ensure that the expected volume of the thermal lesion was concentric and encompassing the tumour. The tip of the electrodes was placed outside the tumour approximately 5mm from the edge of the tumour. After checking that there was enough safe space between the needle and the skin and the chest wall, RFA was performed for 8 to 10 minutes, using intermittent US monitoring. Two sterile ice packs were placed on the skin overlying the lesion to prevent skin burns during the procedure. The breast was lifted to maximize the distance between the skin and the chest wall. After ablation, the track of the electrode through the tissue was specifically ablated and slowly retracted (track ablation mode) to prevent tumor seeding and to achieve complete hemostasis. The maximum local temperature inside the tumor after the RFA was also recorded and grouped depending on whether or not 70°C was reached.

After RFA, lumpectomy was immediately performed (by A.G.T. and E.F.M., with 19 and 32 years of experience, respectively) with or without the axillary approach according to the National Comprehensive Cancer Network guidelines.<sup>20</sup>

#### Pathological evaluation

After lumpectomy the breast specimen was sent to the pathology department. After weighing and inking, the specimen was serially sectioned at 4-mm intervals. The tumour and the ablated area were identified and measured.

One frozen tumour tissue sample of a representative area of the tumour was selected and placed in optimal cutting temperature compound embedding medium, snap frozen in liquid nitrogen and stored at -80°C. Depending on the size of the tumour, 3 to 6 sections were obtained from representative areas of the tumour, adjacent breast tissue and margins and fixed in 10% neutral buffered formalin for 18-72 hours, embedded in paraffin and sectioned.

NADH staining (ref. 107727, Roche) was performed on 8-um frozen sections. This staining causes an oxidation reaction in the cytoplasm of viable cells, producing a dark blue stain, with nonviable cells appearing pale gray in color.<sup>14</sup> COX, also referred to as complex IV, is a component of the electron transport chain in the mitochondria, and is involved in the initiation of apoptosis. SDH is the first enzyme in the succinate oxidase chain, which ends in respiratory reactions. Like COX, it is also a marker of mitochondrial activity. Therefore, the histochemical detection of both enzymes reflects cell viability. For COX staining, cells with normal mitochondrial function appear brown, while those with low activity appear blue, and for SDH staining, cells with normal mitochondrial function appear blue, while those with low activity appear pale gray.<sup>21</sup>

H&E-stained slides were prepared from all the blocks submitted and the tissue block containing the most optimally ablated tissue was selected for CK18 (Ref. GAG18, clone DC10. Agilent-DAKO, Dako Omnis) and CK19 (Ref. GA561, clone RCK108. Agilent-DAKO, Dako Omnis) immunostaining. Cytokeratins are intermediate filament keratins found in the intracytoplasmic cytoskeleton of epithelial tissues. CK18 and CK19 are markers for epithelial tumors and apoptosis.<sup>16,17</sup>

TUNEL assays (Apoptag Plus Peroxidase In situ Apoptosis Kit. Catalogue number S7101. Sigma-Aldrich) are used for the *in situ* detection of DNA degradation in apoptotic and necrotic cells. The labeled cells are visualized on a fluorescent microscope as those with heterogeneously stained (green) nuclei.<sup>22</sup>

NADH-diaphorase, SDH and COX activities were scored as diffusely positive if > 50% of the tumor cells showed positive staining, focally positive if less than 50% of the tumor cells showed staining, and negative if there was no staining.

CK18 and CK19 were categorized into five groups according to the percentage of tumor cells that showed a positive staining for them, as follows: negative: less than 10%, 10%–25%, 26%–50%; positive: 51%–75%, and 76%–100%. The final cutoff to consider a complete RFA by cytokeratin staining was below 25%. The TUNEL staining results were categorized as either negative or positive when death cells were detected.

For the control tests, all the staining techniques were also performed on the non-ablated tissue obtained from the core needle biopsies just before the RFA procedure.

The effects of the RFA and cell viability were assessed by three pathologists (T.S., A.P. and C.C., with 35, 13 years of experience and "in training" respectively).

Statistical analysis Principal component analysis (PCA) for

categorical data was performed as an exploratory analysis to reduce the original set of variables (stains) into a smaller set of uncorrelated components that represented and also summed up the information found in the original variables. PCA is useful in highlighting strong patterns from complex biological datasets. By reducing the dimensionality, a few components rather than several variables are interpreted, and similarities and differences among the variables (e.g., stains) become apparent. We chose the optimal-scaling approach in PCA because it allows variables to be scaled at different levels (e.g., CK18 staining was scaled in five grades and SDH staining in four grades). As a result, nonlinear relationships between the variables can be modeled at different scales. A PCA loading biplot was obtained, on which both the PCA scores of the samples (cases) and loadings of the variables (vectors) were projected. The further away these vectors are from

the origin, the more influence the variable has on the PCA. Loading vectors also indicate how variables correlate with one another: a small angle indicates a positive correlation, while a large angle (e.g., 180°) suggests a negative correlation. The overall internal consistency of the staining methods was assessed using Cronbach's alpha.<sup>23</sup> Categorical variables are described as the number of cases and proportions. Statistical analyses were performed using the SPSS package version 23.0.

#### Results

From September 2013 to February 2017, a total of 20 patients treated for invasive ductal carcinoma of the breast were recruited for this study (Figure 1). The mean size of the tumors was 11 mm, 15 tumors measuring < 15 mm, and 5 tumors  $\geq$  15 mm. The overall mean age of the patient sample was 64 years (46–86 years).

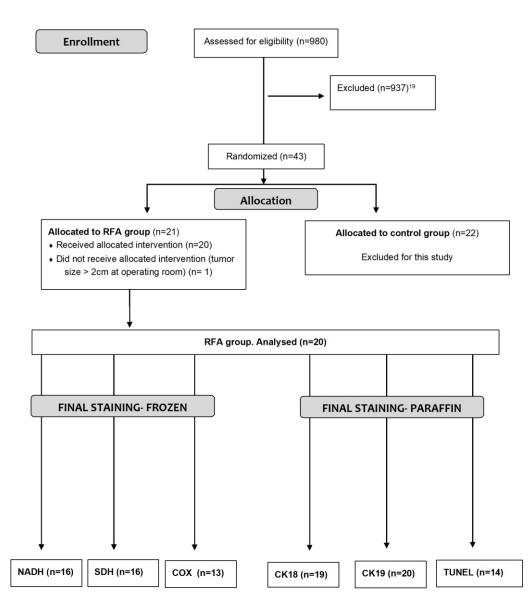
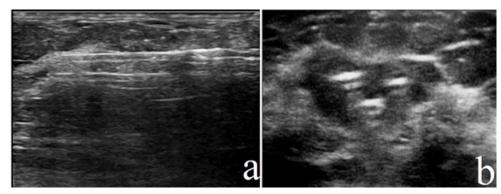


Figure 1. Flow chart shows participants in RFA



**Figure 2.** Ultrasound images, transverse (a) and coronal (b) planes with confirmation in three dimensions of correct placement of the 3 electrodes.

Correct placement of the electrodes inside the tumor was achieved in all the cases (Figure 2 a,b). During the ablation treatment, real-time US examination showed that the target lesion and the surrounding tissues became an area of echogenic foci due to tissue heating and microbubble formation. There were no burns to the skin or chest wall.

On gross examination, the ablated area was observed as an ellipsoid central area of necrotic white tumor tissue with central charring and the needle tracks inside. This was surrounded by nontumor fibro-adipose tissue that looked firm and had changed to a yellowish tan colour. This area was well delineated by a demarcated hyperaemic rim, which represented viable breast tissue (Figure 3 a,b). The median largest ablation diameter was 35 mm (range, 25–60 mm), while the median smallest ablation diameter was 30 mm (range, 20–35 mm).

The criteria for the H&E evaluation of the effects of RFAs on breast cancer have been previously described.<sup>9</sup> In our series, microscopic changes observed with H&E staining of the frozen and fixed section were similar to the ones reported previously with thermal effects in all the lesions: tissue shrinkage, hyperchromatic neoplastic cells with eosinophilic cytoplasm and pyknotic "streaming" nuclei, and secondary artefactual changes, with degenerative changes in the fibrous connective tissue.

Tumor and the adjacent non-tumoral tissue in the

ablated area exhibited the same behaviour regarding morphology on H&E. Stainings with NADH, COX, SDH, CK18 and CK19 were similar in tumor and adjacent non-tumoral tissue whenever non-tumoral tissue was represented in the slide analyzed. However, non-tumoral tissue located in the periphery of the ablated area did not present any change on H&E and showed a preserved histology. All margins of the tumours were adequately ablated.

Since cell death due to thermal effects is timedependent, H&E staining alone cannot be used to evaluate cell death in specimens obtained immediately after an RFA, as with this study. Thus, it is better to assess degenerative changes and cell death a few months after an RFA.<sup>14,24</sup>

Figures 3, 4, 5 and 6 show the microscopic features of the biopsies and ablated tumors treated with the different staining techniques. Frozen sections could not be obtained in 4 of the 20 cases due to technical problems. Thus, NADH and SDH stainings were assessed in 16 of the 20 cases before and after the RFA. All tumors were positive for SDH and NADH before the RFA. After the RFA, however, SDH and NADH positivity was lost in 13 of the 16 tumors (81%). In the three tumors where SDH and NADH positivity was maintained (19%), two were focal and one diffuse. The focal staining occurred around the needle when the track ablation was not feasible. COX staining could only be performed in 13 cases, but their results were comparable with those of NADH staining.

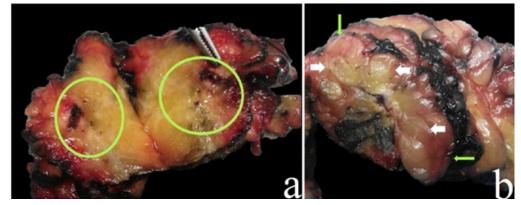


Figure 3. Macroscopic appearance of breast specimen after ablation: central charring of the needle track through the center of the tumor (a) with a surrounding area of yellow, coagulated, adipose and breast tissue (white arrows in b). Red fat is seen in the area outside the zone of thermal destruction (green arrows).

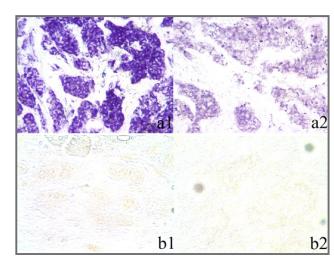


Figure 4. Comparison between NADH and SDH on frozen sections pre (a) and post-radiofrequency (b) (all images, 400x). Pre-radiofrequency diffuse positivity for NADH (a1) and SDH (a2) demonstrates presence of enzymatic activity. Post-radiofrequency negativity for NADH (b1) and SDH (b2) supports absence of viable cells.

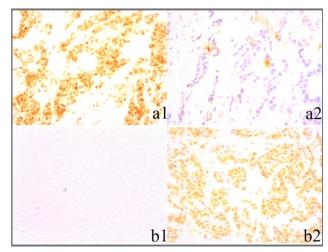


Figure 5. Comparison between COX on frozen sections and TUNEL in paraffine pre (a) and post-radiofrequency (b) (all images, 400x). Pre-radiofrequency section shows diffuse positivity for COX (a1), and absent Tunnel staining (viable) (a2). Post-radiofrequency COX negativity with absence of viable cells (b1), and Tunnel staining with positive nuclear staining in post-ablated tumor (non-viable) (b2)

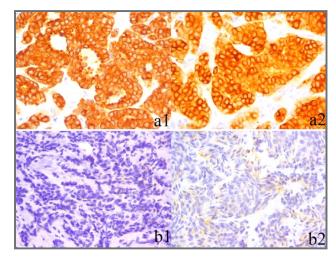
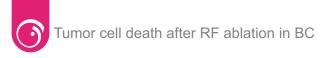


Figure 6. Microscopic features comparing previous biopsy with ablated tumour with Cytokeratin 18 and Cytokeratin 19 pre (a) and after radiofrequency (b) (all images, 400x). Pre-radiofrequency the cells show diffuse immunostaining for Cytokeratin 18 (a1) and 19 (a2).Conversely, post-radiofrequency cytokeratin 18 (b1) and 19 (b2) expression is reduced with focal positive cells highlighted with cytokeratin 18 and complete loss with cytokeratin 19.



Considering the technical difficulties involved in processing frozen tissues, we tried to verify cell death with markers evaluated in paraffin samples. Diffuse CK18 and CK19 staining was found in 19 and 20 tumors, respectively, before the RFA. After the RFA, one case showed CK18 stain between 25.50% of the cells, 6 cases between 10-25%, below 10% in 5 cases and negative in 8. In any case, all tumors showed a lower percentage of positive cells (less than 50%), except one that was still positive for CK18. The TUNEL technique revealed damaged cells in 78% of the tumors assessed, while COX staining indicated a loss of normal mitochondrial function in 84% of the cases studied.

The reliability and internal consistency between the different staining techniques was high, with a Cronbach's alpha of 0.8 (Figure7). TUNEL staining correlated negatively with the detection of mitochondrial enzymes (COX, NADH and SDH), while the staining results of the mitochondrial enzymes and cytokeratins showed a clear but low internal correlation.

The RFA samples (all of the 20 cases; 100%) showed a complete absence of staining of at least one of the following three: NADH, CK18 or CK19. The results are summarized in Table 1. However, there were 3 cases where > 50% of the tumor cells showed mitochondrial enzyme activity after the RFA. These 3 cases were from patients with tumors measuring > 15mm, in whom the maximum temperature achieved at the center of the tumor after the RFA was  $\leq$  70°C. Mitochondrial enzyme activity disappeared in the tumors where the temperature after the RFA was > 70°C (p < 0.001).

#### Discussion

US-guided percutaneous RFA of small invasive breast carcinomas was a feasible and safe technique, as we have demonstrated previously.<sup>19</sup> NADH staining is the standard procedure used for assessing tissue viability following RFA. However, it requires frozen tissue, which is not always available in routine practice, and its accuracy is questionable, especially in the margins of the ablated area, where abundant fatty tissue can make it difficult to obtain a frozen section.<sup>14, 15, 23, 25</sup> NADH staining was completely absent in 81% of our cases, similar to previous findings. Other studies have found viable invasive tumor cells in around 15-21% of RFA cases at the margins of the tumor or lining the needle track.<sup>12, 18, 26</sup> By contrast, one study did not find viable tumor tissues in any of the 23 RFA cases evaluated with NADH staining, both immediately after the RFA and a period after the procedure.<sup>27</sup>

COX and SDH staining are techniques described in RFAs in hepatic tissues.<sup>28,29</sup> In our series, SDH and COX staining was absent in 81% and 84% of the cases, respectively, which was in perfect concordance with the results of NADH staining.

The TUNEL technique performed on paraffin sections has been described for RFAs in skin tissues<sup>30</sup> and hepatocellular carcinoma.<sup>27</sup> In our series, cell death was confirmed by the TUNEL technique in 11 of the 12 cases studied, whose results were also consistent with those of the other staining techniques.

In this study, CK19 showed absence or reduction of expression in all tumors after the RFA, while CK18 in all tumors except one. CK18 and CK19

		1	0	0								
Patient	NADH	NADH	SDH	SDH	COX	COX	Ck18	Ck18	Ck19	Ck19	TUNNEL	TUNNEL
	pre	post	pre	post	pre	post	pre	post	pre	post	pre	post
1	2	0	2	0	2	0	4	2	4	0	1	0
2	2	0	2	0	2	0	4	2	4	2	1	0
3	2	0	2	0	2	0	4	0	4	0	1	0
4	2	0	2	0	2	0	0	0	0	0	1	0
5	N/A	N/A	N/A	N/A	N/A	N/A	4	1	4	0	1	0
6	N/A	N/A	N/A	N/A	N/A	N/A	4	0	4	0	0	0
7	N/A	N/A	N/A	N/A	N/A	N/A	4	0	4	0	1	0
8	2	0	2	0	N/A	N/A	4	0	4	0	1	0
9	2	1	2	1	2	1	4	1	4	1	1	0
10	2	2	2	2	2	1	4	2	4	2	1	0
11	2	1	2	1	1	0	4	3	4	2	1	1
12	2	0	2	0	2	0	4	0	4	0	1	0
13	2	0	1	0	N/A	N/A	4	2	4	2	1	1
14	2	0	2	0	N/A	N/A	4	1	4	0	1	0
15	2	0	2	0	2	0	4	1	4	1	N/A	N/A
16	2	0	2	0	2	0	4	2	4	2	N/A	N/A
17	2	0	2	0	2	0	4	0	4	0	N/A	N/A
18	2	0	2	0	2	0	4	2	4	2	N/A	N/A
19	N/A	N/A	N/A	N/A	N/A	N/A	4	1	4	1	N/A	N/A
20	2	0	2	0	2	0	4	0	4	1	N/A	N/A

Table 1. Results of pathologic stainings

N/A not available

NADH-diaphorase, SDH and COX: 2: >50% diffusely positive; 1: <50% focally positive, 0: negative

CK18 and CK19: 0: less than 10%, 1: 10%–25%, 2: 26%–50%, 3: 51%–75%, 4: 76%–100%.

Tunnel: 0: negative, 1: positive.

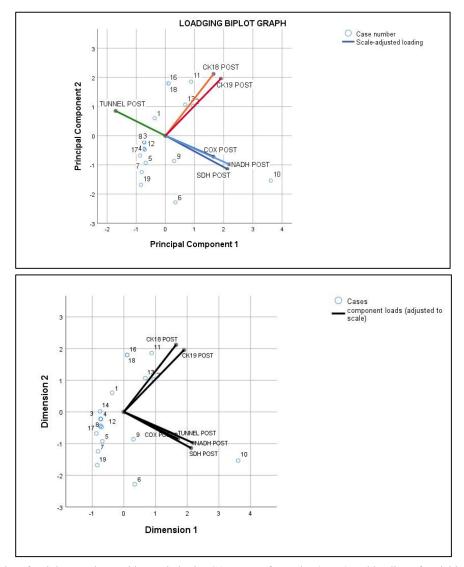
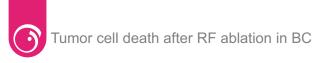


Figure 7. Biplot of staining results: In this graph, both PCA scores of samples (cases) and loading of variables (vectors) are pictured. The further away these vectors are from the origin, the more influence the variable has on PCA. Loading vectors also hint at how variables correlate with one another: a small angle implies positive correlation; a large angle (eg. 180°) suggests negative correlation. Note (a) the negative correlation (180° angle between vectors) between TUNEL staining (which stains apoptosis) and Cox, NADH and SDH (which assess mitochondrial activity). Note also the perfect correlation among mitochondrial enzymes themselves and also along cytokeratins themselves (b). Howerver, correlation between cytokeratins and the rest of enzymes is weak likely because more than 50% of the cases presented residual staining.

staining could be performed in all the cases, except for one in the case of CK18 staining, which was negative in the control sample. It should be noted that approximately 5% to 15% of breast carcinomas may not express CK18/19. Thus, core needle biopsies should be analyzed to confirm the presence of cytokeratins before ablation.<sup>15</sup> Therefore, correlation of cytokeratin staining results with those of other markers of viability is necessary.

The reliability and internal consistency between the staining techniques was high, with the NADH-SDH-COX staining results correlating strongly with the TUNEL results and the CK18 staining results correlating clearly with those of CK19. However, due to high sensitivity in detecting residual cytokeratin activity, there is no strong correlation between mitochondrial enzyme and cytokeratin staining techniques. That is why the cut-off to consider complete ablation was different between mitochondrial enzyme and cytokeratin staining; therefore, the final result could be considered acceptable with both techniques. It is likely that the residual staining of cytokeratines after inmediate death may account for this different behaviour with mitochondrial enzymes. In any case, CK18 and CK19 staining techniques are simple, reproducible, and show good concordance. Thus, they can be used as markers of cell viability. As these techniques can be performed on fixed sections, CK18 and CK19 assessment is simpler and more economical than those using frozen sections. Provided that there is good concordance between a loss of enzymatic activity and the loss of CK18 and CK19 expression, CK18 and CK19 could be considered surrogate



markers of cell viability.

To our knowledge, this study is the first to assess COX, SDH, TUNEL and CK19 staining in the evaluation of cell viability after a RFA in breast cancer, allowing us to compare the techniques and the causes of persistent tumor cell viability. The limitations of this study were the small number of cases and the surgical excision being performed immediately after the RFA that could have led to an underestimation of tumor necrosis.

We observed an absence of cellular viability in at least one of the markers in all our cases, although the possibility of viable cells remaining in the tumor after an RFA cannot be completely ruled out. The specificity of any of the staining techniques in ruling out any remaining non-necrotic tumor tissue was high after the complete inclusion of the resection specimen, although we did perform the assessments immediately after the RFA.

In conclusion, US-guided percutaneous RFA of small invasive breast carcinoma is a feasible and safe technique that could be an option for less invasive treatments. Staining techniques assessing cell viability revealed decreased NADH, COX and SDH staining in frozen sections and positive TUNEL results. CK18 and CK19 staining results in paraffinembedded sections showed an excellent correlation with those of the other markers studied in frozen sections, becoming negative after the RFA. Thus, CK18 and CK19 immunohistochemistry can be used to assess RFA efficacy in paraffin-embedded tissues, which is simpler and more economical than the methods using frozen tissues.

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#### **Conflicts of Interest**

The authors declare that they have no conflict of interests.

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## Association of Coping Strategies with Death Anxiety through the Mediating Role of Disease Perception in Patients with Breast Cancer: A Cross-Sectional Study

Kefayat Salmanian<sup>a</sup>, Fatemeh Sadat Marashian<sup>\*a</sup>

<sup>a</sup> Department of Psychology, Ahvaz Branch, Islamic Azad University, Ahvaz, Iran

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#### ABSTRACT

**Background:** Understanding disease symptoms is among the important psychological and behavioral variables in cancer treatment. The present study aimed to investigate the relationship between coping strategies and death anxiety through the mediation of disease perception in patients with breast cancer in Abadan in 2020.

**Methods:** The study was a descriptive correlation performed by path analysis. The statistical population included all patients with breast cancer in Abadan in 2020 who referred to medical centers for six months. A total of 200 of them were selected as the sample of the study using convenience sampling. Research instruments included the Death Anxiety Scale (DAS), the Ways of Coping Questionnaire (WCQ), and the Brief Illness Perception Questionnaire (Brief IPQ). The proposed model was evaluated using path analysis with AMOS software.

**Results:** The results showed that there was a significant association between emotion-focused strategy and disease perception ( $\beta$ = 0.67, P= 0.002), problemfocused strategy and disease perception ( $\beta$ = -0.08, P= 0.001), disease perception and death anxiety ( $\beta$ = 0.39, P= 0.001), and between emotion-focused strategy and death anxiety ( $\beta$ = -0.26, P= 0.001). There was no significant association between problem-focused strategy and death anxiety. The results of path analysis indicated that disease perception played a mediating role in the association between the emotion- and problem-focused strategies with death anxiety (P=0.01).

**Conclusion:** According to the results of the study, the relationship between coping strategies and death anxiety through the mediation of disease perception had a good fit and can be an important step in identifying the factors affecting the death anxiety of patients with breast cancer.

Key words: Coping strategies, death anxiety, perception of disease, breast cancer, women

#### Introduction

Breast cancer is the most prevalent and the leading cause of death among women worldwide.<sup>1</sup> The experience following a diagnosis of cancer is a far worse and unbelievable experience than with other diseases by disrupting the occupational, socioeconomic, and family life of the patients.<sup>2</sup> Anxiety from fear of

\* Address for correspondence: Fatemeh Sadat Marashian, Ph.D. Address: Islamic Azad University of Ahvaz Science and Research Branch, Farhang Shahr, Ahvaz, Khuzestan, Iran Email: <u>fsadatmarashian@gmail.com</u> death is an effective factor in mental health in patients with cancer.<sup>3</sup> Patients may experience physical symptoms during medical or surgical interventions. Anxiety and distress exacerbate in patients with increased symptoms caused by anxiety from fear of death.<sup>4</sup> The annoying existential concerns that come with death anxiety in patients at an advanced level of cancer, the interaction between physical symptoms, and concerns about the family, age, and self-esteem increase the degree of death anxiety.<sup>5</sup> Death anxiety can result in severe fear and neuroticism which, in turn, negatively affect patients' capabilities and control.<sup>6</sup> Due to its highly ambiguous nature, death is perceived as a threat to many people, particularly in patients diagnosed with serious problems, such as cancer.<sup>7</sup> Lehto and Stein showed that death anxiety is an effective factor in mental health and a significant psychological diagnosis in patients with cancer.<sup>8</sup>

Given the ambiguous, uncontrollable, and immutable nature of death, anxiety is generally related to coping strategies.9 Stress coping strategies refer to continuous changes in behavioral and cognitive efforts of an individual in regulating internal and external desires, which are assessed beyond his/her level of tolerance.<sup>10</sup> Lazarus and Folkman identified two stress-coping methods: 1problem-focused coping that assesses direct activities based on self and environment to eliminate or change threatening conditions, 2- emotionfocused coping that includes activities or thoughts used to control undesired emotions caused from stressful conditions.<sup>11</sup> When a patient struggles with cancer, he/she has to deal with many challenges and goals, which can be medical, physical, emotional, interpersonal, and spiritual.

Understanding the disease symptoms is among the important psychological and behavioral variables in cancer treatment, which is based on the acquisition of information from different sources and patient's beliefs and can affect the mental health and capability of the individual in coming to terms with the disease. Awareness of the disease includes information in five dimensions: identity refers to disease labels and symptoms (e.g., fatigue and weakness), causes of or beliefs about the disease, perceived duration of the disease by the notions of acuity and chronicity; expected disease outcomes and results by economic, social, psychological, and physical effects and effectiveness of control, treatment, and improvement.<sup>12</sup>

Farahbakhsh Beh et al. showed a significant negative correlation between the cognition of disease and social support, and between resilience and death anxiety.<sup>13</sup> Sharifi Saki et al. reported a significant relationship between the properties of psychosis and disease awareness.<sup>14</sup> Bibi and Khalid showed that there is a significant negative relationship between death anxiety and social support, with the latter contributing to a lower level of death anxiety and an increase in the recovery period.<sup>15</sup> Mikulincer and Florian showed that there is a significant relationship between emotion- and problem-focused coping strategies and death anxiety.<sup>16</sup> Monirpour et al. reported that social support and coping strategies could, respectively, predict 16% and 32% of treatment compliance of patients with cardiovascular diseases.<sup>17</sup> Soleimani et al. reported that there is a significant relationship between emotion-focused coping strategy, problem-focused coping strategy, and avoidance strategy and disease perception. They concluded that coping strategies with stress are effective in the perception of the disease in mothers

with children with type 1 diabetes, with better perception of the child's disease leading to the use of more appropriate coping strategies.<sup>18</sup> Hashemi Razini et al. reported that there is a significant correlation between coping strategies and source of control and death anxiety in the elderly.<sup>19</sup>

There is scant Iranian research on the relationship between coping strategies and disease awareness with death anxiety among cancer patients. Moreover, none of these studies investigated the relationship between coping strategies and disease awareness and death anxiety in a patient with breast cancer. Accordingly, the present study sought to investigate the association between coping strategies with death anxiety according to the moderating role of disease perception in patients with breast cancer.

#### Methods

The study was a descriptive correlation performed by path analysis. The statistical population included all patients with breast cancer in Abadan, Iran, in 2020 who referred to medical centers for six months. A total of 200 of them were selected as the sample of the study using convenience sampling. Patients were introduced to the researcher after examination by a specialist physician and receiving a diagnosis of breast cancer. The inclusion criteria included having breast cancer diagnosed by a specialist doctor, age range between 30-50 years, having at least a middle school education, and not being under treatment for a mental health condition. The exclusion criteria included failure to completely answer all the questions. For ethical considerations, the researcher obtained written consent from the participants for participation in the study. The research objectives were explained to the patients and were told they could stop participating in the study at any time. The study was approved by the Ethical Committee of Abadan University of Medical Sciences (code: IR.ABADANUMS.REC.1399.103).

#### Research Instruments

#### 1. The Death Anxiety Scale (DAS)

This scale was developed and validated by Templer in 1970. It is a self-executive questionnaire comprised of 15 correct-incorrect items. The total score of the questionnaire is in the range of 0 and 15, where the higher score indicates a higher degree of anxiety. In the present study, the Persian version of this scale was used.<sup>20</sup> Sharif Nia et al. reported the reliability of this scale at 0.83 based on Cronbach's alpha coefficient. In the present study, Cronbach's alpha coefficient was 0.88 for the scale.<sup>20</sup>

#### 2. Ways of Coping Questionnaire (WCQ)

The WOCQ was developed by Lazaros and Folkman in 1980 and revised in 1985. This 66-item



questionnaire was developed based on cognitivephenomenological theories on tension, estimation, and coping. Moreover, this questionnaire had two major emotion- and problem-focused subscales. Sixteen items of this questionnaire are deviatory and 50 items evaluate the coping style. The WOCQ scoring is done through either raw or relative methods. Raw scores describe the coping effort for each of the eight types of coping styles and are the sum of responses to the items. The relative scores describe the ratio of effort made in each type of coping. The present study used the relative scoring method. In both methods, individuals respond to each item on a 4point Likert scale, which shows the frequency of each strategy as follows: 0 "Does not apply or not used," 1 "Used somewhat," 2" Used quite a bit," and "Used a great deal.".<sup>21</sup> In the present study, the Persian version of this questionnaire was used.<sup>22</sup>Kordi et al. reported a Cronbach's alpha of 0.97 for the questionnaire. Cronbach's alpha coefficient was found to be 0.87 for the questionnaire in our study.<sup>22</sup>

# 3. The Brief Illness Perception Questionnaire (BriefIPQ)

Brief IPQ is a 9-item questionnaire designed to rapidly assess cognitive and emotional representations of illness.<sup>23</sup> The Brief IPQ uses a single-item scale approach to assess perception on a 0–10 response scale. It is developed by forming one question that best summarizes the items contained in each subscale of the Illness Perception Questionnaire-Revised which has over 80 items. The Brief IBQ comprises 5 items on cognitive representation of illness perception: consequences, timeline, personal control, treatment control, and identity. There are 2 items on emotional representation: concern and emotions. One item is related to illness comprehensibility. The last item is

concerned with perceived cause of illness, in which respondents list the three most important causal factors in their illness. For this questionnaire, the general word 'illness' can be replaced by the name of a particular illness such as asthma. The word 'treatment' in the treatment control item can be replaced by a particular treatment such as 'surgery' or 'physiotherapy. In the present study, the Persian version of this questionnaire was used.24 Masaeli et al. reported the reliability of this questionnaire equal to 0.73 based on Cronbach's alpha coefficient. In the present study, Cronbach's alpha coefficient was 0.82 for the questionnaire.<sup>24</sup>

#### Statistical analyses

Data were analyzed by descriptive and inferential statistics such as mean, standard deviation, and Pearson correlation coefficient. To determine the significance of mediating-based relations, the bootstrap method was utilized. The path analysis was used to assess the proposed model. SPSS and AMOS were used for analyzing the data.

#### Results

The participants included 200 women with breast cancer. The demographic variables of the participants are shown in Table 1. Descriptive statistics including mean and standard deviation (SD) and Pearson correlation coefficient of study variables are presented in Table 2.

According to the data in Table 3, the root means square error of approximation (RMSEA=0.28; TLI= 0.535; AGFI= 0.724) showed that the initial model required modification. To this end, the nonsignificant relationship between problem-focused strategy and death anxiety was removed. Figure 1 shows the final model in which the root means square

Demographic variables		Ν	%
	30-35	32	19.00
Age (years)	35-40	67	33.50
0 0 0	40-45	59	29.50
	45-50	42	21.00
	Housewife	161	80.50
Employment status	Employed	39	19.50
	Single	31	15.50
Marital status	Married	169	84.50
	Middle school	96	48.00
Education	High school	74	37.00
	College education	30	15.00

Table 2. Mean, standard deviation (SD), and Pearson correlation coefficients of the study	
variables	

variables	М	SD	1	2	3	4
1. Emotion-focused strategy	44.39	5.25	1			
2. Problem-focused strategy	46.61	3.55	-0.39*	1		
3. Disease perception	74.64	12.79	0.87*	-0.55*	1	1
4. Death anxiety	9.25	2.28	0.55*	-0.25*	0.75*	1

M: Mean; SD: Standard deviation; \*: p < 0.05

Fit indicators	χ2	df	(χ2/df)	GFI	AGFI	IFI	TLI	CFI	NFI	RMSEA
Initial model	12.03	1	12.03	0.902	0.724	0.913	0.535	0.910	0.913	0.28
Final model	2.44	1	2.44	0.993	0.902	0.997	0.970	0.997	0.996	0.06

#### Table 3. Initial and final model fit indicators

GFI: Goodness of Fit Index; AGFI: Adjusted Goodness of Fit Index; IFI: Incremental Fit Index; TLI: Tucker Lewis Index; CFI: Comparative Fit Index; NFI: Normalized Fit Index; RMSEA: Root Mean Square Error of Approximation

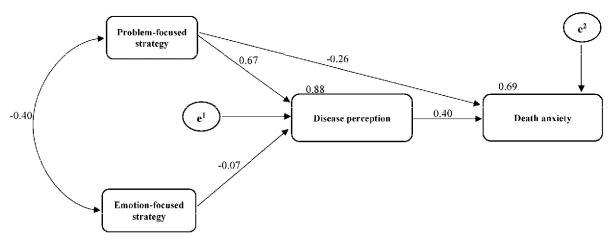


Figure 1. Final modified model

error of approximation (RMSEA= 0.06;  $\chi^2/df$ = 2.44; CFI= 0.997; GFI= 0.993) indicated a good model fit.

Based on the data in Table 4 there was no significant direct correlation between problemfocused strategy and death anxiety ( $\beta$ = -0.09, P= 0.06). The correlation between emotion-focused strategy and disease perception was positive and significant ( $\beta$ = 0.67, P= 0.002). Moreover, there was a negative and significant correlation between problem-focused strategy and disease perception among the patients with breast cancer ( $\beta$ = -0.08, P= 0.001). We also observed a direct and significant correlation between disease perception and death anxiety ( $\beta$ = 0.39, P= 0.001). The correlation between emotion-focused strategy and death anxiety was negative and significant ( $\beta$ = -0.26, P= 0.001). The indirect path from emotion-focused strategy to death anxiety through the mediating role of disease perception was significant ( $\beta$ = 0.408, P= 0.01). Moreover, the indirect path from problemfocused strategy to death anxiety through the mediating role of disease perception was significant ( $\beta$ =-0.607, P=0.02) (Table 5).

#### Discussion

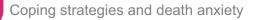
The present study aimed to investigate the relationship between coping strategies and death anxiety through the mediation of disease perception in patients with breast cancer in Abadan. The emotion- and problem-focused strategies had a direct effect on disease awareness in patients with breast cancer. The emotion-focused strategy directly

Table 4. Path coefficients of direct associations between research variables in the initial and final model.
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Path		Initial mode	1	Final model		
	Path type	β	Р	Path type	β	Р
Emotion-focused strategy to disease perception	Direct	0.67	0.001	Direct	0.67	0.002
Problem-focused strategy to disease perception	Direct	-0.08	0.02	Direct	-0.08	0.001
Disease perception to death anxiety	Direct	0.42	0.001	Direct	0.39	0.001
Emotion-focused strategy to death anxiety	Direct	-0.27	0.001	Direct	-0.26	0.001
Problem-focused strategy to death anxiety	Direct	-0.09	0.06	Direct	-	-

Table 5. Results of the Bootstra	n method for inve	estigating indirect	and intermediary naths
Table 5. Results of the Dootstid	p memou for miv	sugaring maneer	and internetitionally pains

Predictor variable	Mediator Variable	Criterion variable	Final model			
			Bootstrap	Lower limit	Upper limit	Р
Emotion-focused strategy Problem-focused strategy	Disease perception Disease perception	Death anxiety Death anxiety	0.408 -0.607	0.352 -0.123	0.457 -0.021	0.01 0.02



affected death anxiety in patients with breast cancer. Moreover, disease perception had a direct effect on death anxiety in patients with breast cancer. This finding is consistent with the results of Hashemi Razini et al.<sup>19</sup> and Mikulincer and Florian.<sup>16</sup> Hashemi Razini et al. showed that there was a significant relationship between coping strategies and locus of control with death anxiety among older adults. They also reported that avoidance and emotion-oriented coping and external locus of control significantly could predict death anxiety.<sup>19</sup> Mikulincer and Florian reported that the accumulation of negative life events was related to high levels of fear of personal death.<sup>16</sup> However coping strategies mediated this relationship. The mindfulness efforts can be either external (problem-focused) or internal (emotionfocused). The problem-focused coping strategy refers to stress management efforts. The emotionfocused coping strategy refers to efforts made to reduce the emotional stress a person feels. According to Skinner et al.<sup>25</sup>, strategies are different types of coping styles, which may be related to highly different results. It is very important to comply with stressful events such as cancer. The coping style is a common method to deal with a stressful event and comply with it. In addition to biological mechanisms, psychological factors can affect psychosomatic diseases such as breast cancer. What makes this chronic disease psychologically important is its broad range of neuropsychiatric aspects.

Coping strategies are among the individual psychological variables, which may have an important role in death anxiety. Coping refers to an individual's efforts to manage the strategic requirements of a specific situation.<sup>26</sup> One of the common strategies to manage challenging situations is making efforts to deal with stressors and alter them. Emotion-focused coping is concentrated on the emotional outcomes of a stressful situation. This strategy includes different approaches, such as not thinking about the problem (denial or avoidance) and opening up the problem to others.

The direct effect of a problem-focused strategy on death anxiety was not significant in patients with breast cancer. Regarding coping strategies and death anxiety, it can be said that death anxiety in a two-way interaction not only is among the results of selection and use of effective coping strategies in compliance with change and stress but also creates healthy psychological space through which proper identification and evaluation of stressful situations can be made to select an effective coping strategy. In this regard, due to specific disease conditions and their harmful physical and psychological effects, cancer patients fail to use problem-focused coping strategies, which include constructive measures to deal with stressful conditions and eliminate/change the sources of stress. This failure inhibits them from

coping with undesired situations properly.<sup>27</sup>

A low level of stress is among other characteristics of people who use an effective problem-focused coping strategy. Low level of emotional stress enables a person to use cognitive and dynamic skills to cope with problems, thereby achieving more satisfaction. On the other hand, denial and passivity are two characteristics of individuals with ineffective coping styles. Denial of a stressful situation can result in avoidance and passive behavior in dealing with stressful situations, thereby causing an inability to use potential capabilities and initiative. With this coping style, the problem remains unsolved, and the degree of dissatisfaction increases. The characteristics of denial and passivity, and their consequences in an ineffective coping with stressful conditions increase problems and dissatisfaction levels through reducing self-confidence.

The emotion- and problem-focused coping strategies have an indirect effect on death anxiety in patients with breast cancer through disease perception. This finding is consistent with the results of Soleimani et al.<sup>3</sup> and Basharpoor et al.<sup>28</sup> To explain this finding, it can be said that patients with breast cancer suffer from a high level of stressors.<sup>29</sup> On the other hand, coping methods are mindfulness efforts to manage internal and external stress.<sup>30</sup> Death anxiety and stress are among the most common concepts in human communities and affect many people. Stress seems to be an essential component and an inevitable result of human interaction with the environment. However, what causes a difference in patients' performance is the way they fight the disease. The majority of patients with breast cancer can manage their health at an acceptable level and continue to live in a highly stressful environment. The coping styles are among the key concepts in dealing with stress. Given the inevitable nature of stress, the use of appropriate coping styles can protect the person against severe stresses. The ability to cope with stress and identify a proper method to deal with stressful changes allows the patients to determine the sources of stress and the way they affect their lives, and take an appropriate stance for reducing pressure and stress and achieve calmness. Psychologists believe that even the treatment of the most severe physical diseases requires changing individuals' response patterns.

The emotion- and problem-focused coping strategies have an indirect effect on death anxiety in patients with breast cancer through disease perception. Future studies are recommended to consider the cause-and-effect relationship of these variables in the form of experimental designs. Future studies are also recommended to perform experimental designs at a more general level considering socio-cultural and economic conditions of different segments of society. Hospitals are also recommended to implement specific psychological programs for psychotherapists, psychiatric nurses, midwifery consultants, and rehabilitation programs for patients with breast cancer.

The present study was performed on patients with breast cancer in Abadan. Caution should be applied in generalizing the results to other communities in different time and place situations due to different cultural conditions. The participants sometimes showed a lack of interest and motivation in answering the questions. Another limitation of this study was failure to consider the duration of the participants' disease.

#### **Conflict of Interest**

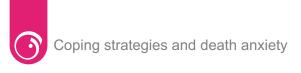
The authors declare that there is no conflict of interest.

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# Trends in Adjuvant Chemotherapy Use in Endocrine-Sensitive, HER-2 Negative Breast Cancer, With 1 to 3 Positive Nodes: A Single-Centre Study

José Manuel Baena Cañada<sup>\*a,b</sup>, Carlos de la Torre Hita<sup>a,b</sup>, Marta Bernal Gómez<sup>a,b</sup>, Alicia Campini Bermejo<sup>a,b</sup>, Salvador Gámez Casado<sup>a,b</sup>, Lourdes Rodríguez Pérez<sup>a,b</sup>, Alicia Quílez Cutillas<sup>a,b</sup>, Julio Calvete Candenas<sup>a,b</sup>, Sara Estalella Mendoza<sup>a,b</sup>, Encarnación Benítez Rodríguez<sup>b,c</sup>

<sup>a</sup> Medical Oncology Department, Hospital Universitario Puerta del Mar, Cádiz, Spain

<sup>b</sup> Instituto de Investigación e Innovación Biomédica de Cádiz (INiBICA), Spain

° Provincial Cancer Register, Regional Health Authority, Cádiz, Spain

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#### ABSTRACT

**Background:** There is a tendency to decrease the intensity of breast cancer treatments, e.g. omitting adjuvant chemotherapy in endocrine-sensitive and HER-2 negative patients. The purpose of this study was to analyse changes in the frequency of the indication of adjuvant chemotherapy and the differences in survival over time for this subtype of breast cancer, with 1–3 positive nodes.

**Methods:** The study was based on descriptive, observational, retrospective, single-institution research between 2004–10 and 2011–18, on endocrine-sensitive, HER-2 negative breast cancer, stage pN1 (1–3 nodes). The analytical tests carried out for a comparison of the frequency of chemotherapy use the chi-square test with Fisher's exact test. Survival data in both periods are presented.

**Results:** A total of 236 patients were included, 66 for the period 2004–10, and 170 for 2011–18. More patients were treated with hormone therapy alone in 2011–18: hormone therapy alone 10/66 (15.20%) for 2004–10, and 83/169 (49.10%) for 2011–18; chemotherapy-hormone therapy 56/66 (84.80%) for 2004–10, and 86/169 (50.90%) for 2011–18 (P=0.0001). For 2004–10, the 5-year overall survival probability was 100%. For 2011–18 it was 98.20% (95% CI 95.65–100). For 2004–10, 5-year disease free survival (DFS) was 96.9% (95% CI 92.7–101). For 2011–18 it was 87.7% (95% CI 81.8–93.5) (P=0,040). For 2004–10 the 5 year distant relapse free interval was 96.9% (95% CI 92.5–101.2). For 2011–18 it was 93% (95% CI 88.1–97.9) (P=0.312).

**Conclusion:** A decrease in the indication of adjuvant chemotherapy according to the clinical risk is confirmed in endocrine-sensitive, HER-2 negative breast cancer, with 1-3 positive nodes, over the period 2011–18 compared to 2004–10. Based on the results, 5-year DFS is slightly worse in the 2011–18 period.

#### Introduction

In recent years, there has been a tendency to deescalate breast cancer treatment, by shortening or omitting adjuvant chemotherapy, reducing the extent

\* Address for correspondence: José M. Baena-Cañada, MD Address: Medical Oncology Department, Hospital Universitario Puerta del Mar, Avenida de Ana de Viya 21, 11009 Cádiz, Spain Email: josem.baena.sspa@juntadeandalucia.es of axillary lymph node surgery or reducing the cycles of radiotherapy.<sup>1</sup> Standard adjuvant systemic treatment for women with hormone-sensitive and HER-2 negative breast cancers is based on hormone therapy. Some women obtain additional benefit from chemotherapy, while others can avoid this. International consensus guidelines recommend adjuvant chemotherapy in this subtype of breast cancer in women with 4 or more positive lymph nodes, including those with lobular and grade 1 or luminal A carcinoma.<sup>2</sup> However, it is not recommended for women with negative lymph nodes and tumors smaller than 1 cm.<sup>2</sup>Between these extremes are those women with pN1 axillary lymph node involvement (1 to 3 metastatic nodes).

Data on the benefit of adjuvant chemotherapy in early breast cancer are derived from meta-analysis of the Early Breast Cancer Trialists' Collaborative Group (EBCTCG) with 100,000 patients randomised in 123 trials.<sup>3</sup> This benefit is independent of clinical and pathological factors such as lymph node involvement, oestrogen receptor status, age, and use of hormone therapy.<sup>3</sup> Clinical tools such as Adjuvant! Online<sup>4</sup> and PREDICT Plus<sup>5</sup> have been developed based on patients' primary tumors and their clinical-pathological markers such as age, tumor size and grade, and the number of lymph nodes with metastases, which help in deciding whether adjuvant chemotherapy is indicated. However, the degree of accuracy provided by clinical-pathological markers in indicating adjuvant chemotherapy is far from perfect, especially if patients have hormonesensitive, HER-2 negative disease and 1–3 positive nodes.<sup>6</sup> For this reason, gene expression signatures have been studied in node-positive patients in order to discern which patients would benefit from adjuvant chemotherapy and which might avoid it.

In the Mindact trial, 6693 women, 21% of whom were node-positive, were randomised using discordant clinical or genomic risk (*MammaPrint*) to assign chemotherapy or not. In high clinical risk patients, 46.20% can be spared chemotherapy if their genomic risk is low, with no significant increase in the risk of distant recurrence.<sup>7</sup> The lack of statistical power and the short follow-up mean that the evidence from this trial is limited for node-positive patients; therefore, the benefit of chemotherapy cannot be excluded.

A retrospective analysis of the SWOG-8814 trial showed that using chemotherapy did not improve distant metastasis-free survival or overall survival in 146 patients with Recurrence Score (RS) (Oncotype DX, 21-gene signature) <18 (low genomic risk) or RS 18-30 (intermediate genomic risk). Once again, we encounter the limitations of low statistical power as a result of the small sample size, and its retrospective nature, for confirming that low genomic risk predicts no benefit from adjuvant chemotherapy in node-positive patients.<sup>8</sup> Other retrospective analyses show excellent survival rates among node-positive and RS <18 patients without adjuvant chemotherapy.<sup>9</sup> Lastly, the results of the retrospective analysis of the ATAC trial<sup>10</sup>, and the prospective analysis of the PlanB trial<sup>11</sup> also support omitting chemotherapy, although with the same limitations of short follow-up and low sample size.

Other gene signature tests, such as Prosigna (PAM50) and EndoPredict, also provide retrospective evidence for identifying patients at low

risk of metastasis in node-positive women treated with only adjuvant hormone therapy and no chemotherapy.<sup>12-14</sup>

For both node-negative and node-positive patients, the oncologists' recommendations on receiving adjuvant chemotherapy have decreased significantly over time, with no substantial change in clinical practice guidelines.<sup>15</sup> Incorporating genomic risk assessment bears much of the responsibility for this reduction in chemotherapy use<sup>16</sup>, despite the lack of evidence, as we have seen, from prospective and randomised trials.<sup>6,16</sup>

The aim of our study is to analyse changes in the frequency of the indication of adjuvant chemotherapy over the past 15 years in patients with hormonesensitive, HER-2 negative, 1–3 node positive breast cancer. The authors' hypothesis is that the relative frequency of use of adjuvant chemotherapy has decreased over time, with no impact on survival and without the incorporation of genomic risk being responsible for this, since its authorisation in our environment excludes patients with positive nodes.

#### Methods

This is a descriptive, observational, retrospective, single-centre study, carried out in the Medical Oncology department of a university hospital. It was registered on the website of the Spanish Agency for Medicines and Health Products (AEMPS) with code number JBC-EPI-2020-01. The study protocol was approved by the Cadiz Research Ethics Committee. It was conducted in accordance with the principles of the Declaration of Helsinki and its subsequent updates. Since it is a retrospective study using data contained in clinical records, with no intervention or risk to the patients, their informed consent was not necessary. The Cadiz Research Ethics Committee authorised the absence of informed consent.

The cohort selected attended the hospital over the past 15 years (1 January 2004 to 31 December 2018). The patients were identified from the Medical Oncology department's database. The data on patients and treatment received from Medical Oncology health records were collected retrospectively. The Spanish National Register for Deaths was consulted to find out the patients' vital status and date of death. The data was collected between September 2019 and February 2020.

Missing data was kept to a minimum by good study planning and careful collection. A proactive database design was created to reduce or detect data entry errors. The methodology used for data collection and entry was disseminated in writing and a chat room was set up for communication between researchers. A single researcher (EBR) reviewed the database to improve its quality and correct any errors detected.

The participants were women and men with breast cancer undergoing surgical treatment for pathological

infiltrating carcinoma, oestrogen and/or progesterone receptor positive, HER-2 negative and 1-3 nodes with metastases (including micrometastases), with a tumour size of less than 5 cm. Those treated with adjuvant hormone therapy alone, no chemotherapy, or without any adjuvant systemic treatment were eligible for inclusion in the study, as were those treated in combination with chemotherapy and hormone therapy. Patients assessed as having genomic risk, those treated with neoadjuvant systemic therapy, those not treated surgically and those with distant metastases were excluded. The independent patient-related variables analysed were: age, sex, performance status (measured by the ECOG scale)<sup>17</sup>, menopause status and comorbidity (measured by the Charlson scale).<sup>18</sup> The independent breast tumour-related variables analysed were tumour size, number of axillary nodes with metastasis, number of total axillary nodes analysed, tumour stage (American Joint Committee on Cancer -AJCC-, 8th edition), histological grade, histological type, oestrogen and progesterone receptor status (considered positive if immunohistochemical expression was equal or greater than 1%), and Ki67 proliferative index. The independent treatmentrelated variables were type of surgery, type of adjuvant hormonal treatment and type of adjuvant chemotherapy treatment, and year of treatment.

The dependent variables analysed were overall survival (OS), disease-free survival (DFS) and distant recurrence-free interval (DRFI).<sup>19</sup> The OS was calculated by measuring the time between surgery and one of the following events: death from breast cancer, death from any cause other than breast cancer, or

death from an unknown cause. The DFS was calculated by measuring the time between surgery and one of the following events: local or regional infiltrating recurrence, distant metastasis, death from breast cancer, death from any cause other than breast cancer, death from an unknown cause, contralateral infiltrating breast carcinoma, or invasive non-breast cancer. The DRFI was calculated by measuring the time between surgery and one of the following events: distant metastasis or death from breast cancer.<sup>19</sup>

 $(\cdot)$ 

We compared the use of chemotherapy and all the other variables between two time periods (2004-10 and 2011-18). We have presented the survival data, shortening the observation time to 5 years. In this way, we increased the accuracy of the results, and we attempted to manage the different follow-up duration between the two time periods when we made formal comparisons.

As this was a retrospective study, all patients from the last 15 years were included. There were estimated to be around 250 patients with these characteristics in this period. A descriptive analysis of the variables was carried out. For the qualitative variables, the absolute and relative frequency, the mean, median, and standard deviation for the quantitative variables were used. The analytical tests carried out were: the chi-square test with Fisher's exact test for a comparison of the qualitative variables; the t-student test for quantitative variables, the Kaplan Meier method to calculate survival, and the Log-Rank test for comparison of curves. SPSS version 15 was used for statistical analysis of the data. In the statistical analysis, p<0.05 was considered to indicate statistical significance.

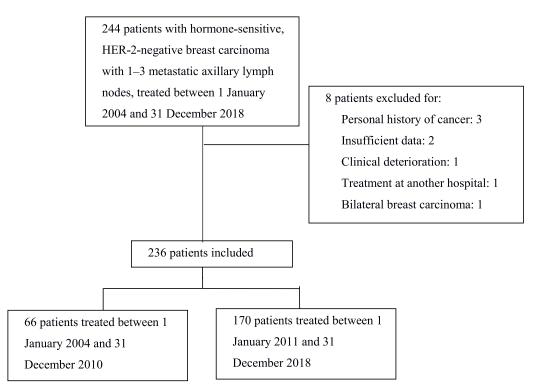


Figure 1. Study flow chart



### Table 1. Characteristics of the 236 patients

Patient characteristics	N	%	2004-10 N (%)	2011-18 N (%)	P Value
Age (median and range) Sex	55 (28-84)		54.63 (28-82)	55 (29-84)	0.831 0.518
Female	234 2	99.2	66(100)	168(98.8)	0.010
Male Menopause status		0.8	0 (0)	2 (1.2)	0.559
Premenopausal	96 138	40.7 58.5	25(37,9) 41(62,1)	71(42,3) 97(57.7)	
Postmenopausal Male	2	0.8	(02,1)	57(57.7)	0.050
Performance status (Measured using the ECOG scale	e) 128	54.2	44(66.7)	84(49.4)	0.058
0 1	103	43.6	21(31.8)	82(48.2)	
2 Comorbidity (Measured using the Charlson scale)	5	2.1	1(1.5)	4(2.4)	0.307
0	151 60	64 25.4	40(60.6) 17(25.8)	111(65.3) 43(25.3)	
1	17	7.2	8(12.1)	9(5.3)	
2 3	4 4	1.7 1.7	$1(1.5) \\ 0(0)$	3(1.8) 4(2.4)	
>3 Disease stage					0.040
IA	155 61	66.2 25.8	45(68.2) 21(31.8)	110(65.5) 40(23.8)	
IB IIA	16	6.8 0.8	0(0) 0(0)	16(9.5) 2(1.2)	
IIB	2 2	0.8	0(0)	2(1.2) 2(1.2)	
Unknown Tumor	1	0.4	0(0)	1(0.6)	0.393
pTis	29	12.3	7(10.8)	22(13)	
pTla-pTlb pTlc	110 93	46.6 39.4	37(56.9) 21(32.3)	73(43.2) 72(42.6)	
pT2 pTx	3	1.3	0(0)	1(0.6)	0.521
Palpability of the tumor	162	68.6	45(68.2)	117(68.8)	0.521
Palpable Non-palpable	74	31.4	21(31.8)	53(31.2)	
Tumor size in cm (median and range)	1.80 (0.30-5)		2.01(0.7-5)	2.05 (0,3-4.8)	0.753
Number of analyzed axillary nodes (mean and SD)	10.39 (6.69) 1.50 (0.71)		14.24 (8.91) 1.63 (0.82)	8.81 (4.73) 1.46 (0.63)	0.031 0.445
Number of positive axillary nodes (mean and SD) Lymph node ratio (mean and SD)	0.14 (0.10)		0.11 (0.07)	0.16 (0.12)	0.750
Histopathological type	202	86	49(74.2)	153(90.5)	0.03
Ductal Lobular	27 6	11.5	15(22.7) 2(3)	12(7.1) 4(2.4)	
Other	1	2.6 0.4	2(5) 0(0)	1(0.6)	
Unknown Histological grade	77	32.6	23(34.8)	54(31.8)	0.229
1	131	55.5	39(59.1)	92(54.1)	
2 3	28	11.9	4(6.1)	24(14.1)	
Oestrogen receptors	235 1	99.6	66 (100)	169 (99)	
Positive Negative	1	0.4	0(0)	1(0.60)	
Progesterone receptors	228	96.6	63(96)	165(97)	
Positive Negative	8	3.4	3(4)	5(3)	
Ki67 proliferative index	10 (1-90)			18,83(1-90)	
Median and range ≤20	123	52.1	0(0)	123(72,4)	
>20 Unknown	38 75	16.1 31.8	$0(0) \\ 66(100)$	38(22,4) 9(5.3)	
Breast surgery	129	517	45(68.2)	84(49.4)	0.013
Conservative Mastectomy	107	54.7 45.3	21(31.8)	86(50.6)	
Axillary surgery					0.000
Sentinel lymph node biopsy Axillary lymph node dissection	75	31.8	0(0)	75(44.1)	0.000
Year of treatment	161	68.2	66(100)	95(55.9)	
2004–2010 2011–2018	66 170	28			
Adjuvant systemic treatment	170	72			0.000
Hormone therapy	93 142	39.4 60.2	10(15.2) 56(84.8)	83(48.8) 86(50.6)	
Chemotherapy-Hormone therapy None	142	0.4	0(0)	1(0.6)	
Hormone therapy Temovifon	114	48.3	26(39.4)	88(52.1)	0.000
Tamoxifen Tamoxifen-goserelin	5	2.1	3(4.5)	2(1.2)	
Aromatase inhibitor Tamoxifen/Aromatase inhibitor	54 62	22.9 26.3	26(39.4) 11(16.7)	28(16.6) 51(30.2)	
None	1	0.4	(10.7)	1(0.6)	0.201
Chemotherapy Anthracyclines	8	3.4	5(8.9)	3(3.5)	0.301
Anthracyclines and taxanes	95 39	40.3	38(67.9)	57(66.3)	
Other None	94	16.5 39.8	13(23.2)	26(30.2)	
Radiation therapy	180	76.2	50 (75.7)	130 (76.4)	0.840

#### Results

The medical records of 244 patients were reviewed. Of these, 8 were excluded for various reasons. In the end, 236 were valid for analysis: 66 of these attended between 1 January 2004 and 31 December 2010, and 170 between 1 January 2011 and 31 December 2018 (Figure 1). Information on patient characteristics, their tumours and treatment received is summarised in Table 1. Median patient follow-up was 59.50 months (range: 2–185 months). Between 2004-10, the median patient follow-up was 142 months (3-185), and between 2011-18, it was 40 months (2-104). A summary of the events can be found in Table 2.

Of these 236 patients, 93 (39.4%) were treated with hormone therapy, 142 (60.2%) with

Table 2. Events in	n the 236	patients
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chemotherapy-hormone therapy and 1 (0.4%) did not receive any systemic adjuvant treatment. When we looked at the time period from 2004 to 2010 and compared it with the period from 2011 to 2018, we saw that more patients were treated with hormone therapy alone than chemotherapy-hormone therapy in the 2011-18 period. In the earlier period, 2004-10, 10/66 (15.2%) received hormone therapy and 56/66 (84.8%) chemotherapy-hormone therapy, and between 2011 and 2018, 83/169 (49.1%) received hormone therapy and 86/169 (50.9%) chemotherapy-hormone therapy (P=0.0001). The same differences were detected when the comparison was made year by year (P=0.0001). In Figure 2, the number of patients treated with chemotherapy in each year is presented.

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Events	Ν	%	2004-10 N (%)	2011-18 N (%)
Loco-regional recurrence	6	2.5	2 (3)	4 (2.4)
Local recurrence after conservative surgery	4	1.7	1 (1.5)	3 (1.7)
Local recurrence after mastectomy	1	0.4	1 (1.5)	
Lymph node recurrence	1	0.4		1 (0.6)
Metastasis <sup>1</sup>	15	6.4	5 (7.5)	10 (5.8)
Invasive second primaries	11	4.7	4 (6)	7 (4.1)
Contralateral breast	6	2.5	2 (3)	4 (2.4)
Non-breast	5	2.1	2 (3)	3 (1.7)
Contralateral ductal carcinoma in situ	1	0.4		1 (0.6)
Deaths	10	4.2	4 (6)	6 (3.4)
For breast carcinoma	5	2.1	2 (3)	3 (1.7)
For other reasons	4	1.7	2 (3)	2 (1.2)
Heart failure	2	0.8	1 (1.5)	1 (0.6)
Endocarditis	1	0.4		1 (0.6)
Sepsis	1	0.4	1 (1.5)	
Unknown	1	0.4		1 (0.6)

<sup>1</sup> Initial metastatic locations

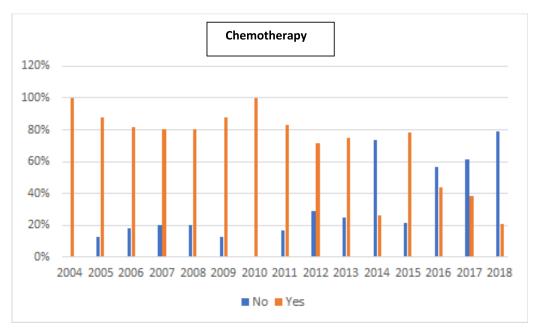
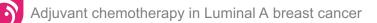


Figure 2. Use of adjuvant chemotherapy per year of treatment in patients with hormone-sensitive, HER-2 negative, and 1-3 node positive tumors



For all patients, the probability of OS, DFS and DRFI at 5 years was 98.9% (95% CI 97.3–100.4), 91% (95% CI 86.8–95.1) and 94.6% (95% CI 91.2–97.9) respectively. And at 10 years: 90.7% (95% CI 84.6–96.7), 70.6% (95% CI 60.9–80.2) and 87.9% (95% CI 81.2–94.5) respectively. And at 15 years, 88.8% (95% CI 81.7–95.8), 66.7% (95% CI 56.3-77.0) and 87.9% (95% CI 81.2–94.5), respectively.

In patients treated over the period 2004–10, the estimated probability of OS at 5 years was 100%. In patients treated over the period 2011-18, the estimated probability of OS at 5 years was 98.2%

(95% CI 95.6–100). The median OS was never reached. In this case, no comparison was made because no event occurred in the 5 follow-up years in the group of cases diagnosed between 2004-10. Figure 3 shows the OS curves.

In patients treated over the period 2004-10, the estimated probability of DFS at 5 years was 96.9% (95% CI 92.7–101). In patients treated over the period 2011–18, the probability of DFS estimated at 5 years was 87.7% (95% CI 81.8–93.5). The median DFS was never reached. The differences were statistically significant (P=0.040). Figure 4 shows the DFS curves for the 5 years.

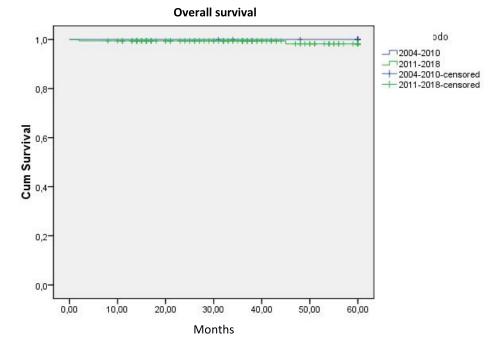


Figure 3. 5-year overall survival curve for 66 patients treated during 2004–10 and in 170 patients treated during 2011–18

**Disease-free survival** 

#### riodo 1.0 \_\_\_\_2004-2010 2011-2018 -2004-2010-censored 2011-2018-censored 0.8 Cum Survival 0,6 0.4 0,2 0,0 0,00 10,00 20,00 30,00 40,00 50,00 60,00 Months

Figure 4. 5 year disease-free survival curve in 66 patients treated during 2004–10 and in 170 patients treated during 2011–18

**Distant recurrence-free interval** 

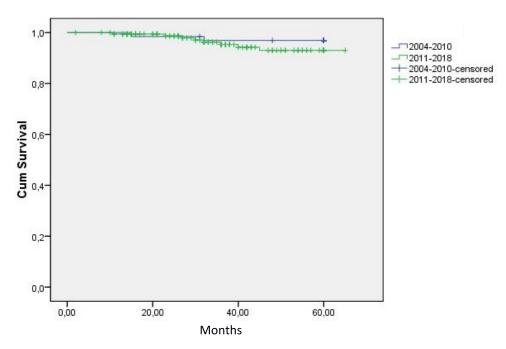


Figure 5. 5 year distant recurrence-free interval curve in 66 patients treated during 2004–10 and in 170 patients treated during 2011–18

In patients treated over the period 2004–10, the probability of DRFI estimated at 5 years was 96.9% (95% CI 92.5–101.2). In patients treated over the period 2011–18, the estimated probability of DRFI at 5 years was 93% (95% CI 88.1–97.9). The median DRFI was never reached. The differences were not statistically significant (P=0.312). The DRFI curves are shown in Figure 5.

#### Discussion

The results of this study provide evidence that this subgroup of patients have few events and enjoy long survival. These results have been obtained by indicating the type of systemic treatment based on classic prognostic factors, comorbidity, and the patient's decision. Overall, this is a population with a good prognosis (median tumour size 1.8 cm, median number of nodes with metastases 1 and a proliferative rate of 10%) for which chemotherapy treatment could constitute overtreatment.<sup>6</sup> Events related to breast cancer recurrence (locoregional recurrences, breast cancer metastases and deaths) were the most frequent, but the frequency of nonrecurrence events (second primary tumours and deaths from other causes) was not negligible.

Oncologists are indicating increasingly less chemotherapy for this subgroup of patients.<sup>20</sup>Although incorporating genomic risk assessment is behind this reduction, in our study it is not justified by such assessment, since in Andalusia the determination of genome platforms in 2016 was only authorised for patients with negative nodes.<sup>21</sup>

There is growing concern regarding the overtreatment of breast cancer patients as results

have improved over time and there is a sense that the use of chemotherapy has decreased in recent years, although little is known about how the use of chemotherapy and the recommendations of oncologists have changed. There are some studies that have found that for both node-negative/ micrometastasis and node-positive patients, the use of chemotherapy and oncologists' recommendations for its use have decreased significantly over time, with no substantial change in clinical practice guidelines to justify it.<sup>15</sup> Kurian et al. show that the use of adjuvant chemotherapy in patients with stage I-II disease decreased from 26.6% to 14.1% and from 81.1% to 64.2% in node-negative and nodepositive patients, respectively, between 2013 and 2015.15 Previous studies have shown a decrease in the use of concomitant adjuvant chemotherapy with the increased use of genomic tumour profiles in patients diagnosed between 2006 and 2013.<sup>22</sup>

Once the results were known, the concern of our study was not focussed on overtreatment but on undertreatment, since we detected a slight advantage in the DFS of the women treated over the period 2004–10. However, it is risky to attribute this slight benefit to the use of more adjuvant chemotherapy where there is an imbalance in some patient characteristics between the two time periods (more cases of lobular carcinoma in the first period, more cases of stage IIA and IIB in the second period, plus a higher rate of mastectomy, more conservative axillary surgeries and higher use of tamoxifen than aromatase inhibitors in the second period). Survival improvement in a comparison of late versus early period could also be attributed to the Will Rogers phenomenon of stage migration.<sup>23</sup> In the first period, 100% of the patients had axillary dissection, but in the second period this figure was only 55.9%. That implies patients considered as having 1-3 positive nodes in the second period are a heterogeneous group, more likely to contain patients with 4 or more positive nodes, as compared to patients of the first period. The large number of patients (living or nonevent patients) censored at the end of the follow-up due to a reduced follow-up of many patients treated in the second period, without guaranteeing a minimum of 5 years, is a bias that underestimates the event and needs to be taken into account. Consequently, it would be reasonable to compare only 5-year survival, where, incidentally, the differences are not so great (96.9% DFS for the period 2004-10 and 87.7% in 2011-18, with no differences in OS and DRFS). However, analysing 5-year survival alone for a disease such as hormonesensitive breast cancer, where late events are frequent, should be considered short. It is difficult for us to extend this to 10 years when only 30% have a follow-up of more than 5 years and approximately 1% have a follow-up for 100 months.

There was also concern where the decrease in chemotherapy use based on the incorporation of genomic risk assessment is confirmed.<sup>6</sup> The RxPONDER trial, now closed for recruitment of patients with hormone-sensitive, HER-2 negative breast cancer with 1–3 positive nodes and an RS of less than 25 who were randomly assigned to receive chemotherapy, will conclusively answer the question of whether adjuvant chemotherapy is necessary in this breast cancer subgroup.<sup>24</sup> The OPTIMA trial for patients with hormone receptor-positive, HER-2negative, and 1-9 positive nodes or tumours larger than 3 cm, also randomly assessed chemotherapy assignment based on genomic risk.<sup>25</sup> Pending their results, no specific recommendations can be made. However, the inclusion criteria of the RxPONDER and OPTIMA trial are being used in clinical practice, prior to publication of their results, to suggest that patients abstain from chemotherapy.<sup>16,26</sup> Nonadherence to the recommendations of clinical practice guidelines and the incorporation of treatments or technology before the definitive results of clinical trials are available is common in Oncology. In a recent study, the highest percentage of treatment non-compliance in breast cancer patients was observed with chemotherapy (18%) and this treatment non-compliance was associated with worse survival.<sup>27</sup>

The main limitation of this study is the small sample size and its retrospective nature, with possible errors made during the selection of patients or during the collection and measurement of variables or errors in the comparison of groups, as well as in the generalisation of results in other populations. We have already commented on the bias of the large number of patients censored for the comparison of survival curves due to the short follow-up of the patients treated in the period 2011–18.

Over the years there has been a decrease in the indication for adjuvant chemotherapy according to the clinical risk in hormone-sensitive, HER-2 negative and 1-3 node positive breast cancers. This reduction cannot be attributed to the incorporation of genomic risk assessment. DFS was slightly worse over the period 2011–18, when less chemotherapy was indicated.

For hormone-sensitive, HER-2 negative, and 1-3 node positive breast cancer patients, the oncologists' recommendations on receiving adjuvant chemotherapy have decreased significantly over time. The decrease in chemotherapy use is based on the incorporation of genomic risk assessment, with no substantial change in clinical practice guidelines.

Over the years, a decrease in the indication for adjuvant chemotherapy is confirmed but this reduction cannot be attributed to the incorporation of genomic risk assessment in our study. The concern of our study was not focussed on overtreatment but on undertreatment, since DFS was slightly worse over the period when less chemotherapy was indicated. No specific recommendations can be made. However, the RxPONDER and OPTIMA trial inclusion criteria should not be used in clinical practice, prior to publication of their results, to suggest that patients abstain from chemotherapy.

#### Acknowledgment

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#### **Conflict of Interest**

There is no conflict of interests to declare.

#### **Compliance with Ethical Standards**

This study was approved by The Ethics Committee of Cádiz. It was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. As it is a retrospective study based on the data contained in the health histories of patients and there is no intervention or risks to them, there is justification for not requesting informed consent. The Research Ethics Committee authorised the absence of informed consent.

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# What Do We Mean When We Ask for More Metastatic Breast Cancer Research?

Heather Douglas<sup>a</sup>, Catherine Hays<sup>b</sup>, Kimberly Badovinac<sup>\*c</sup>

<sup>a</sup> Patient Advocate, Ottawa, Ontario, Canada

<sup>b</sup> Patient Advocate, Rocky View County, Alberta, Canada

 $^\circ$  Canadian Partnership Against Cancer, Toronto, Ontario, Canada

ARTICLE INFO

#### ABSTRACT

priorities.

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Key words: Breast cancer, metastasis, research priorities, patient engagement

#### Introduction

Breast cancer is incurable and deadly once it metastasizes. Extrapolating from US sources, an estimated 15,000 to 18,000 people are living with metastatic breast cancer (MBC) in Canada, from Stage IV de novo diagnoses or from metastatic recurrences after previous Stage 0-3 diagnoses and treatment.<sup>1</sup> Each year, over 5,000 Canadians die from MBC.<sup>2</sup> The genesis of this paper was a concern among MBC research advocates about the lack of research progress that offers potential cures or

\*Address for correspondence:

Kimberly Badovinac, MA, MBA

Address: Canadian Partnership Against Cancer, 145 King Street West, Suite 900, Toronto, Ontario, Canada, M5H 1J8 Tel: +1 (416) 619-6265 quality long-term survival. The purpose of this paper is three-fold: (1) to

Background: Almost all deaths from breast cancer are due to metastasis.

People living with metastatic breast cancer (MBC) and their loved ones have been

concerned about the lack of research progress. The purposes of this paper were to

analyze breast cancer research spending in Canada, and to evaluate whether MBC

research was aligned with patient priorities. The results from the MBC Priority

Setting Partnership (MBC PSP) were used as an approximation of patient

projects were identified and mapped to the patient priorities.

providers is needed to address research gaps.

Methods: The data source was the Canadian Cancer Research Survey. MBC

**Results:** This analysis found that 18% of breast cancer research investment was directed to MBC, with a large proportion of this research investment focused on the biology of metastasis. Four of the top 10 MBC PSP priorities had not been addressed: optimal sequence of therapy, role of continuous versus intermittent treatment, benefits of early palliative care, and best methods for patient education.

Conclusion: These figures provide a baseline from which any increases in

MBC research and improved alignment to patient priorities can be measured. A

cooperative effort by funders, researchers, patients, caregivers, and health care

provide an analysis of breast cancer research spending in Canada, (2) to provide an analysis of MBC research spending in Canada, and (3) to assess how the MBC research investment aligns with patient priorities, closely approximated by results from the Metastatic Breast Cancer Priority Setting Partnership (MBC PSP). Patients, patient representative groups, and caregivers represented 60% of the contributors to the MBC PSP.<sup>3</sup>

#### Methods

The data source for this descriptive analysis was the Canadian Cancer Research Survey (CCRS)<sup>4</sup>, a longitudinal database of cancer research grants and awards funded by 42 Canadian governmental and non-governmental organizations and programs. The

Email: <u>kimberly.badovinac@partnershipagainstcancer.ca</u>

database contains over 26,000 projects from January 1, 2005 to December 31, 2018. It is updated annually and the 2019 data are in progress. The CCRS is estimated to capture about 60 to 80% of the peer-reviewed cancer research funded by Canadian organizations. All projects are coded to cancer site(s) and to the Common Scientific Outline (CSO)<sup>5</sup>, an internationally used typology of cancer science.

The database was first filtered for projects coded to breast cancer where at least 50% of the research was relevant to breast cancer (N=4,469). Next, keyword searches (metast\*, invasion/invasive, migration, stage 4, cancer spread) combined with CSO codes were used to identify MBC projects (N=905). Finally, each MBC project identified through the search was manually reviewed by a single reviewer (and a subset by a second reviewer) and assigned to one (or none) of the top 10 MBC PSP research priorities.

#### Results

Across the 14 years examined, 18% of the investment in breast cancer research was related to MBC (range 14 to 22%) (Figure 1). MBC investment accounted for less than 3% of the overall cancer research investment captured in the CCRS.

The results showed that 84% of the MBC research investment for 2014-2018 was aligned with the MBC PSP priorities (Table 1). The causes of breast cancer metastasis (priority 4a + 4b) represented over 65% of investment, but little was related to the brain-blood barrier, the second part of this priority (4b). Notably, there was no investment in four of the priorities, namely: optimal sequence of therapy (priority 5); continuous versus intermitted treatment (priority 7); the benefit of early palliative care (priority 8); and the best practices of patient

education (priority 9).

#### Discussion

At 18%, the proportion of MBC research among Canadian funders included in the CCRS varies from the 7% reported by the Metastatic Breast Cancer Alliance for  $2000-2013^6$ , to the 58% (for the Breast Cancer Research Program of the U.S. Congressionallydirected Medical Research Program to 15% (U.S. National Institutes of Health) for the fiscal year 2016 reported in a poster from the 2019 San Antonio Breast Cancer Symposium prepared by advocates from the U.S.-based National Breast Cancer Coalition.<sup>7</sup> These differences may reflect the emphases of funders' research funding opportunities and/or the areas of expertise of the researchers successful in these grant competitions. Overall, the low proportion of MBC research compared to the breast cancer total may reflect obstacles such as a lack of in-vivo models, barriers to resource sharing and collaboration, and procurement of tissue/samples throughout disease progression.<sup>8</sup>

A limitation to this work is that CCRS database does not include all research spending in Canada. The database does not include industry-supported clinical trials, BC Cancer (not a contributor to CCRS), and sources from outside Canada (in particular, the U.S. Department of Defense's Congressionally Directed Medical Research Programs and Susan G. Komen).

Since 2018, several promising competitions have been announced and are not yet reflected in the CCRS. Stand up to Cancer Canada, CIHR, and Canadian Cancer Society announced the SU2C MBC Dream Team winner in 2019, with funding up to \$6 million.<sup>9</sup> The Cancer Research Society and the Quebec Breast Cancer Foundation announced a \$1 million competition in late 2020. The call was

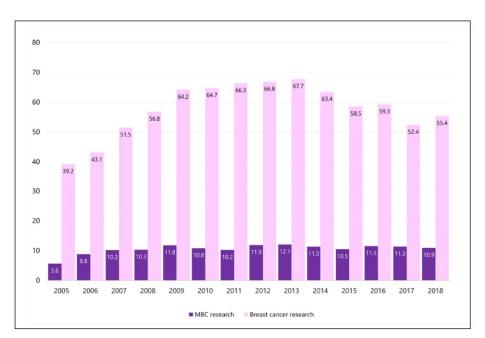


Figure 1. Breast cancer research investment in Canada included in the CCRS, 2005–2018 (CAD million)

influenced by the MBC PSP priorities and projects must focus on personalized medicine, immunotherapeutic approaches, or overcoming drug resistance.<sup>10</sup> In March 2021, Rethink Breast Cancer and Pfizer Canada announced a \$200,000 competition for strategies to improve the quality of care of MBC patients, which has the potential to address two of the unfunded MBC PSP research priorities (early palliative care and patient education).<sup>11</sup> In summary, this analysis provides a baseline from which any increase in MBC research spending and progress on the alignment of research with patient priorities can be measured and targeted funding opportunities may be needed to address obstacles and identified gaps, those priorities which are not currently funded. Including all stakeholders – patients, caregivers, health care providers, researchers, and funders – appears to be critical to ensure these and evolving priorities are promoted and pursued.

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 Table 1. Research investment by MBC PSP Priority

	CA	۸D	%	6
Priority	2014-2018	2018 only	2014-2018	2018 only
1. Identification of biomarkers, or intrinsic features of the tumor that can be used to guide treatment decisions	2,531,140	578,916	4.55	5.30
2. Role of immunotherapy in treatment for MBC	2,399,899	332,571	4.32	3.05
3. Delaying and overcoming treatment resistance	2,094,554	538,428	3.77	4.93
4a. Identifying what causes (i.e., cellular, genomic changes) breast cancer cells to metastasize	37,286,204	6,511,822	67.09	59.62
4b. Identifying what changes in breast cancer cells allow them to penetrate the blood-brain barrier	408,263	31,292	0.73	0.29
5. Optimal sequence of therapy in MBC	0	0	0.00	0.00
6. Role for local therapy (radiation or surgery to sites of metastatic disease) in MBC	1,260,597	101,648	2.27	0.93
7. Role for continuous treatment with systemic therapy vs intermittent treatment	0	0	0.00	0.00
8. Early palliative care for MBC patients	0	0	0.00	0.00
9. Best methods of education for MBC patients around treatment options and decision making	0	0	0.00	0.00
10. Role for non-invasive, more accurate methods for detecting spread of disease (including following curative-intent treatment)	525,075	100,700	0.94	0.92
Total	46,505,732	8,195,377		
\$ invested not in top 10	9,068,901	2,725,927	16.32	24.96
Total MBC Research Investment	55,574,633	10,921,303	100.00	100.00

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#### **Conflict of Interest**

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# Impalpable Breast Cancer and Service Delivery During the COVID-19 Pandemic – the Role of Radiofrequency Tag localization

Jonathan Strickland<sup>a,b</sup>, Beatrix Elsberger<sup>a,b</sup>, Gerald Lip<sup>a,b</sup>, Mairi Fuller<sup>a,b</sup>, Yazan A Masannat\*<sup>a,b</sup>

<sup>a</sup> Aberdeen Royal Infirmary, NHS Grampian, Aberdeen, Scotland, UK

<sup>b</sup> School of Medicine, University of Aberdeen, Aberdeen, Scotland, UK

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#### ABSTRACT

**Background:** Radiofrequency tags are used to localize breast lesions for surgery. During the Covid-19 pandemic, these offered the flexibility of inserting the Tags days or weeks before surgery. This made logistics of planning theatres lists easier, especially with most of the lists having been moved off site.

**Methods:** In the 7 weeks following the first lockdown in the UK, we reviewed all planned admissions for breast surgery looking at the types of surgery offered, type of localization used and assessed which cases would not have been able to go ahead had radiofrequency tags not been available.

**Results:** Out of 85 planned admission, 83 had surgery, 11 were for re-excision of margins and 72 for their first breast surgery excision (mastectomy or breast conservation). Out of the 54 that had breast conserving surgery, 40 needed localization, out of whom 27 had radiofrequency tags. Looking at theatre order list and location of surgery, 20 out of the 27 would not have had their surgery had radiofrequency tags not been available, which is 50% of the patients needing localization.

**Conclusion:** Radiofrequency tags are new devices used for breast lesion localization that offer a much-needed flexibility especially as seen during the Covid-19 pandemic.

#### Introduction

The SARS-COV-2 (Severe Acute Respiratory Syndrome Coronavirus 2), also known as Covid-19 pandemic has prompted a significant global change in the provision of health care services. In the UK, elective surgery was suspended and although emergency surgery and urgent cancer surgery was intended to continue with 'business as usual', there were issues with logistics and capacity, impacting urgent cancer treatment plans across all four nations in the UK. This was mainly due to the reduction in

\* Address for correspondence: Yazan Masannat, MD Consultant Oncoplastic Breast Surgeon The Breast Unit, Ward 315, Orange Zone, Aberdeen Royal Infirmary, Foresterhill Campus, Aberdeen, Scotland, UK, AB25 2ZN Tel: +44 (0) 1224 552 739, Email: <u>vazanmas@hotmail.com:</u> <u>vazan.masannat2@nhs.scot</u> the number of hospital beds, theatre availability and redeployment of staff to the acute sector and critical care. Additionally, changes in practice to mitigate Covid-19 transmission risk impacted on clinical capacity.<sup>1</sup>

The increased risk posed by Covid-19 resulted in changes to management plans for many cancer patients. In breast cancer in particular, this included avoidance of neoadjuvant chemotherapy with a move to surgery first, and for some the use of temporary endocrine blockade, until a safe surgical window became available. Furthermore, the type of surgery offered also changed with less choice to both patients and clinicians. Most immediate total breast reconstructions were put on hold and many of the more complex mammoplasty techniques were modified to simpler operations on the cancer side alone, avoiding symmetrising procedures. This prompted a radical change in the approach to surgery with new guidelines emerging aimed at helping decision making.1-3

In breast surgery, theatre lists have considerable logistical requirements, in particular joint radiological planning with localization of impalpable tumours. The standard approach for many decades has been the insertion of a guide wire into the lesion on the day of surgery.<sup>4</sup> With the change in service delivery due to the pandemic, the logistics of breast surgery theatre list planning became more complex, specifically taking account of the need for radiologists, along with the support of radiographers to wire localize breast lesions. With the uncertainty regarding staffing due not only to redeployment but with the potential for staff being off sick or selfisolating, lists with wire localizations could not be planned with any certainty. Furthermore, many National Health Service (NHS) trusts moved their cancer surgery off site, to a 'cold site', and thus remote from their main radiology departments where localizations would take place. Locally, our NHS Trust, NHS Grampian, was able to partner, early on, with the private healthcare provider BMI Albyn Hospital to maintain elective cancer services but keeping some theatre capacity at Aberdeen Royal Infirmary (ARI), which is the main NHS Site for the higher risk patients.

New tumour localisation techniques have been emerging to replace wire localisations including radioactive125-Iodine Seeds<sup>5</sup>, Magseed<sup>6</sup>, and radiofrequency (RF) tags.<sup>7, 8</sup> Previous publications have demonstrated their safe use in clinical practice. Our unit started to trial RF tags in the beginning of 2020. These are small tags but larger than the usual marker coil with a RF transmitter in them. They are mounted on a needle applicator and inserted, image guided into the tumour site which can be done days or even weeks prior to surgery. Intraoperatively, the localizer portable handheld reader and probe are used to identify the location of the RF tag to aid the surgical excision.

In our unit, these new localization techniques were pivotal in reconfiguring our service during the pandemic because of the the flexibility offered. When the UK moved from the "contain" to the" delay" phase in mid-March 2020, RF tags were seen as an important part of the breast surgical unit strategy planning to provide the flexibility needed. In this retrospective case series, we looked at the effect of using RF tags for breast lesion localization to accommodate the change in service delivery during the first lockdown.

#### Methods

This is a retrospective case series of all patients with planned admission for breast cancer surgery at NHS Grampian from the week beginning the 23rd of March and for the 7 weeks thereafter. This particular time point was used as the UK entered the 'lockdown phase', placing many implications on NHS service delivery.

Patients' records were reviewed retrospectively and the data collected included patients age, type of surgery performed breast conserving surgery (BCS) vs Mastectomy) and hospital site. Patients with BCS were categorised into either simple wide local excision (WLE) or therapeutic mammoplasty (TM). For BCS patients, we looked at whether localization was required or not, the type of localisation used (standard wire or RF tag technique), day of insertion of device and the order of theatre list. When planning our theatre lists, patients that needed wire localization received it in the afternoon part of the list, as the wires are inserted at the main hospital on the day on surgery by our radiology colleagues. By the time the procedure is done, the check mammogram is performed and checked, the TC-99 is injected and the patient is transferred to BMI Albyn hospital it is already midday.

For the patients that had a RF tag inserted we looked at whether their surgery would have been able to go ahead on that day had they not had that type of localization as all the RF tags were inserted a week or more prior to surgery.

#### Results

Overal, 85 patents were listed on 29 theatre lists in the 7-week period. All patients were female with a median age of 59 years (33-90). Out of the 85 patients listed for surgery, 83 patients had operations, 28 patients (34%) at ARI and 55 patients (66%) at BMI Albyn hospital. The two patients cancelled were both BMI Albyn patients; the first was thought to be borderline conservable initially and the operating surgeon discussed neoadjuvant endocrine therapy to downstage her, while the second was postponed as her husband displayed symptoms of Covid-19 infection.

Among the patients, 11 had re-excisions of margins from previous BCS and 72 had their first breast surgery +/- axillary surgery; 18 out of 72 procedures were simple mastectomies (25%) and the remaining procedures were BCS; 11 out of the 54 BCS (20%) were therapeutic mammoplasties and 43 had simple WLE +/- glandular mobilization; 40 out of the 54 breast conserving procedures needed localization (74%) while 14 were palpable; 13 were localized using wires inserted on the day of surgery and 27 were RF tag guided inserted days in advance.

Out of the 27 that had RF tag localization, 20 would not have been able to have had their surgery that day had it not been for the tag. This is due to logistical issues presented by the alternative of wire localization which requires radiology cover and factoring in timing for this and also TC-99 injection at ARI. If the RF tag had not been in place prior to the day of surgery, the patient would have had to be on an afternoon list and with the case mix of predominantly impalpable breast cancers, this would have left

morning lists underutilized and patients incurring a delay in their surgery.

#### Discussion

The Covid-19 pandemic has had significant worldwide impact, exacerabated in many situations by lockdowns and the continuing of only essential services. This has had a drastic effect on all aspects of health care services with a reduction and in many cases cancelling of elective surgery to accommodate an overwhelming number of unwell patients. Unsurprisingly, this has also impacted on cancer services.9 A 'new normal' has emerged, resulting in creative new ideas and ways of working to deliver safe and efficient services. Minimising face to face consultations and the resultant decrease in hospital footfall with the use of phone and/or video appointments, planning workspaces and clinic flow to allow for social distancing and the introduction of protocols based on the latest and continually emerging evidence are all examples of how units have had to change.<sup>2,3</sup>

In our unit, all surgeons were already trialling RF Tags as a new localisation technique prior to the Covid-19 lockdown and feasibility for this was established quickly. Our unit is one of the first units in the UK and Europe to adopt this, with the first European cohort published only earlier this year.<sup>8</sup> The need to move most of the breast cancer surgery to a cold site stipulated a fast up-scaling of this as it gave us the flexibility and enabled us to accommodate sudden changes of dedicated theatre days, to adapt to short notice cancellations and to efficiently plan theatre lists. The RF tags were inserted days and sometimes weeks before surgery providing the freedom of placing patients at any order of a theatre list at short notice on any day of the week. Redeployment or shortage of radiology staff on the day of surgery was not of any concern as RF tags were implanted in the breast days before surgery. This also provided an opportunity to review the position of the tag in relation to the impalpable lesion on check mammograms and to discuss issues with the breast radiologist.

As demonstrated in the results section, three quarters of the patients that had their first cancer operation had BCS, the majority requiring localization. Due to logistical constraints around radiology cover and timing, half of the patients needing localisation would not have been suitable for surgery on their scheduled day. While they would ultimately have had a traditional wire localization (on the day of surgery), this would have been at a later date with the risk of further cancellation depending on staffing levels. The RF tag enabled the most efficient use of staff and service time.

In conclusion, the use of RF tags in the localization of impalpable breast lesions offers several benefits to patients, clinician and the

healthcare service provider. They give the flexibility to theatre list timing and location that cannot be assured with traditional wire localization. In our experience, fast-tracked by the Covid-19 pandemic, RF tag insertion is a suitable alternative localisation technique. More prospective studies are required to allow adequate comparison to other localisation devices.

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Management of Cancer Patients During the Novel Coronavirus Disease 2019 (COVID-19) Pandemic: An International Collaborative Group. Oncologist. 2020;25(6):e936-45. Case Report

#### DOI: 10.32768/abc.202183251-254 Metaplastic Breast Cancer with Chondroid Differentiation

Garima Daga\*<sup>a</sup>, Rajeev Kumar<sup>a</sup>

<sup>a</sup> Department of Surgical Oncology, Rajiv Gandhi Cancer Institute, New Delhi, India

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#### Introduction

Metaplastic breast cancer (MBC) is an extremely rare subtype identified in 2000. It represents 0.2-1% of breast cancer and is typically composed histologically of poorly differentiated invasive ductal carcinoma coexisting with areas of squamous or mesenchymal differentiation.<sup>1</sup> The pathologic diagnosis of MBC is difficult due to heterogeneity. Aggressive biological parameters like high histological grade are more frequently found in MBC compared to invasive ductal carcinoma which drives a more aggressive treatment. Mastectomy rates are higher due to larger tumour size at the time of presentation despite lower incidence of axillary lymph node involvement.<sup>2</sup> They typically do not express estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor

\* Address for correspondence: Garima Daga, MD Address: Department of Surgical Oncology, Rajiv Gandhi Cancer Institute, New Delhi, 9930484364, India Email: narsinghbaba@yahoo.co.in

ABSTRACT Backgrour

**Background:** Metaplastic breast cancer (MBC) cancer is a rare subtype of breast carcinoma and carries a worse prognosis. Chondroid differentiation is the rarest among all its histologic subtypes. We report a case of MBC with chondroid differentiation and review its clinicopathological details, genetic basis, and management.

**Case presentation:** A 56-year female presented with right-sided large breast lump. She noticed this lump 4 months before presenting. Trucut biopsy was suggestive of invasive ductal carcinoma. She underwent breast conservation surgery and histology was consistent with MBC with chondroid differentiation, pT2N3aM0. Tumour was triple-negative for ER, PR, and Her-2- neu receptors. Adjuvant treatment with chemotherapy followed by radiotherapy was given and she has been doing fine during 11 months of follow-up.

**Conclusion:** The MBC is an uncommon subtype with heterogeneity in biological and morphological features and its knowledge is paramount while evaluating a breast lump. Understanding the pathologic and molecular basis is imperative in developing the targeted therapy to improve outcomes.

receptor-2 (HER2), which is suggested to be a reflection of absence of extensive glandular component. This cancer is considered as a subgroup of basal like breast cancers when classified by gene expression and carry a poor prognosis due to lack of response to hormonal therapy. MBC with chondroid differentiation is the rarest among all histologic subtypes of breast cancer.<sup>3</sup> It has worse prognosis compareed to infiltrating ductal carcinoma, even when adjusted for stage, with a 3-year overall survival rate of 48-71% and 3-year disease-free survival rate of 15-60%.<sup>4</sup> We report a case of metaplastic carcinoma with chondroid differentiation.

#### **Case Presentation**

A 56-year-old woman presented with a lump in right breast in upper outer quadrant. There was no history suggestive of nipple discharge. Family history was non-contributory. On examination, a hard non-tender mass measuring approximately 4 x 5 cm was noted in upper outer quadrant of right breast extending into axillary tail. Mass was not fixed to overlying and underlying structures. There were no obvious skin changes. A lymph node was







palpable in the right axilla. Mammography was suggestive of a single lesion in the upper outer quadrant with few nodes in the axilla. The PET-MRI Fusion study was done revealing a well-defined rim enhancing hypermetabolic altered signal intensity lesion in upper outer quadrant at 9 -11 o' clock in anterior depth measuring 4.3 x 4.2 x 4.5 cm. It showed nodularity along the periphery and internally with washout pattern of enhancement. Lesion was 2.2 cm, 1.5 cm and 7 cm away from nipple, skin and chest wall, respectively. A few enlarged hypermetabolic axillary lymph nodes, the largest measuring 2.1 x 1.7 cm, were noted. There was no evidence of disease elsewhere. Fine needle aspiration cytology revealed a poorly differentiated carcinoma consistent with mammary duct origin. Biopsy of mass revealed invasive ductal carcinoma in a background of dense stromal fibrosis. The patient underwent right-sided breast conserving surgery with latissimus dorsi flap reconstruction.

Histology revealed a tumour measuring  $3.8 \times 2.9 \times 1.2$  cm solid cystic lesion containing haemorrhage and papillary excrescences. Skin and nipple were negative for tumour. Histological type was a metaplastic carcinoma with chondroid differentiation, as shown in Figure 1 a, b. The tumour was triple negative on immunohistochemistry as oestrogen receptor (ER), progesterone receptor (PR), and HER-2 neu were negative, as shown in Figure 2 a, b. Tumour cells showed some degree of anisocytosis with a nuclear pleomorphism score of 3, and mitotic figures in tumour cells were frequent with an average of 8 mitoses or more per square mm. Overall Nottingham score was 9 (Tubule formation (3) + Mitotic Count (3) + Nuclear Pleamorphism (3) = 9), with the grade being 3. Lymphovascular emboli were present along with nodal involvement with extracapsular extension. Totally, 14 lymph nodes were involved out of 28 (pT2 N3aM0).

Adjuvant chemotherapy with AC-T (Adriamycin, Cytoxan, and Taxol) was initiated. She developed taxol-induced sensory neuropathy leading to early discontinuation of Taxol. She also received radiotherapy and has been under surveillance with no evidence of recurrence for 11 months.

#### Discussion

The MBC is a rarely encountered tumour and constitutes less than 1% of all malignant breast tumours. World Health Organization (WHO) classifies MBC into (1) epithelial type and (2) mixed type. Epithelial type of MBC is further classified into squamous cell carcinoma, adenocarcinoma with spindle cell differentiation and adenosquamous carcinoma. Mixed type is further classified into carcinoma with chondroid metaplasia, carcinoma with osseous metaplasia, and carcinosarcoma. Prognosis of

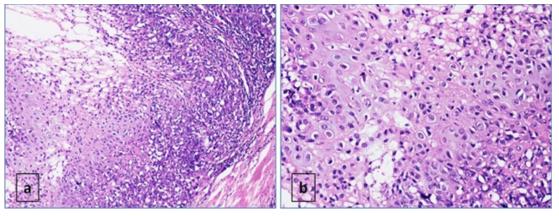


Figure 1. Hematoxylin and Eosin stained slide showing metaplastic breast carcinoma with chondroid differentiation, a. 20x, b. 40x.

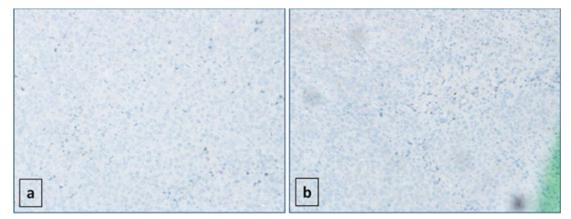


Figure 2. Immunohistochemistry negative for Estrogen, a and HER2 Neu, b.

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each of these varies widely. Typical histologic picture is comprised of poorly differentiated infiltrating ductal carcinoma coexisting with areas of squamous or mesenchymal differentiation. Chondroid differentiation is the rarest among all above varieties and carries the worst prognosis.<sup>5</sup>

MBC usually affects the females over 50 years old. The common clinical presentation is a palpable and firm large breast mass usually greater than 3 cm. The history is usually of short duration and around 20% cases present with skin tethering. Our patient presented with a right sided large breast lump. No skin changes were observed. Large tumour size and hence higher T stage is explained by rapid growth kinetics in poorly differentiated tumours.<sup>6</sup>

Diagnosis is usually inconclusive on fine needle aspiration biopsy due to large size and tumour heterogeneity and is established mainly after excisional biopsy or resection. Trucut biopsy in our case was suggestive of infiltrating ductal carcinoma but final histology showed MBC with chondroid differentiation.

Spread to axillary lymph node is less common despite large tumour size and high histologic grade. The paucity of lymph nodal involvement was attributed to the presence of mesenchymal elements. Higher incidence of axillary lymph node metastasis has been reported in squamous subtype by Huvos et al.<sup>7</sup> Even with rarity of lymph nodal involvement, axillary dissection cannot be avoided as diagnosis is sometimes not clear. In our case, axillary clearance was done and 14 out of 28 lymph nodes were positive. Despite low rates of axillary involvement, MBC has high potential for distant metastases via hematogenous route, mostly to lung and bone.<sup>8</sup>

Treatment is largely on lines of invasive ductal carcinoma. Large tumour size is responsible for high rates of mastectomy but rates of breast conservation surgery and mastectomy are similar to other tumours if corrected for tumour size. Breast conservation therapy with adjuvant radiation can be considered if the tumour size is less than 5 cm. If tumour size exceeds 5 cm, total mastectomy is suitable.<sup>9</sup>

Response to conventional chemotherapy is limited but as per current guidelines, adjuvant treatment is the same as invasive ductal carcinoma.<sup>10-12</sup> This cancer is associated with poor prognosis and common poor prognostic factors are younger age, skin involvement, lymphovascular invasion, high Ki67 scores, nodal involvement and squamous cell carcinoma in lymph nodes. Positive basal marker and cancer stem cell expression in tumor cells are independent indicators for poor prognosis. Some immunohistochemical characteristics like EGFR overexpression, EGFR gene amplification, and focal staining of CK14 have been reported to be associated with decreased disease free survival.<sup>13</sup>

In molecular terms, MBCs usually cluster with triple-negative breast cancers (TNBCs) preferentially

with basal-like or claudin-low molecular subtypes and frequently harbour mutations in TP53 gene. MBC has markers of epithelial-mesenchymal transition and cancer stem cells responsible for production of chemotherapy resistant cells capable of dedifferentiation and propensity for invasion. Overexpression of epithelial-mesenchymal transition inducers like vimentin and SPARC has been found to be associated with higher grade and triple negative status in MBC.<sup>14</sup> The recent literature is also suggestive of this. Studies investigating genetic basis of MBC which explore potential therapeutic targets are the way forward. Gene expression profiling of tumor holds great promise in developing targeted therapy for MBC in future.<sup>15</sup>

In conclusion, the limited knowledge of MBC is due to its rarity and heterogeneity in biological and morphological features as well as various classifications and different treatment strategies. It is imperative to keep MBC in differential diagnosis while evaluating any breast lump and giving due treatment. In a small and selected group of patients treated according to cancer stem cell characteristics, the results are encouraging; hence, more efforts are needed to explore potential molecular targets and improve outcomes.

#### **Ethical consideration**

The informed consent was obtained.

#### **Conflict of Interest**

The authors declared no conflict of interests.

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Figure 2. Immunohistochemistry negative for Estrogen, a and HER2 Neu, b.





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# Contralateral Axillary Lymph Node Enlargement in a Woman with Silent Silicone Breast Implant Rupture 30 Years After Breast Cancer Diagnosis: A Lesson to Be Learnt

Helen J. Trihia\*<sup>a</sup>, Efthymia Souka<sup>a</sup>, Gabriela Stanc<sup>a</sup>, George Galanopoulos<sup>a</sup>, Eleftheria Ignatiadou<sup>a</sup>, Ioannis Provatas<sup>b</sup>

<sup>®</sup> Departments of Pathology, Metaxas Cancer Memorial Hospital, Piraeus, Greece

<sup>b</sup> Breast Unit, Metaxas Cancer Memorial Hospital, Piraeus, Greece

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#### ABSTRACT

**Background:** Silicone lymphadenopathy is a recognized complication of silicone implant rupture. It occurs when silicone droplets migrate from breast implants to lymph nodes, resulting in the formation of granulomas (known as siliconoma) and lymph node enlargement. The ipsilateral axillary lymph nodes are most commonly involved but it can also affect contralateral axillary, supraclavicular, internal mammary and mediastinal lymph nodes.

**Case presentation:** A 60-year-old woman with a history of left breast cancer who had undergone modified radical mastectomy (MRM) followed by left breast reconstruction with implant (30 years ago) presented with right axillary lymph nodes enlargement. An excisional biopsy of the two larger lymph nodes was performed to rule out malignancy. Pathologic examination showed features of silicone lymphadenopathy. Further examination with Ultrasound and MRI confirmed breast implant rupture.

**Conclusion:** Silicone lymphadenopathy following breast augmentation and reconstruction primarily affects the ipsilateral axillary nodes. Contralateral lymph node involvement is rare and may occur several years after breast cancer diagnosis and can be the first sign of breast implant rupture. Although, the need to exclude malignancy in such cases is of outmost importance, silicone lymphadenopathy should also be considered in the differential diagnosis.

#### Key words:

Breast carcinoma, silicone lymphadenopathy, contralateral axillary lymphadenopathy

#### Introduction

Breast implants have been in use since the early 1960s.<sup>1</sup> Every year, thousands of women undergo implant surgery for augmentation or reconstruction following mastectomy. As the age of the implant increases, so does the risk of silicone leaking. This is responsible for most local complications as well as for silicone migration beyond breast tissues. Therefore, the number of women who develop palpable axillary masses can be expected to increase. Unilateral axillary lymphadenopathy is a worrisome finding in women with implant-based breast

\* Address for correspondence: Helen J Trihia, MD, MSc, PhD Address: 51 Mpotassi st., 18537 Piraeus, Greece (work) Tel: 6970918556 Email: eltrix@otenet.gr reconstruction since it can be the first sign of breast carcinoma recurrence. Most palpable axillary lymphadenopathies in patients with implants are silicone granulomas due to leakage and migration of silicone particles through lymphatics. This case report stresses the fact that similar considerations should be taken in a case of contralateral axillary lymph node enlargement in women with breast implants. On this occasion, diagnostic investigation must be meticulous since both silicone granulomas and breast cancer metastases may coexist in the same lymph node.

#### **Case presentation**

A 60-year old woman with a history of left breast cancer 30 years ago who had undergone modified radical mastectomy (MRM) followed by implantbased breast reconstruction 12 years later presented



with right axillary. lymphadenopathy. The woman had undergone total mastectomy and axillary lymph node dissection level I and II. The pathology report of cancer was invasive ductal carcinoma, T=1cm, Grade 2, with no nodal involvement in 26 dissected lymph nodes (LNs). She further underwent oophorectomy and chemotherapy with 3 cycles VAC (vincristine, adriamycin, cyclophosphamide). The reconstruction was done twelve years after diagnosis; therefore, it was delayed one stage. The implant was compounded with a the latissimus dorsi musculocutaneous flap. The type of prosthesis was SilitexR® Low Bleed Gel-filled, round moderate profile, size: 275cc, rounded raw surface (Mentor Company).

Current clinical and radiological examination (CT, US, Mammography, MRI) showed no local or regional recurrence, apart from enlarged, movable, rounded, painless axillary lymph nodes, of maximum diameter of 2 and 1 cm, in the contralateral axilla.

The patient underwent excisional biopsy of two larger LNs to rule out malignancy. On pathologic evaluation, the specimen consisted of two fibro-fatty fragments, which included two lymph nodes with 1,3cm and 3,5cm of larger dimension.

#### Histological Features

Both lymph nodes demonstrated extensive

involvement by diffuse follicular hyperplasia with interspersed foamy histiocytes with clear, bubbly, vacuolated cytoplasm corresponding to silicone and foreign body type giant cells with refractile, nonbirefrigent particles, with a small peripheral rim of preserved lymphoid cells (Figure 1a, b). Asteroid body was evident inside giant cell (Figure 1-c). Therefore, the diagnosis of silicone gel lymphadenopathy was made.

#### Clinical Features

After histological diagnosis and requesting the clinical history of the patient, the clinician confirmed the history of breast silicone augmentation mammoplasty. Further review with Ultrasound and MRI confirmed breast implant rupture. The patient was referred to a plastic surgeon for removal of the implant. She refused any further treatment due to social-economic factors.

#### Discussion

Silicone gel implants have been widely used for breast augmentation and reconstruction since 1963 and are made of silicone shells filled with either saline or silicone gel.1 Rupture is a late complication and consists of intracapsular rupture (when the gel remains within tissue capsule surrounding the implant), extracapsular rupture (when the gel moves outside the capsule but remains within the

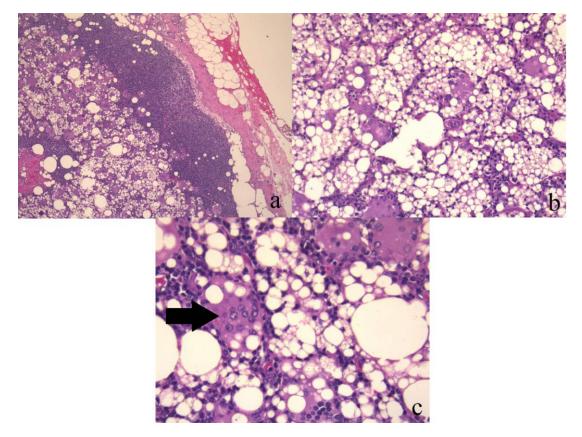


Figure 1. a, b. Lymph node involvement of the medullary sinuses by interspersed foamy histiocytes with clear, vacuolated cytoplasm and foreign type giant cells (H&E, in different magnifications, X100, X200) c. Multinucleated histiocytes with occasional intracytoplasmic asteroid body (arrow) (H&E, X400)

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breast tissue) and migrated gel (when the gel moves beyond the breast).<sup>2</sup> Silicone particles can migrate through tissues following overt breast implant rupture or slow gel 'bleed' through an apparently intact outer implant shell. The exact prevalence of implant rupture remains unclear and is estimated to be between 0.3% and 77%.<sup>3</sup> The incidence increases with implant duration<sup>4</sup> and depends on the site of implantation (most likely if subglandular as opposed to retropectoral), the presence of local tissue contractures and type of implant used.<sup>2</sup> The sensitivity of physical examination for detecting silicone implant rupture may be as low as 30%5, although the diagnosis is easier when capsular contracture is present.<sup>°</sup> Magnetic resonance imaging (MRI) techniques have made the diagnosis of previously undetected implant rupture possible.<sup>7</sup> The FDA advises removal of ruptured breast implants<sup>8</sup>, but silicone lymphadenopathy does not warrant treatment unless it is symptomatic or interferes with breast cancer detection." Silicone leak can remain confined to the breast or spread to draining axillary lymph nodes, and all across the body<sup>10-14</sup>, and even to remote organs, lung parenchyma, chest wall muscles, where silicone leads to foreign body inflammation<sup>15</sup> <sup>19</sup>, and sometimes mimics neoplastic disorders on imaging studies.<sup>20, 21</sup> Subcutaneous siliconomas have also been reported in more distal areas such as the abdominal wall, inguinal region and lower limbs.<sup>2</sup> This occurrence can be attributed to the fact that silicone polymer is lipid soluble, which facilitates its migration in fatty tissues. Once outside the confines of the implant, silicone particles may be transported to regional lymph nodes by macrophages and generate a granulomatous reaction which may present as lymphadenopathy with the ipsilateral axillary lymph nodes being most commonly involved.<sup>11</sup> Involvement of ipsilateral intramammary, internal mammary, supraclavicular as well as contralateral internal mammary and axillary lymph nodes has also been reported.<sup>10</sup> Although studies have analyzed the pathologic features of silicone lymphadenopathy and accuracy of imaging modalities in detecting breast implant rupture, there are relatively few reports that describe the clinical correlates and the distribution of involved lymph nodes in patients with ruptured silicone breast implants. The latter was attempted by Fernando Collado-Mesa et al.<sup>10</sup>, who described for the first time the silicone spread to mediastinal lymph nodes and the use of endobronchial ultrasound (EBUS)-guided biopsy to confirm it.

The lymphatic drainage of the breast occurs through three principal routes: the axillary, transpectoral, and internal mammary pathways.<sup>23</sup> The axillary lymph nodes involvement is easily explained by the major lymphatic drainage system of the breast toward the axilla. More than 75% of the lymph drainage, particularly from the outer quadrants, drains to the ipsilateral axillary lymph nodes. The remainder drains to either the internal mammary lymph nodes, the opposite breast inner quadrants or to the inferior phrenic nodes (particularly from the lower quadrants).<sup>24</sup> The intramammary involvement, first reported in 1994<sup>12</sup>, can be explained by the other important lymphatic drainage system of the breast.<sup>25</sup> Silicone migration may occur through the same routes but may also spread in retrograde direction or use other pathways. once the jugular-subclavian venous confluence has been reached. Silicone migration can occur in a retrograde direction through collateral pathways when the normal lymphatic flow is obstructed because of scarring from surgery, including lymph node dissection.<sup>25, 26</sup> These include contralateral internal mammary<sup>13</sup> and mediastinal lymphatics.<sup>15, 16</sup> Our case demonstrates that in a patient with disrupted lymph drainage due to prior mastectomy and axillary lymph node dissection, silicone particles can migrate in a retrograde fashion and reach the contralateral axilla. Notably, silicone migration can occur due to gel bleed with intact envelope in the absence of implant rupture.<sup>27</sup> Therefore, patients with silicone lymphadenitis can be asymptomatic and a history of silicone breast implant may be all the history that is provided. In our case, there was no knowledge of the clinical history at the time of diagnosis. Most implant ruptures are not clinically apparent nor are they readily visible on routine mammographic/sonographic imaging. MRI is the most accurate imaging modality to evaluate the integrity of breast silicone implants. However, lymph node morphology is better evaluated by ultrasound. Current FDA recommendations for silent implant rupture screening are breast MRI implant protocol three years following implant placement and every two years thereafter.

Cytological and pathological findings of silicone lymphadenopathy are well described.<sup>28, 29</sup> Foreign body giant cells with birefrigent, granular material and one or more asteroid bodies located peripherally in the cell cytoplasm, are described cytologically.<sup>28</sup> Differential diagnosis includes other granulomatous disorders, which can be easily excluded if birefrigent particles are found within the macrophages in an appropriate clinical setting. Fat necrosis and lipogranuloma are among differential diagnoses too. Most cases of fat necrosis occur postoperatively or after radiation therapy, usually within a periareolar or superficial location.

Fine needle aspiration of palpable lesions in the axilla and breast after breast augmentation is useful in differentiating between cancer recurrence and silicone granulomas.<sup>29</sup> It is well known that FNA is an accurate and cost-effective method of ruling out malignancy and diagnosing implant disruption in patients with silicone prostheses presenting with an axillary mass. Although cytological investigation

can produce an unequivocal diagnosis and thus help alleviate patient's anxiety and lead to patient's confirmation, excisional biopsy is advisable to exclude concomitant malignancy.

Histologically, silicone lymphadenopathy involves accumulation of silicone gel, firstly in the medullary sinuses (unlike metastasis, which primarily involves the lymph node cortex). The histologic appearance can vary widely, ranging from no involvement to global involvement of the lymph node. Histologic features include diffuse follicular hyperplasia with interspersed histiocytes with clear, vacuolated cytoplasm. Foreign-body type giant cells, some containing refractile material, may accumulate in areas where clusters of clear cells have formed empty vacuoles<sup>9, 30</sup> As there is no histochemical or immunohistochemical procedure that can stain silicone, a definitive identification of silicone in lymph nodes and other tissues can be confirmed by electron microbe analysis using transmission or scanning electron microscopy.<sup>31</sup>

Although silicone migration to the contralateral lymph nodes has been described in the literature, in most cases there was also symmetrization with bilateral mammoplasty and bilateral breast implant insertion.<sup>10</sup> This is in contrast to our case where only unilateral ipsilateral breast implant insertion was done.

The development of lymphadenopathy, particularly in patients with a history of breast cancer, raises concern regarding new or recurrent malignancy. Imaging is important in distinguishing reactive lymphadenopathy related to silicone deposition from metastatic disease, since some of these patients may have a history of breast cancer. MRI of the breast is the imaging study of choice in the diagnosis of silicone breast implant rupture for most women.<sup>7</sup> Alternatively, mammography, breast ultrasonography, and breast CT may diagnose silicone breast implant ruptures when MRI is contraindicated.

Silicone within lymph nodes can appear dense on mammogram, can have a snowstorm appearance on ultrasound, may demonstrate color mapping on (dual energy) DECT, and can be hyperintense on silicone-sensitive MRI sequences. The most accurate method to distinguish reactive versus metastatic lymphadenopathy is using ultrasound, as it can show a classic snowstorm ("sandstorm") appearance in cases of silicone deposition within the node.<sup>32</sup> Silicone-sensitive MRI may not always exhibit high signal intensity as silicone may variably infiltrate the node.<sup>33</sup>

In addition, PET scanning may demonstrate positive FDG uptake in silico3ne-induced lymphadenopathy and further heighten the suspicion for malignant disease.<sup>34</sup> FNA can lead to the correct diagnosis. Nevertheless, confirmation by excisional biopsy should be done to exclude coexistent malignancy, specifically in a patient with a history of breast carcinoma.<sup>35</sup> Once malignancy is excluded, treatment consists of conservative approach or excision of the affected lymph nodes with an excellent prognosis.

In this case report, we presented a rare case of silicone migration to the contralateral axillary lymph nodes post mastectomy and reconstruction with silicone implant. Silicone axillary lymphadenopathy due to leakage from silicone breast implant is a rare occurrence that presents 6-10 years after implant placement.<sup>9</sup> The actual incidence and prevalence are unknown with less than 180 cases noted in the literature.<sup>36</sup> To the best of our knowledge, there have been only 5 case reports concerning silicone migration to the contralateral lymph nodes.<sup>10, 11, 36, 37</sup> Factors that lead to aberrant lymphatic flow include prior breast or axillary surgery or irradiation, bulky tumor in breast or heavy burden disease in the ipsilateral lymph nodes.<sup>38, 39</sup> Our case and the few published similar cases indicate that involvement of the contralateral lymph nodes can happen due to aberrant drainage and not necessarily via hematogenous spread. The implications of these findings are important as they can be the underlying mechanism in the case of metachronous contralateral axillary metastasis (CAM), in the absence of a contralateral breast cancer or an ipsilateral breast cancer recurrence (IBCR), therefore representing a regional event rather than a systemic disease. After treatment of breast cancer, 3,6% to 6% of patients present with contralateral axillary lymph node metastasis.<sup>40</sup> According to AJCC staging manual, CAM is considered an M1, stage IV disease, even in the absence of distant organ metastasis, such as bone, liver or lung. Studies have shown that patients with CAM have a better prognosis than patients with distant stage IV metastatic disease<sup>41-44</sup> and better OS when CAM is subjected to surgical and systemic treatments with a curative intent.<sup>43</sup> Therefore, arguments have been made that CAM should be classified as locally advanced (N3) disease, rather than metastatic (M1, stage IV) disease.<sup>45</sup>

In conclusion, axillary lymphadenopathy in any patient with a history of breast cancer should raise the concern for recurrence. However, migration of silicone to the regional lymph nodes in patients with implant-based breast reconstruction is a well-known condition too. This is not always limited to the corresponding axillary lymph nodes and can also affect the contralateral axillary lymph nodes. Our case demonstrates that in a patient with disrupted lymph drainage due to prior mastectomy and axillary lymph node dissection, silicone particles can migrate in a retrograde fashion and reach the contralateral axilla. On encountering enlarged lymph nodes in a patient with silicone breast implants, the possibility of silicone lymphadenopathy should be considered, even in the case of contralateral axillary lymph node

involvement. Biopsy is the only definite way to rule out malignancy.

#### **Ethical considerations**

Written informed consent was obtained from the patient.

#### **Conflict of Interest**

The authors have no conflict of interests to declare.

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#### Editorial





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### Surgical Oncology Myth or Reality

Remy J Salmon\*\*

<sup>a</sup> GCS Cognac Jay, Saint Jean de Dieu Clinic, Paris, France

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Darwin said that "the species that survive are not the strongest or the most intelligent, but they are the ones that will be able to adapt."

In addition, in an article of the Lancet, volume 22, issue 2, p182-189, February 01, 2021 titled "Global demand for cancer surgeons: Evaluation of the optimal number of surgeons and anesthesiologists between 2018 and 2040" concerning 143 countries of low, medium and high-income levels, we are provided with predictions that concern us all. The model used in this study estimates that the number of cancer cases requiring surgery will increase by 5 million (52%) from 2018 (9,065,000) to 2040 (13,821,000). The largest relative increase will mainly affect 34 low-income countries, where the level of equipment is the lowest. To balance these figures with high-income countries would require an increase in the number of surgeons by four and anesthetists by 5.5. However, this does not mean that the number of surgeons in high-income countries is and will remain optimal.<sup>1</sup>

In the context of multidisciplinary meetings, surgeons are also faced with "internal competition" with other players in cancer care. The term "oncologist" has gradually drifted into medical oncology. It is true that the gigantic investments provided by the Big Pharms, in order to overcome this global scourge, somehow justify their current preeminence and that no scientific meeting could take place without active support from the industry. But let's take a step back and take the example of breast cancer.

\* Address for correspondence: Remy J Salmon, MD Address: GCS Cognac Jay, Saint Jean de Dieu Clinic, Paris, France Email: dr.rjsalmon@gmail.com Halsted published his original article on radical mastectomy in 1907. This intervention underwent many variations in the 20th century. With the development of radiotherapy, the concept of conservative treatment appeared after the Second World War, followed by irradiation.<sup>2</sup> It took several decades and prospective randomized trials, in Europe, as in the USA, to show identical survival between mastectomies and conservative treatments. These results were definitively acquired at the end of the 20th century, thanks to adequate radiotherapy equipment.

Along with progress in more conservative surgical resections, the concept of adjuvant chemotherapy emerged, with the work of G. Bonnadonna in Italy<sup>3</sup> and B. Fischer in the USA.<sup>4</sup>

Then came the work of Guiliano, allowing us to limit lymph node dissection and their morbidity to the identification of a single sentinel node<sup>5</sup>, and that of M.C. King identifying the mutations predisposed to the occurrence of breast cancer BRCA1-2, then more recently their variants, bringing together constitutional genetics and surgical prevention.<sup>6</sup> Besides, the TNM given classification has way to molecular classification, etc. Oncoplasty, immediate or secondary reconstruction, and lipofilling have more recently changed the management of our patients. Robotic surgery, still limited by financial constraints, is slowly taking its place in our surgical therapeutic arsenal.<sup>7,8</sup>

The purpose of this editorial is not to provide a history, necessarily incomplete, on the techniques and on the men and women who allowed these advances but to recall the place of surgeons in the management of solid tumors and especially the breast.

Even today, surgeons are still the ones who most often see patients first, and their questions remain the same:

Doctor! are you going to take my breast out?



#### Will I be receiving chemotherapy?

So far, it is still the surgeons who will answer the first question.

For the second, several choices are possible depending on their age and their mode of exercise.

1) Either they operate first, and they discuss the file afterwards with the postoperative results and then see paragraph 2.

2) Or they present the file to the multidisciplinary consultation meeting, which in a "democratic" way, will make the best decision suited to a given patient. But anyone who has attended one of these meetings at least once knows that in a human group, there are always "dominant males", not always males!

Admittedly, the majority of files are seen quickly because they correspond to treatment protocols validated in the institution, at local, regional, national and even international levels. These protocols are developed and updated regularly in consensus conferences and, in fact, are very similar, regardless of the place of treatment. But there is a sizeable proportion of cases that do not fit, and that's when the real battle begins. Each will defend his chapel, his specialty, egos clash with publications, molecular signatures, the panel of genes, etc. Depending on the country, the culture, the final decision is taken on criteria of variable objectivity, which makes the analysis of the results of certain publications from across the Atlantic in particular difficult to understand. Surgeons historically know that solid tumors do not heal until they have been completely removed. The story circulating is that surgeons have complete answers within an hour, at little cost (except in robotic surgery!) where it takes several months at great expense to try to get a more or less complete answer, and that we can, in any case, assess only through a surgical act.

3) The third and probably the best solution would obviously be the one taught to me by one of my old masters in surgery who said, "you have to know the pathology that you are going to take care of as well as the other doctors with whom you are going to be confronted; anatomo-pathologists, radiologists, radiotherapists, etc. Because if you are not as "strong" as them in their specialty, they will ask the surgery indications of you, and then it is you who would be in trouble with your patients. Of course, you will make enemies, but you will be respected by those who will be happy to have a real interlocutor ".

In oncology, no one will ask you to do contouring before radiotherapy or to know the dose per m2 of this or that drug, any more than radiotherapists or medical oncologists need to know the brand of the automatic forceps, nor the size of the thread you are going to use to close the skin. On the other hand, if you do not know the side effects of a chemotherapy protocol, the intraoperative bleeding and the consequences will not be the same depending on the date you perform an operation on the patient.

In France today, interns preparing for the specialty of oncology must spend a semester in radiotherapy as part of the medical oncology course, but there are no plans to offer them a semester in surgery. This is an anomaly that lasts and does not look set to change in the years to come.

Out of ignorance rather than ill will (we hope!) in the case of a loco-regional alternative, our medical oncologist colleagues always tend to choose methods, which they think are non-invasive through ignorance of technical procedures. In a recent example of metastatic breast cancer to the liver, the metastases were in segments III and IV. The oncologist in charge of this patient proposed a radio frequency, where a focused irradiation type cyberknife. He was surprised to know that these two metastases could be removed by laparoscopy, with reduced hospitalization time and ability to provide precise histology with documented and healthy excisional margins.

To conclude, it is the surgeons who, as Darwin said, will create their future for themselves. By dint of being concerned with technique, some of them have forgotten the pathology and, in a way, the patients. The efforts to be made are not so important to retain or regain the place they deserve in multidisciplinary teams. These efforts will also have the merit of giving back to their care a human dimension that is well worth these efforts. They should not forget that in France, they have not been barbers since 1268, when the brotherhood of Saint Côme was created by LouisXI. They took the same college exams as their fellow medical oncologists until the day they chose the surgical route, and this route, contrary to what some medical oncologists believe, did not atrophy their brains.

There are currently societies for oncological surgery in many countries, and we can only recommend that our colleagues with a predominant oncological activity join these different societies by definition because of the transversality inherent in oncology, and in societies of the pathology of the organ.

#### **CONFLICT OF INTEREST**

The author declares no conflicts of interest.

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# Neuroanatomic Map for Nerve Block Design in Breast Surgery

Mingkeng Hsieh<sup>a</sup>, Yiying Chiang\*<sup>a</sup>

<sup>a</sup> Department of Anesthesiology, China Medical University Hospital, Taichung, Taiwan

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We have read with interest a recent publication of Puentes-Gutiérrez and colleagues about post-breast surgery pain syndrome.<sup>1</sup> Perioperative pain has been identified as one of the risk factors for post-breast surgery pain syndrome.<sup>2</sup> There has been increasing literature regarding locoregional anesthesia techniques to cope with perioperative pain after breast surgery. In the PROSPECT guideline published in 2020, paravertebral block was recommended as the first choice with or without wound infiltration and pectoral nerve block was considered an alternative to paravertebral block.<sup>3</sup>

However, we would like to point out that paravertebral block, which acts on the intercostal nerves, is effective for pain from skin and the intercostal muscles. Pectoral nerve block which acts on the lateral and medial pectoral nerves is effective for pain from pectoralis major and minor muscles. Paravertebral block and pectoral nerve block could work better together instead of replacing each other.

Pectoral nerve block can be achieved by infiltrating local anesthetics between the pectoralis major muscle and the pectoralis minor muscle as well as between the pectoralis minor muscle and the serratus anterior muscle.<sup>4</sup>

As the target of paravertebral block, the intercostal nerves give rise to their lateral cutaneous branches which go forward subcutaneously after penetrating the chest wall muscles near the mid-axillary line and supply the skin over the arm pit as well as the lateral

\* Address for correspondence:

Yiying Chiang, M.D. Address: Department of Anesthesiology, China Medical University Hospital, No.2 Yude Road, Taichung, 40447, Taiwan. Tel: +886422052121 Fax: +886422052121 Email: chiang.yiying@gmail.com and inferior aspects of the breast. The anterior cutaneous branches of the intercostal nerves arise more distally near the lateral border of the sternum and course back to supply the skin over the medial and superior aspects of the breast. Paravertebral injection blocks both branches of the intercostal nerves and numbs the skin of both the axilla and anterior chest. After paravertebral block, only a small area of skin of the chest around the clavicle, which is innervated by the supraclavicular nerve, is spared. Paravertebral block requires repositioning the patient, is more skill demanding, is contraindicated in patients with bleeding tendency, and carries the risk of pneumothorax.<sup>5</sup> Thoracic epidural block and erector spinae plane block (injection between the erector spinae muscle and the transverse process of the spine) also have similar problems. Serratus anterior plane block could be a simple technique to block the lateral cutaneous branches of the intercostal nerves by infiltrating local anesthetics between the latissimus dorsi muscle and the serratus anterior muscle within the axillary fossa.<sup>6</sup> The anterior cutaneous branches of the intercostal nerves could be blocked by parasternal subcutaneous infiltration or transversus thoracic plane block (injection between the innermost intercostal muscle and the internal intercostal muscle).

Multimodal analgesia for perioperative pain has been strongly recommended to enhance recovery after breast surgery.<sup>3,7</sup> Comprehensive locoregional anesthesia minimizes the requirement of sedation or the depth of general anesthesia, reduces perioperative pain, facilitates early mobilization, and reduces opioid requirement and associated side effects such as constipation and post-operative nausea and vomiting. Table 1 classifies related nerve block options according to their level of spinal nerve origins. Although distant from each other proximally, their peripheral analogs could be approached around the surgical field in supine position (Figure 1). In our

3

practice, we perform serratus anterior plane block and/or transverse thoracic plane block in supine position pre-operatively based on anticipated skin incision instead of paravertebral block if the intercostal muscles are not involved. But if the intercostal muscles are involved, paravertebral block or other locoregional anesthesia techniques targeting the intercostal nerves may be required. We also consider pectoral nerve block when pain from the pectoral muscles is a concern. Besides image findings, it would be helpful to identify pain from the pectoral muscles if there is referred pain in C5-T1 dermatome because the pectoral nerves arise from brachial plexus. According to preoperative image studies, pre-existing pain and its referral pattern, as well as anticipated surgical procedure, a patienttailored locoregional anesthesia plan based on the understanding of neuroanatomy is recommended.

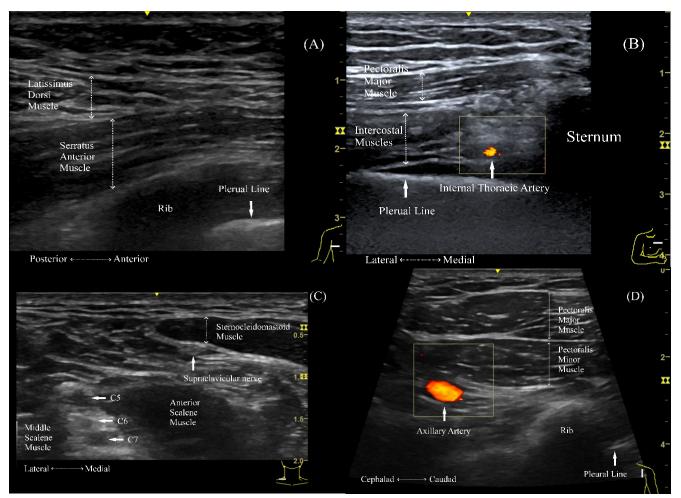
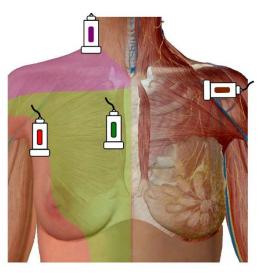


Figure 1. Surface and ultrasound anatomy in different techniques (Image courtesy of Visible Body with modification): A: The red probe illustrates the site of injection for serratus anterior plane block which blocks the cutaneous sensation of the red area innervated by the lateral cutaneous branches of the intercostal nerves. B: Serratus anterior plane block could be done by injection between the latissimus dorsi muscle and the serratus anterior muscle or deep to the serratus anterior muscle. The green probe illustrates the site of injection for transversus thoracic plane block which blocks the cutaneous sensation of the green area innervated by the anterior cutaneous branches of the intercostal nerves. C: The internal thoracic artery helps to identify the target plane of transversus thoracic plane block between the innermost intercostal muscle and the internal intercostal muscle. The purple probe illustrates the site of injection for supraclavicular nerve block which blocks the cutaneous sensation of the purple area innervated by the supraclavicular nerve. D: The supraclavicular nerve emerges beneath the sternocleidomastoid muscle. The brown probe illustrates the site of injection for pectoral nerve block which blocks the sensation of the pectoralis major and minor muscles. Pectoral nerve block could be done by injection between the pectoralis major muscle and the pectoralis minor muscle and deep to the pectoralis minor muscle.



Origin	Peripheral nerve	Sensory innervation	Proximal block	Peripheral block	
C3/C4	Supraclavicular nerve	Skin of the lower neck, shoulder and around clavicle	Cervical plexus block	Subcutaneous local infiltration or supraclavicular nerve block	
C5/C6/C7	Lateral pectoral nerve	Major and Minor	Brachial	Pectoral	
C8/T1	Medial pectoral nerve	pectoral muscles	plexus block	plexus block	
T2/T3	Intercostobrachial nerves	Skin of axilla and medial upper part of the arm	Paravertebral,	Serratus anterior	
T3/T4/T5/T6	Intercostal nerves (lateral branches)	Skin of lateral and inferior aspects of breast	epidural, erector spinae plane block	plane block	
T3/T4/T5	Intercostal nerves (anterior branches)	Skin of medial and superior aspects of breast		Subcutaneous local infiltration or transverse thoracic plane block	

#### Table 1. Neuroanatomic map for nerve block design.

#### **CONFLICT OF INTEREST**

The author declares no conflicts of interest.

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# An Insight into the Role of Bee Venom and Melittin Against Tumor Cells: A Review of Breast Cancer therapy

Roha Tariq<sup>a</sup>, Amna Liaqat<sup>b</sup>, Usama Ahmed Khalid\*<sup>c</sup>

<sup>a</sup> Department of Biotechnology, Lahore College for Women University, Lahore, Pakistan

<sup>b</sup> Department of Healthcare Biotechnology, Atta ur Rahman School of Applied Biosciences, National University of Sciences and Technology, Islamabad, Pakistan

<sup>c</sup> Department of Biotechnology & Genetic Engineering, Kohat University of Science and Technology, Kohat, Khyber Pakhtunkhwa, Pakistan

ARTICLE INFO	ABSTRACT
Received: 09 February 2021 Revised: 25 May 2021 Accepted: 04 June 2021	<b>Background:</b> Breast cancer is the most common and life-threatening cancer in females characterized by the abnormal proliferation of tumor cells in lobules and ducts. For years, many anti-breast cancer drugs have been tested with some of them showing severe health problems and drug resistance. Recently, different biological and pharmacological actions of bee venom have been indicated to play anti-bacterial, anti-viral and anti-inflammatory role against different cancers specially breast cancer. <b>Methods:</b> This review study is based on PubMed, Google Scholar and PubMed search. Search terms used were Melittin, Breast cancer and Honey Bee Venom. <b>Results:</b> Many studies have shown that a positively charged C-terminal sequence of mellitin facilitates plasma membrane contact and antitumor action. Precise targeting and selective activity of melittin has been found in recent studies as it suppresses the activation of growth factor receptors in HER2-enriched and triple-negative breast cancer that are generally difficult to treat. Significantly, it leaves healthy cells intact. The most striking feature of melitin is the pore formation property. Monomers of melitin bind to the plasma membrane of cancer cells in a
	collective manner and start forming pores ultimately bringing cell lysis.
Keywords: Breast cancer, Melittin, combinational therapy, Honeybee venom	<b>Conclusion:</b> Since melittin has a very selective action against the HER-2 related tumors, a combinational therapy of melittin and HER-2 targeted agents could be a very potent strategy in breast cancer. This review reflects the importance of honey bee venom and melittin as a potential therapy for aggressive breast cancer.

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#### INTRODUCTION

Although advancements and innovations have calmed human lifestyles, this technological age is somehow responsible for emerging diseases which are

\* Address for correspondence: Usama Ahmed Khalid, MD Department of Biotechnology & GE, Kohat University of Science and Technology, Pakistan Tel: +923434656343 Email: <u>usama.ahmed.khalid@gmail.com</u> very difficult to deal with. Cancer is one of the leading, widespread, and lethal diseases with various complications.<sup>1</sup> When it comes to the most common cancers in women, breast cancer is predominant with high prevalence ratio<sup>2</sup> and ranks second in number worldwide.<sup>1</sup> Mostly, the cells in lobule and ducts divide in an uncontrolled manner resulting in breast cancer, while a few cells in other portions of the breast also contribute in this regard.<sup>3</sup> However, in some cases, breast cancer cells from glandular portions cross the



duct and lobular wall barrier and their entrance into the surrounding tissues proves to be fatal.<sup>4</sup> The severity of breast cancer depends on the analysis of the status of the cancer cells and then the "stage" of cancer is nominated. Declaration of breast cancer stages is based on the invasive and non-invasive manner of cells and ranges from 0-IV.<sup>5</sup> As per report cases, it is an alarming situation that the breast cancer is strengthening its root in America, Africa and Asia.<sup>6</sup> Majority of countries are on red line with high mortality rates in women and breast cancer is one of the causes of it.<sup>7</sup> Mortality rate can be considerably reduced if breast cancer is detected in the initial stages and that is only possible when the patient instantly gets checked upon the appearance of visible symptoms.<sup>6</sup> Multiple factors are directly or indirectly involved in the origin of breast cancer. Some women who had breast cancer in the past or have family history are susceptible to this disease.<sup>8,9</sup> Family history is directly linked to abnormal genetic makeup. While considering the genetic causes, two genes, namely BRCA1 and BRCA2, are the prominent ones. Mutations present in them are precursor to large scale breast cancer.<sup>10</sup> To tackle the different aspects of cancer, scientists and researchers are looking for novel and combined therapeutic approaches. Considering the usefulness of honeybee compounds at biological levels, its use to treat cancer is under research.

Honeybee is widely being used for the welfare of human beings in the form of various products. The main usages of honeybee compounds include therapies of various diseases. Among different compounds of honeybee, "Honeybee venom" has shown promising effects in treatment. The term used for the treatment of various diseases by using bee compounds is "apitherapy". Important compounds of honeybee venom are Melittin, Apamin, peptides, Adolapin and Phospholipase A<sub>2</sub>. Different diseases like Alzheimer, Parkinson, viral infection, bacterial infections, and different type of cancers have been treated by honeybee compounds.<sup>11</sup> Despite the many uses of bee venom, its molecular effects with respect to breast cancer treatment have not been properly understood. As breast cancer is prevailing in women worldwide, efficient strategy regarding its treatment is urgently needed.<sup>12</sup> Honeybee venom and its main compound melittin are proved to be good agents for cancer treatment by managing different conditions of tumor like initiation of apoptosis, inhibition of cell proliferation and cell growth and control of metastasis.<sup>13</sup> Melittin is the essential component of bee venom. This can be inferred from the fact that dry weight of bee venom contains 40-50 % of melittin. Melittin is a cationic and amphipathic peptide which perform its activity by attaching onto the negatively charged membrane. On attaching, melittin forms pores in the membrane and destabilizes it.<sup>14, 15</sup>

In this era of multifactorial diseases, a quick and efficient treatment strategy is utmost necessary. Breast cancer is the result of various complicated cellular processes, so understanding these complications is imperative for the discovery of new treatment and therapeutic strategies.<sup>16</sup> This review paper will give insight regarding the novel therapeutic approaches to breast cancer by using bee venom and melittin.

#### **METHODS**

Search terms used for this review includes "cancer", "breast cancer", "honeybee venom", "melittin" and a combination of these terms. The data for this review was collected through different search databases including PubMed and Google Scholar. Collected data were correctly citated. Here, we have demonstrated, by a comprehensive literature review, the role of honeybee venom and its component named mellitin in effectively inducing cell death mainly in HER2enriched and triple-negative breast cancer. All the images used in this review were retrieved from "Pixabay", an open source of non-copyrighted images.

#### **RESULTS AND DISCUSSION**

#### Therapies in use for Breast Cancer

Effective therapy and management of breast cancer are the two most crucial steps for its eradication. Surgical attempt depends upon the types of tumor and stage of breast cancer.<sup>17</sup> Radiation therapy is sometimes linked with surgical attempts, as in some cases, it is necessary to irradiate the tumor site after surgery using radiation. In the case of breast cancer, radiation therapy is usually sought only after surgery. However, radiations should be strong enough to wipe out cancer cells.<sup>3, 18</sup> In addition, chemotherapy is also used for treatment in the case of serious risk of cancer. This therapy can be attempted both before and after surgery depending on the type of cancer cells. Targeted drugs are also in practice for breast cancer therapy, but its repercussions cannot be ignored.<sup>17</sup> As the days go by, science is leading the search to new techniques for diagnosing and treating the disease. Discovery of novel biomarkers and advancement in the genomics and transcriptomics assessment are giving an insight into the production of personalized therapies. Three prominent biomarkers of breast cancer, i.e., Estrogen Receptor (ER), Progesterone Receptor (PR) and Human Epidermal Growth Factor Receptor 2 (HER2), decide the nature and precise targeted therapy for breast cancer. However, based on these biomarkers, we can get an insight into the optimal therapeutic approach. In the case of Triple Negative Breast Cancers (TNBCs) which are lagging in the expression of these receptors still have no approved targeted treatment available.<sup>19</sup> This indicates that new agents and treatment strategies that may increase the efficacy



of conventional chemotherapeutic drugs are instantly needed to acquire effectiveness against cancers. Some of the common therapeutic approaches that are in practice for breast cancer are shown in Figure 1.

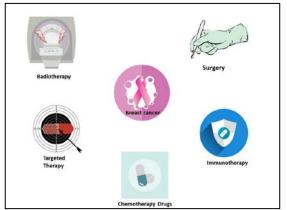


Figure 1. Main therapeutic approaches currently in practice for breast cancer treatment

#### Alternative way for treatment of breast cancer

High prevalence and death rate of breast cancer have placed this disease at the top of life threatening diseases in women.<sup>20</sup> About 80% of the patients' treatment ultimately fail due to the side effects and resistance developed against the anticancer drugs administered to them.<sup>21</sup> A promising way to impede the development of cancer cells without any side effects is to use oriental medicine such as bee venom (BV).<sup>22</sup> Several studies have discussed the anti-cancer effects of BV on lung, liver, renal, prostate, breast, and cervical-cancer cells.<sup>23</sup>

#### Biotoxins: A Natural Remedy

In recent years, a substantial growth has been witnessed in the treatment of breast cancer using natural substances especially biotoxins.<sup>24</sup> It is also supported by an extensive investigation carried out by a group of scientists that various animal toxins have demonstrated an exceptional antitumor activity against innumerable illnesses.<sup>25</sup> These venoms include scorpion venom<sup>26</sup>, Bee Venom (BV)<sup>27</sup>, sea anemone toxin<sup>28</sup>, snake venom<sup>29</sup>, and some other animal toxins. The factor which makes these biotoxins a valuable biological resource is that they are produced and secreted in the venom gland of the living organisms comprising pharmacologically functional constituents that might be having potential therapeutic significance.<sup>30</sup> Through complex pathways, these resources employ outstanding anticancer properties exerted by their novel compounds and play a key part in regression of the cancer.<sup>31</sup>

#### Anticancer role of Bee Venom (BV)

One such natural resource is a Bee Venom (BV), produced from the venom gland of the honey bee (Apis mellifera) containing approximately eighteen bioactive compounds.<sup>32</sup> These include peptides (melittin, adolapin, apamin, polamines, histamine, and mast cell-degranulation peptide), enzymes (phospholipase A2, hyaluronidase), and other amines and non-peptide components.<sup>27, 33</sup> In Asian countries especially Korea, BV has been used to treat various human diseases.<sup>34</sup> Furthermore, in other countries it is widely used for various skin problems, rheumatism<sup>35</sup>, arthritis<sup>36</sup>, and chronic pain.<sup>37</sup> Many researchers have depicted amazing effects of BV on diverse range of human cancerous cells such as breast cancer<sup>38</sup>, lung cancer<sup>39</sup>, ovarian cancer<sup>40</sup>, melanoma<sup>41</sup>, bladder cancer<sup>42</sup>, leukemia<sup>43</sup>, and so on. For that reason, biotoxins present great potential as antitumor pharmaceutical products in cancer therapy.

#### Melittin and its anticancer properties

Melittin (MEL) is the primary active component of bee venom, responsible for 40-60% of its dry weigh.44-46 It is a linear, strong, cationic and amphiphilic peptide entailing 26 amino acids with 6 positive charges at physiological pH.47 Its chemical formula is C131H228N38O32, weighing 2847.5 Da and is hemolytic and strongly cardiotoxic.<sup>48</sup> The most extensive literature on MEL has reported that it performs several biological functions such as antibacterial49, antifungal19, antiviral50, and antiparasitic.<sup>51, 52</sup> Apart from that, a large number of research studies emphasize its antitumor effect in glioblastoma<sup>53</sup>, leukemia<sup>54</sup>, cervical cancer<sup>55</sup>, nonsmall-cell lung cancer<sup>56</sup>, and pancreatic cancers with a greater cytotoxic strength in cancer cells in comparison to non-transformed cells.<sup>57</sup> Moreover, it has an exponential role in inhibition of cancer cell growth and clonogenicity, inducing apoptosis or suppressing tumor metastasis, signifying that it might be an excellent substitute for managing cancer.<sup>13</sup>

#### *Role of Honeybee venom in treating aggressive Breast Cancer cells*

Recently, a detailed and comprehensive study carried out by Duffy et al. has been published in the Journal, Precision Oncology. It establishes the role of bee venom and melittin in suppressing the activation of growth factor receptors in HER2-enriched and triple-negative breast cancer. Irrespective of many years into study for the exact functioning and preciseness of this venom, molecular mechanism and selectivity for the bio molecular constituents of honeybee venom are still unclear to an extent especially in breast cancer, the most widespread cancer in women across the globe.<sup>12, 58</sup> It is all about the depiction of the powerful and effective induction of cell death by melittin specifically in the aggressive triple-negative and HER2-enriched breast cancer subtypes. Research reveals the actual system supporting the anticancer selectivity of melittin and summarizes the management approaches used to tackle aggressive breast cancers.<sup>58</sup> As a thorough understanding of the molecular basis and preciseness of bee venom action against cancer cells is crucial, it is important to manufacture and optimize new active therapeutics from a natural product that are not only readily available but also economical to develop in different countries worldwide. The anticancer effect of mellitin and bee venom on different cancer cells has been studied previously which is shown in Table 1.

Treatment condition	Cancers	Cell lines	Dose	<b>Results/ Mechanisms</b>	Reference
	Lung cancer	NCI-H1299 cells	1, 10 μg/mL	Induction of apoptosis in NCI-H1299 human lung carcinoma cells Apoptosis induction by	39
condition	Mammary carcinoma	MCF7 cells	7.5, 12.5 μg/mL	mitochondria- dependent pathway	38
	Prostate cancer	LNCaP cells, DU145 cells, PC- 3 cells	1, 5, 10 μg/mL (in vitro)	Decreasing cell growth via activation of caspase pathway	59
	Melanoma	A2058 cells	0.5, 1, 2, 4 μg/mL	Apoptosis induction through calcium reliant & caspase-independent pathway	41
	Ovarian cancer	A2780cp cells 4	8 μg/mL 8 μg/mL (24 h)	Induction of apoptosis	60
	Gastric cancer	KI735M2	2.8,11,14.2 µg/mL 10 µg/mL (24 h)	Repressed cell multiplication in vitro Initiating apoptosis with	61
	Leukemia	U937 cells	0.5, 1, 2, 3 μg/mL	Bcl-2 and caspase-3 as key regulators by down-regulation of ERK and Akt signal pathway	43
	Cervical carcinoma	HeLa cells	0.7125, 1.425, 2.85, 7.125 or 14.25 µg/mL (72 h)	Impeding cell growth, cell propagation, and clonogenicity of HeLa cells, via inhibition of calmodulin	62
	Osteosarcoma	MG63 cells	0.5, 1, 2 μΜ	Induction of cell apoptosis by phospholipase A2- independent Ca2+ entry	63
	Prostate cancer	LNCaP cells, DU145 cells, PC- 3 cells	0.5,1, 2.5 μg/mL 2.9, 1.5 and cancer 1.8 μg/mL (72 h)	Suppressing cell growth by activation of caspase pathway through inactivation of NF-kB	59

Table 1. Anticancer effects of mellitin and bee venom on different cancer cells



Treatment condition	Cancers	Cell lines	Dose	Results/ Mechanisms	References
	Hepatocellular cancer	MHCC97L cells, MHCC97H cells	4, 8 μg/mL (in vitro), 80 μg/ kg (in vivo) respectively	Inhibiting cell metastasis by suppressing Rac1-dependent pathway	64
	Breast cancer	MCF-7 cells	0.5, 1, 2 μg/mL	Inhibiting cell proliferation and attack by inhibiting PI3K/Akt/mTOR signaling pathway	65
Melittin	Renal cancer	Caki-1 cells	1, 2, 3 μg/mL	Suppressing PMA- induced invasion and migration by inhibiting MMP-9 expression	66
	Esophageal cancer	ECA109 cells, TE13 cells	0.5, 1 μM 1.88, 1.64 μM (24 h) respectively	Radio-sensitizing esophageal squamous cell carcinoma with induction of	67
	Skin cancer SCC12 Inhibite	apoptosis Inhibited cell proliferation in vivo	19		
	Retinoblastoma MEL	Y79	10–500 ng/mL	Induced cell apoptosis via AA pathway	68

Effect of melittin and combined compounds in triple negative breast cancer and HER-2 enriched cell lines

Triple negative breast cancer and HER-2 enriched tumors are among the most aggressive forms of tumors. These tumors do not express genes such as estrogen receptors or progesterone receptors but show high level of genomic instability, invasiveness and repetition in comparison to other cells of breast cancer. Mutations in tumor suppressor genes are most prevalent.<sup>69</sup> For this reason, treatment against such cancer cells is very difficult to attain. TNBC and HER-2 cells show the absence of a lot of important molecular targets.<sup>70</sup> It also shows poor prognosis as compared to other subtypes of cancers in females.<sup>71</sup> Melittin extracted from honeybee venom shows a very targeted and selective action in TNBC and HER-2 aggressive cancer cells. It attacks the cell surface by disturbing the phosphorylation process at receptors. Ligand induced phosphorylation is especially targeted. This compound also suppresses the activation of HER-2 which is overexpressed in breast cancer.58

Melittin displays a wide range of effective properties such as being anti-fungal, anti-bacterial and anti-cancerous. The most striking feature of melittin is its ability to form pores. This compound binds with the negatively charged phospholipids present in the membrane. Binding forms pores through which atomic ions can easily pass. It also shows the surfactant activity in creating pores. The usual problem in treating cells is the non-targeted attack on body cells with no differentiation between normal cells and undifferentiated cells. Melittin is nearly a 100% target specific treatment strategy. However, in both TNBC and HER-2 cancer cells, melittin targets cancer cells and even aggressive cancer cells. As compared to other compounds, melittin causes a minimum damage to the normal cells because the membrane potential of cancerous cells is larger due to the outflow of ions and molecules through pores.<sup>24</sup>

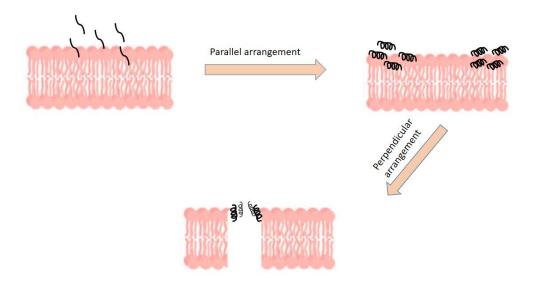
Signaling pathways are also disturbed in triple negative breast cancer and HER-2 cancer cells such as the P13K/AKT as well as mTOR. Alterations in these pathways disturb a lot of downstream gene expression in cascades. Resultant genomic instability is at its peak, causing aggressive cancer cells. Melittin has auspicious potential in normalizing the expression levels of genes involved in progression of tumor formation.<sup>58</sup>

# Mechanism of action of melittin in TNBC and HER-2 breast cancer cell lines

Melittin is known to have remarkable positive effects against cancers especially breast cancer using different mechanisms to initiate cancer cells killing. Among many strategies, one of the most accepted mechanisms is "model for pore forming peptides" on cancer cells surfaces. According to this model, monomers of melittin get attached to the cell membrane. However, these monomers do not act independently but show a collective action where all the monomers attack the receptors simultaneously. Moreover, melittin has the capacity of acting upon the cell membrane at even lower concentrations. In such conditions, melittin forms pores that allow the conduction of only atomic ions.<sup>72</sup>

Structural conformation of melittin alters while binding to the cell membrane. Binding occurs within no less than milliseconds and results in amphipathic alpha helical confirmation. The resulting structure settles with parallel or perpendicular to plane of membrane. In the parallel conformation, melittin does not get activated, while perpendicular confirmation is of particular importance to anti-cancerous effect.<sup>73</sup>

The action of melittin is accomplished in two steps. Firstly, the melittin monomers at low concentration bind to the cell membrane in the parallel fashion, and during this process the compound is kept in the inactive state. Secondly, the arrangement is shifted from parallel to perpendicular manner, and hence causing the activation as shown in the Figure 2. Activation leads to pores formation. Mechanism of conversion of parallel to perpendicular conformation is yet to be understood clearly. Another important aspect is that melittin has a strong affinity towards the phosphatidylcholine of membranes due to the cationic form of melittin. Concentration of melittin is crucial for estimating the action rate; interactions however, the strong towards phosphatidylcholine PC heads suggest that ratio of concentration of melittin to lipid molecules is a determining factor for anti-cancerous activity and studies have been conducted to understand this aspect of melittin.<sup>73, 74</sup>.



**Figure 2.** This model represents pore formation by melittin in membrane. Monomers of melittin accumulate on cell membrane and orient in parallel arrangement. Upon reaching a threshold concentration, it undergoes a shift from parallel to perpendicular arrangement. This perpendicular arrangement is crucial for pore formation activity. Figure 2 is adapted with permission from van den Bogaart *et al.* (2008).

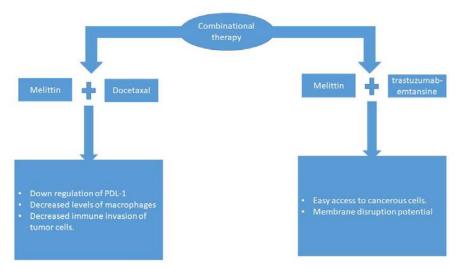
Meanwhile, scientists are working to get a detailed insight into the binding mechanism of melittin to cell membrane. Since melittin has strong affinity towards selective regions of membrane, Duffy *et al.* conducted a study to identify those segments of melittin that show maximum potential for binding. The results showed that melittin forms the attraction and bond with the negatively charged cell membrane through its positively charged C terminus. The binding is facilitated as the C terminus is carrying a positive charge which assists in the formation of an alpha helix. Binding leads to the creation of pores and ultimately the lysis of the cancerous cells. The evidence of functional involvement of C terminus of melittin in anti-cancerous pore formation was confirmed when the scientist designed a negatively charged C terminus of melittin and checked for pore formation ability. None of the cells appeared to show any signs of cell lysis and the effect can be reused by replacing the negatively charged terminus again by positively charged C terminus and formation of alpha helix.<sup>58</sup>



#### Melittin and Combinational therapy

Effectiveness of a treatment depends upon two factors; maximum efficacy along with minimum side effects and targeted action. The precise and careful combination of therapeutic strategy can provide the patient with maximum desirable benefits, giving least recurrence and toxicity.<sup>75</sup> One recent combinational therapy involves the robust and synergistic anti-

cancerous effect of melittin and docetexal, showing favorable effects on breast cancer cell lines. Melittin in combination with docetexal causes the down regulation of PD-L1 and lessens the immune evasion process of cancerous cell. It also causes the levels of tumor associated macrophages to decrease.<sup>76</sup> Combinational therapeutic strategy is shown in Figure 3.



**Figure 3.** The enhanced activity of melittin in combination with several anti-tumor drugs can be seen. Docetaxal and trastuzumab-emtansine in combination with melittin result in anti-cancerous activities such as decreased immune invasion, easy access as well as membrane disruption of cancer cells.

Melittin has a very selective action against the HER-2 related tumors and so a combinational therapy of melittin and HER-2 targeted agents could be a very potent strategy. Melittin along with monoclonal antibodies, trastuzumab-emtansine and antibody-drug conjugates can have desirable effects as melittin can enhance the efficacy by assisting in easy access of drugs to cancerous cells through membrane disruption. Melittin could also be delivered through targeted nanoparticle approaches such as those previously reported with "nanobees".<sup>77</sup>

#### CONCLUSION

In conclusion, this review article focused on the role of Honey Bee Venom and its component melittin in rapidly inactivating two types of breast cancer cells which are otherwise difficult to treat. Breast cancer is the most prevailing cancer amongst women all over the world. Although different treatment options are available it is crucial to come up with an alternative therapy which carries no side effects. For hundreds of years, humans have been utilizing honey and venom from the *Apis mellifera* honeybee as medicine. Quite recently, scientists have exhibited the targeted effect of mellitin and honeybee venom in suppressing the growth factor receptor activation in HER2-enriched and triple-negative breast cancer. It is also lethal to a variety of tumors such as melanoma, pancreatic, ovarian and lung cancers in lab tests. This treatment has a surprising effect on the reduction of the chemical messages of cancer cells essential for the cell growth and division which other therapeutics are unable to carry out. Honeybee venom is accessible worldwide and offer economical and easily available treatment solutions for developing countries. As melittin holds the potential to treat the breast cancer in future, it is important to carry out further research to find out whether venom of some genotypes of bees are more effective. Moreover, studies should be conducted in future to evaluate the ideal method of provision of melittin, level of toxicity and average accepted dose. It will not only open avenues for study of advanced treatment strategies for breast cancer but also make it possible to explore the miraculous properties of natural remedies found in the world.

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#### **CONFLICT OF INTEREST**

Authors declare no conflict of interest.



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#### **Review Article**



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## Surgical Prevention of Breast Cancer-Related Lymphedema: Delayed Distal Lymphaticovenicular Anastomosis– An Alternative to the Classic LYMPHA Technique

Lynn M Orfahli<sup>a</sup>, Tony C.T. Huang<sup>b</sup>, Wei F Chen\*<sup>a</sup>

<sup>a</sup> Department of Plastic and Reconstructive Surgery, Cleveland Clinic Foundation, Cleveland, United States <sup>b</sup> Division of Plastic Surgery, Department of Surgery, Mayo Clinic, Rochester, United States

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Keywords: lymphedema, supermicrosurgery, lymphaticovenicular anastomosis, lymphatic reconstruction, distal delayed LVA ABSTRACT

Breast cancer-related lymphedema (BCRL) is a devastating potential complication of axillary lymphadenectomy and radiotherapy. Several effective surgical treatment measures now exist, including lymphaticovenicular anastomosis (LVA), vascularized lymph node transplant (VLNT), and vascularized lymph vessel transplant (VLVT) for fluid-predominant disease, and liposuction and radical excision for solid-predominant disease. Super-microsurgical LVA is of particular interest, owing to its minimally invasive nature and highly favorable outcomes in the hands of experienced supermicrosurgeons. As LVA techniques are refined and improved, interest is rising in utilizing it to prevent the manifestation of disease in the first place. Lymphatic microsurgical preventive healing approach (LYMPHA), also known as immediate lymphatic reconstruction (ILR), is the most widely used approach. It involves performing axillary LVA immediately following axillary lymphadenectomy. While preliminary results are favorable, the high-pressure proximal axillary venous branches used in ILR and the site's vulnerability to damage from radiotherapy endanger the long-term patency of these anastomoses. Moreover, a theoretical oncologic concern exists regarding creating a direct conduit for the remaining malignant cells in the axilla into the circulation. Finally, coordinating ILR with axillary lymphadenectomy creates significant logistical challenges. Delayed, distally-based LVA (DD-LVA) has emerged as an alternative method that avoids these issues. This article presents an overview of the development of preemptive lymphatic reconstruction, and the senior author's approach to the novel technique of DD-LVA.

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#### INTRODUCTION

A variety of treatment strategies are now available to target fluid-predominant lymphedema. In cases where functioning lymphatic vessels can be found,

* Address for correspondence	*	Address	for	corres	pondence
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Wei F Chen, MD, FACS

Professor of Plastic Surgery, Head, Regional Microsurgery & Supermicrosurgery, Co-Director, Center for Lymphedema Research and Reconstruction. Address: Department of Plastic and Reconstructive Surgery, Cleveland Clinic Foundation, 9500 Euclid Avenue, Desk A60, Cleveland, OH 44195. Tel: +1 216 444 5415 Fax: +1 216 444 9414 Email: weifchen@hotmail.com supermicrosurgical lymphaticovenicular anastomosis (LVA) is a suitable option. When the disease process causes excessive lymphatic injury, healthy lymphatic tissue can be microsurgically transferred in the form of vascularized lymph node transplant (VLNT) or its more novel successor, vascularized lymph vessel transplant (VLVT).<sup>1, 2</sup> As experience with LVA has grown, surgeons have expanded its indications to include nascent or subclinical lymphedema. Its most commonly known form, lymphatic microsurgical preventative healing approach (LYMPHA), involves performing proximal LVA immediately following mastectomy and axillary lymph node dissection.<sup>3</sup>



However, this technique is not truly "preventive" or "prophylactic," because an injury to the lymphatic system has already occurred.<sup>4-6</sup> Therefore, immediate lymphatic reconstruction (ILR) has been adopted by some as an alternative name.<sup>7-9</sup> While preliminary results are favorable, concerns exist surrounding scheduling issues, unfavorable proximal lymphovenous pressure gradients, oncologic safety, and anastomotic injury from postoperative radiation.<sup>10</sup> Delayed, distally-based LVA (DD-LVA) is an emerging alternative method that allows surgeons to avoid these issues. In this article, the senior author outlines his approach to LVA for subclinical, or asymptomatic, lymphedema, supplemented with a focused review of its development, techniques, outcomes, and controversies.

#### **RESULTS AND DISCUSSION**

## *Classic and modified techniques of immediate lymphatic reconstruction*

As surgical treatment of lymphedema has been refined and improved, interest arose in using it to prevent the manifestation of this disease. In 2009, Boccardo, *et al.* published their initial report on immediate proximal LVA following axillary lymphadenectomy. Initially termed LYMPHA, it involved mapping limb-draining lymphatics with isosulfan blue and anastomosing them to branches of the axillary vein.<sup>3</sup> At 4-year follow-up, 4% of their 74-patient cohort had developed lymphedema, versus an estimated incidence rate of 20-40% in this population at large. Rates of lymphorrhea and lymphocele, complications associated with increased regional intralymphatic pressure, were also reduced.<sup>11-13</sup>

As surgeons began to adapt this technique into their own practices, a push arose to move away from the term LYMPHA. Lymphadenectomy is inherently injurious to the lymphatic system—a concept confirmed by postoperative indocyanine green (ICG) lymphographic studies of asymptomatic limbs demonstrating significant rates of subclinical lymphatic dysfunction.<sup>4-6</sup> Therefore, many surgeons began describing this procedure as an immediate lymphatic reconstruction (ILR), rather than preventive or prophylactic.<sup>7-9</sup>

Boccardo and colleagues' results have been replicated by several retrospective and prospective studies. A 2018 meta-analysis of 4 papers found that patients who underwent upper- or lower-extremity ILR had a relative risk of 0.33 for developing lymphedema when compared to controls (P < 0.0001).<sup>14</sup> A broader 2019 meta-analysis of 19 papers found a 12% reduction in lymphedema incidence after axillary lymph node dissection (ALND) and a 23.1% reduction in lymphedema incidence after ALND combined with radiotherapy  $(P=0.029 \text{ and } 0.004, \text{ respectively}).^{15}$  While these results are encouraging, the data should be interpreted

with caution. The incidence of lymphedema peaks 2 years after axillary lymph node dissection; however, many published studies utilized follow-up periods of less than 2 years. Additionally, the most common screening methods for lymphedema were volumetric measurements, circumference measurements, or clinical evaluation—none of which are adequately accurate diagnostic measures, especially in early-stage disease.<sup>5, 15, 16</sup> More robust and long-term follow-up protocols are warranted to gain a true understanding of the impact of this procedure.

Immediate reconstruction combines oncologic surgery and lymphatic surgery into one event, which can be more convenient for patients. However, oncologic surgeons cannot predict the necessity of a lymphadenectomy pre-operatively. Coordinating ILR in these uncertain circumstances can cause significant scheduling difficulties for the lymphatic supermicrosurgeon. Delaying lymphatic reconstruction in a staged fashion post-lymphadenectomy alleviates this logistical burden, allowing for more optimal scheduling of this delicate procedure. While delaying LVA does require that patients return for a separate procedure, this minimally invasive technique can be performed under local anesthesia with sedation; healthy lymphatics abound in subclinical lymphedema and only one or two small incisions are needed. The literature on the outcomes of delayed LVA is limited. In 2016, Yamamoto, et al. published a study of 14 patients with subclinical lower extremity lymphedema (ICG dermal backflow [DB] Stage 1) who underwent preemptive LVA at the groin. One year postoperatively, 6 remained at DB Stage 1 and 8 were downstaged to Stage 0 (p<0.001); subjective symptomatology was significantly reduced (p= 0.008).17

Classic ILR entails the use of a proximal site for anastomosis. However, this is associated with several theoretical concerns. From an oncological safety standpoint, performing LVAs in a cancer-containing field could create a direct passage for any remaining malignant cells to enter the systemic circulation. Additionally, many lymphadenectomy patients require postoperative radiotherapy; long-term LVA patency is questionable given the resultant axillary or inguinal fibrosis in these patients. Moreover, the high-pressure proximal axillary and inguinal venous branches used in proximal ILR can result in unfavorable LVA pressure gradients-the very issue that led to the inconsistent outcomes of LVA's predecessor, traditional microsurgical lymphovenous bypass (LVB) attempted in the 60s and 70s (18-23)<sup>18-23</sup>, and the subsequent switch to distal locations for supermicrosurgical therapeutic LVA.<sup>24-28</sup> If proximal anastomoses are not expected to be patent long-term following therapeutic LVA, should this be expected following preemptive



LVA? Utilizing distal sites for lymphatic reconstruction facilitates the selection of small, low-pressure venules, leading to favorable pressure gradients and anterograde lympho-venous flow. In addition, the superficial location of the distal lymphatics facilitates easier supermicrosurgical manipulation. This is especially advantageous in delayed reconstruction, as scarring and inflammation from recent lymphadenectomy and/or radiotherapy increases the difficulty of dissecting proximal lymphatic beds. Finally, creating distal LVAs keeps any potential remaining cancer cells well out-of-range of these lympho-venous connections.

After experiencing the above issues with classic IRL, the senior author began performing delayed, distally-based LVA (DD-LVA) for preemptive lymphatic reconstruction. Delaying this procedure resolves the logistical issues inherent in performing ILR following sentinel lymph node biopsy and lymphadenectomy. Because LVA is minimally invasive, it can be performed under local anesthesia as an outpatient surgery to avoid subjecting the patient to a second round of general anesthesia. By moving the anastomoses distally, damage from high-pressure proximal veins and adjuvant radiotherapy can be avoided, and the theoretical oncologic risk is mitigated.<sup>10</sup> The limited literature on DD-LVA has demonstrated favorable preliminary results. In 2014, Onoda and colleagues described preemptive LVA of the asymptomatic contralateral limbs of ten patients with unilateral lower extremity lymph-edema. All anastomoses were created via a single incision over the ankle. Complications were limited to one case of lymphorrhea that necessitated suturing of the surgical site. Six months postoperatively, all patients remained at their preoperative Campisi stage (50%: Stage 0, 50%: Stage 1A).<sup>29</sup> While longer follow-up with robust diagnostic tools is needed to establish the efficacy of DD-LVA, these preliminary results demonstrate that this is a safe and promising alternative to prevent the consequences of this devastating disease.

#### DD-LVA technique – our current technique of choice

The senior author's technique for DD-LVA is as follows  $^{10,\,30\text{-}32}$ :

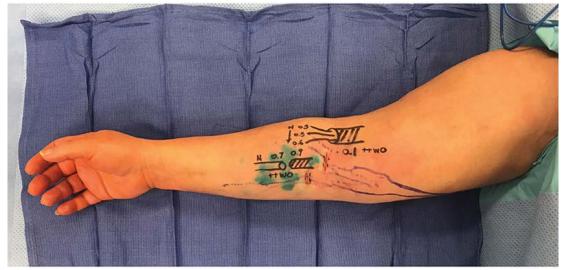
As with any therapeutic intervention for lymphedema, proper patient evaluation and selection are paramount to the success of DD-LVA for subclinical disease. Patients with clinically overt disease (i.e., significant swelling, severe dermal backflow patterns on ICG, skin changes, cellulitis, etc) will require stratification to therapeutic LVA, VLNT, VLVT, or debulking surgery, as previously described.<sup>2, 32</sup> DD-LVA is indicated following lymphatically injurious events such as axillary/groin lymph node dissection or adjuvant radiation therapy. Patients undergo ICG lymphography 1 month postoperatively/post-radiation in order to establish a baseline. Frequently, lymphatic injury is already detectable at this time, despite absence of swelling upon visual inspection or bioimpedance spectroscopy (BIS) measurement. Some of these patients will already be experiencing prodromes of lymphedema, such as sensations of heaviness, tightness, or generalized discomfort. Thus, establishing baseline symptomatology with a thorough history and any of the several quality of life (QoL) questionnaires is recommended.<sup>33-38</sup>

In overt lymphedema, the overall quality of available lymphatics is often less than ideal, especially as one moves proximally, owing to the proximal-to-distal progression of this disease.25-27 This reality necessitates planning numerous incisions, starting at the ankle or wrist, to ensure a sufficient quantity of LVAs.<sup>24, 28</sup> In contrast, in subclinical lymphedema, sufficient healthy lymphatics can easily be found with only one or two incisions, and surgeons have more freedom to choose an ideal site that reliably offers high-flow lymphatic vessels. Thus, when performing DD-LVA, the senior author prefers areas adjacent to the elbow. The movement of the elbow, combined with compression between fascial layers, theoretically enhances the pumping of lymphatic fluid.<sup>39,40</sup> Superficial lymphatic vessels are mapped by injecting ICG just distal to the elbow. An infrared vein finder is used to locate veins adjacent to the mapped lymphatics, and one or two 2-3 cm incisions are marked in proximity to both (Figure 1). Following this, isosulfan blue is injected 2 cm distal to each marked incision to further enhance identification of lymphatic vessels. Following incision, mapped vessels are skeletonized using meticulous supermicrosurgical dissection, which ends once the underlying deep fascia is reached. The healthy state of the dissected lymphatics can be confirmed by visualization of peristalsis and of lymph fluid leak after vessel transection. The high-quality lymphatics and low-pressure distal venules used in DD-LVA create favorable lymphovenous pressure gradients. Because lymphatic pressure exceeds venous pressure, any anastomotic configuration should be successful. Thus, the technically straightforward end-to-end anastomosis is often chosen. If awkward vessel positioning, vessel number mismatch, or vessel size mismatch are encountered, sophisticated anastomotic more configurations (Figure 2) may be more appropriate.

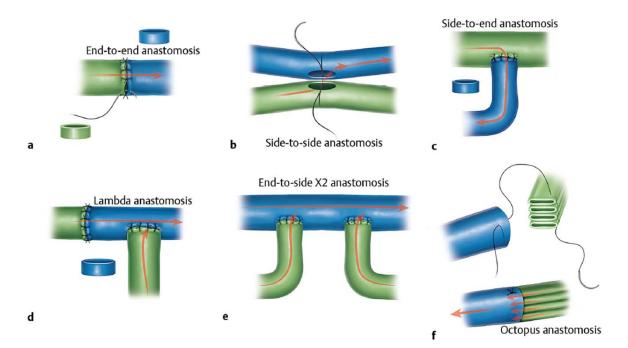
Anastomosis is performed with 12-0 nylon on a 50  $\mu$ m needle; for vessels 0.5 mm in diameter or larger, 11-0 nylon can be used. If the vessel lumen is too small to accommodate supermicrosurgical forceps tips, the needle tip should be used to evert the vessel edge against the side of the forceps to prevent



backwalling (Figure 3). A 7-0 monofilament nylon suture can be utilized as a stent if needed. Sutures are placed until a watertight anastomosis is achieved and to ensure that lymphatic pressure exceeds venular pressure. Following anastomosis, patency is confirmed with ICG lymphography and/or the "washout" sign (absence of blood in the venous lumen); because lymph vessel contractile function is preserved in subclinical lymphedema, this sign is quickly and easily observed. The low number of incisions and high-quality vessels allow a relatively technically straightforward procedure; thus, operative time is generally less than 1 hour. The procedure is performed on an outpatient basis. For most patients, no narcotic pain medication is needed.

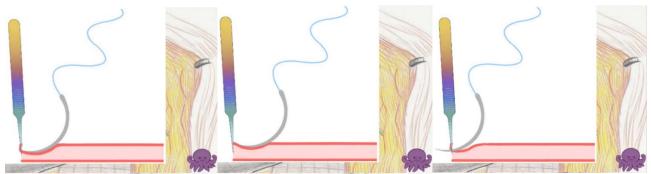


**Figure 1.** Following lymphatic mapping (green) with ICG lymphography and venous mapping (blue) with an infrared vein finder, 2-3 cm incisions (red) are marked near the elbow in proximity to these two structures. Dissected lymphatics are graded as normal, ectatic (mild injury), contracted (moderate in jury), or sclerotic (severe injury) – in subclinical lymphedema, a sufficient quantity of healthy/normal lymphatics should be available for use. Because lymphatic vessels are sufficiently healthy, only one to two incisions are needed to create an adequate number of anastomoses. Following anastomosis, patency is confirmed with positive "washout" signs (absence of blood in the venous lumen); because lymph vessel contractile function is preserved in subclinical lymphedema, this sign is quickly and easily observed. The final anastomoses created in this patient are depicted in the diagram on the arm. Measurements of each vessel (in mm) are marked (erratum: the proximal-most vein, marked 0.1, measured 1.0 mm). ICG: indocyanine green, N: normal, ++WO: briskly positive washout sign, Tubes without fill and lines: the utilized lymphatics, Tubes with dashed line fill: the utilized veins.





**Figure 2.** End-to-end anastomosis is often sufficient in LVA for subclinical lymphedema. However, several LVA configurations are available to maximize drainage pathways in the face of awkward vessel positioning, vessel size mismatch, or vessel number mismatch. Named in a lymphatic-to-vein convention: A) Simple end-to-end anastomosis; B) Side-to-side anastomosis; C) Side-to-end anastomosis; D) Lambda anastomosis; E) Double end-to-side anastomosis; F) Octopus anastomosis. We recommend that surgeons train in all of these configurations in order to efficiently address the difficult vessel positioning and size and number mismatch commonly encountered during LVA. LVA: Lymphaticovenicular anastomosis.



**Figure 3.** If the vessel lumen is too small to accommodate the insertion of supermicrosurgical forceps tips to prevent backwalling, this alternative method can be employed. A) The side of the forceps is used as a barrier to prevent movement of the vessel wall. B) the needle tip is used to evert the vessel edge at a point  $\sim$ 2 needle diameters from the edge against the side of the forceps. C) Once the edge is everted, the needle is driven through the wall.

Postoperatively, following a month of bandage compression, all patients are fitted with a 30-40 mmHg circular knit compression garment. They may also commence other components of complex decongestive therapy (CDT) at this time, which can include manual lymphatic drainage (MLD) and physiotherapy.<sup>41</sup> A 2015 Cochrane review examining the various components of CDT in subclinical lymphedema could not draw any firm conclusions about their efficacy; however, it is a low-harm intervention that we anecdotally find to be useful in this subgroup.<sup>42</sup> Obesity impairs lymphatic function. Not only is it a major risk factor in the development of breast-cancer related lymphedema, but it has been shown to be an independent cause of lymphedema as well.43 Therefore, weight control with diet and exercise should be incorporated into post-operative management, with the goal of achieving height- and age- appropriate weight.44 The benefits of exercise are twofold, as it also enhances lymphatic drainage through muscle contraction.45 However, not all exercise produces similar results. In our experience, many patients report that swimming is helpful but that walking exacerbates symptoms. This is likely because walking places arms below the level of the heart and creates centrifugal forces through arm swing. Ultimately, each patient's lymphatic drainage and

production is unique; regular post-operative followup is required to monitor changes and adjust nonoperative treatment accordingly.

#### CONCLUSION

The efficacy of the procedure is monitored based on the relief of prodromal symptoms (heaviness, tightness, and discomfort) and with ICG lymphography at 3, 6, and 12 months postoperatively. Compression garments are discontinued at 3 months postoperatively if 1) the patient reports relief of prodromal symptoms and 2) ICG lymphography demonstrates sufficiently improved or resolved lymphatic injury.

Now that lymphedema can be controlled with surgical intervention, focus has expanded to the prevention of the manifestations of this devastating disease. DD-LVA offers a promising method for the prevention of symptomatic lymphedema without the theoretical risks and logistical obstacles associated with ILR. As more insight is gained into the pathophysiology of lymphedema and long-term outcomes are collected, effective patient selection and treatment planning can continue to be refined for more effective control of lymphedema.

#### **CONFLICT OF INTEREST**

The author declares no conflicts of interest.

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Breast Cancer Recurrence Survival Among Iranian Patients; A 17-Year Retrospective Cohort Study

Amirreza Ehsani<sup>a</sup>, Nahid Nafissi<sup>\*a</sup>, Mohammadamin Joulani<sup>b</sup>

<sup>a</sup> Department of Surgery, Iran University of Medical Sciences (IUMS), Tehran, Iran.

<sup>b</sup> Student Research Committee, Iran University of Medical Sciences (IUMS), Tehran, Iran

ARTICLE INFO	ABSTRACT
Received: 02 April 2021 Revised: 31 July 2021 Accepted: 04 August 2021	<ul> <li>Background: Nowadays breast cancer (BC) is the most common cancer in women. More than 1.5 million cases are detected yearly. Survival of patients is dependent on several factors. Metastasis and cancer recurrence of different types and in different locations have various outcome.</li> <li>Methods: This is a retrospective cohort study to describe survival of patients after diagnosis of breast cancer based on receptor subtypes and sites of metastasis among Iranian population. A total number of 2051 females with breast cancer were evaluated and among these, 138 patients with recurrent BC were investigated.</li> <li>Results: The 1-year survival of local, bone, visceral and brain metastasis, bone and 64.99%, 63%, 32.83%, and 21.57%, respectively. Based on sites of metastasis, bone and</li> </ul>
Keywords: Breast Cancer, recurrence, metastasis, survival	local metastasis showed the best survival while brain and visceral metastasis had the worst survival and prognosis. <b>Conclusion:</b> Our study showed that Her2 enriched positive BCs had the worst survival, this may be due to Trastuzumab uncovered insurance till 10 years ago in our country. Also, drugs related to luminal A and B which are used to improve their survival and hormonal therapy could be associated with their better prognosis in comparison to triple negative receptor subtype. But this study showed that triple negative BC had better survival.

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#### **INTRODUCTION**

Nowadays breast cancer (BC) is the most common cancer in women.<sup>1</sup> More than 1.5 million cases are detected yearly.<sup>2</sup>

BC has been known as a heterogeneous disease which might have various clinical outcomes.<sup>3</sup> Medical therapies can be preventive in earlier stages<sup>4</sup>.

in spite of all kinds of therapies still patients with breast cancer are at high risk of relapse after 5 years<sup>5</sup>. Adjuvant chemotherapy can decrease the risk of recurrence during first 5 years following to diagnosis<sup>6</sup>.

Address for correspondence: Nahid Nafissi, MD Address: Rasool Akram Hospital, Sattarkhan St., Tehran, 14456 13131, Iran Email: nahid.nafissi@gmail.com Prognostic factors which can almost anticipate the chance of early recurrence of breast cancer are limited.<sup>7</sup> As a result of several studies the most important breast cancer prognostic factors are tumor size and number of involved lymph nodes.<sup>8</sup>

Size of the tumor, histological grade, HER2 receptors and the estrogen progesterone levels are known factors that have also value for prognosis of patients with risk of breast cancer recurrence.<sup>9</sup>

Moreover, studies suggested that extended tamoxifen therapy (extra 5 years) in women with breast cancers at premature stages can lower the risk of late recurrence.<sup>10, 11</sup>

although so many trials had been performed on factors which might be capable of displaying prognosis of BC and its recurrence, all the known markers used

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currently or recently introduced cannot precisely address the timing of metastatic recurrence.<sup>12</sup>

We are focusing on understanding the breast cancer recurrence and survival among Iranian population. Describing survival of patients after diagnosis of breast cancer based on receptor subtypes and sites of metastasis which provide trustworthy information to categorize breast cancer severity according to receptor subtypes and sites of metastasis. To achieve this goal, we analyzed data acquired from previous studies and develop a horizon through controlling the risk of recurrence.

#### METHODS

In this Retrospective Cohort Study, A total number of 2051 female patients with breast cancer who were referred to Hazrat-E-Rasul Akram hospital and Khatam-Ol-Anbia hospital were followed from October 2003-2020.

Among these patients, 138 cases with recurrent breast cancer were included and non-recurrent patients were excluded. Each patient is followed every 6 months in order to update the medical record for at least 5 years. Patients who were male, had lost data or those who had insufficient follow up period also were excluded. Patient's information was extracted from their clinical records in Rasool Akram and Khatam-Ol-Anbia hospitals. Variables include demographic data ; age at the diagnosis of breast cancer, number of pregnancies, number of abortions, family history of breast cancer, marital status, breast feeding duration, and taking oral contraceptives also pathology and surgical findings such as type of tumor pathology, tumor size, number of total lymph nodes, status of sentinel node, perineural or vascular invasion, calcification, necrosis, estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), percentage of KI67 antigen, status of receiving neoadjuvant therapy, and surgery procedure are recorded. Likewise, overall and disease-free survival (DFS) status, recurrence location is investigated at the end of the follow up.

Three types of tumor pathology were reported as invasive ductal carcinoma (IDC), invasive lobular carcinoma (ILC), ductal carcinoma in situ (DCIS). Three surgical procedures were as follow; breast conserving, modified radical mastectomy (MRM) and subcutaneous mastectomy with reconstruction. DFS (disease-free survival) in this study was the time interval between the diagnosis of breast cancer and its recurrence. Also, recurrence site was categorized based on their importance local, bone, visceral and brain recurrence. The Kaplan-Meier survival estimate, survival rate and Log-rank test were performed and reported. This study was done based on declaration of Helsinki, and all the patients filled and signed the consent form to participate in this study and this trial has been approved by the ethical committee of Iran University of Medical Sciences (IUMS). All of the collected data were analyzed by STATA version 13 and level of significance was considered <0.05. Descriptive analysis was presented as mean ( $\pm$ SD) for quantitative variables, frequency and percentage for categorical variables.

#### RESULTS

The mean age of patients was  $47.95\pm12.01$  years at diagnosis and according to the history of patients there was a positive family history for breast cancer in 36 patients (26.1%). 26 patients (18.8 %) mentioned no history of breast feeding and 13 (9.4%) patients were married but had no child and 12 (8.7%) were not married. Demographic data, tumor details and recurrence information were shown in tables 1-3.

Breast tumor features in different patients analyzed based on pathology report. Mean size of tumors among all eligible cases was  $3.06\pm1.70$ centimeters. Total number of resected lymph nodes in all cases was  $10.33\pm6.49$  and among these resected lymph nodes  $6.97\pm5.69$  were involved by difference breast cancers.

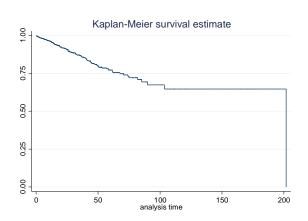
**Table 1.** Baseline characteristics of 138 patients with

 recurrent breast cancer

Variable	2
Quantitative	Mean±SD
Age	47.95±12.01
at Diagnosis	years old
Pregnancy Times	2.86±1.42
Abortion Times	1.34±0.48
Qualitative	Number (%)
Family History Positive	36 (26.1%)
Marital Sta	itus
	12 (8.7%)
	13 (9.4%)
	113 (81.9%)
Breast Feed	ling
	26 (18.8%)
	8 (5.8%)
	16 (11.6%)
	39 (28.3%)
	49 (35.5%)
Oral Contrac	eptive
	113 (81.9%)
	6 (4.3%)
	7 (5.1%)
	12 (8.7%)

Variable		Variable		
Quantitative	Mean±SD	Multicentric	11 (8%)	
Tumor size Centimeter	3.06±1.70	Neoadjuvant	22 (15.9%)	
Total lymph nodes Numbers	10.33±6.49	ER Positive	87 (63%)	
Positive lymph nodes	6.97±5.69	PR Positive	84 (60.9%)	
Numbers	017720105	HER <sub>2</sub> Positive	28 (20.3%)	
Qualitative	Number (%)	KI67 Percent	29.17±20.04	
Pathology		Grade		
Invasive DC	98 (71%)	1	5 (3.6%)	
Invasive LC	26 (18.8%)	2	56 (40.6%)	
Mixed Invasive	2 (1.4%)	3	77 (55.8%)	
DCIS	11 (8%)	Stage		
DCIS + LCIS	1 (0.7%)	2A	31 (22.5%)	
Sentinel node		2B	17 (12.3%)	
Not Done	36 (26.1%)	3A	27 (19.5%)	
Done & Positive	57 (41.3%)	3B	4 (2.9%)	
Done & Free	45 (32.6%)	3C	24 (17.4%)	
Perineural invasion Positive	29 (21%)	4	35 (25.4%)	
Vascular invasion	66 (47.8%)	Туре		
Positive		Luminal A	79 (57.2%)	
Calcification Positive	26 (18.8%)	Luminal B	11 (8%)	
Necrosis Positive	40 (29%)	Triple Negative	31 (22.5%)	
Surgery type		Her2 Enrich	17 (12.3%)	
Breast Conserving	83 (60.1%)	Invasive DC; invasive ductal carcin	noma, Invasive LC; invas	
Subcutaneous Mastectomy with Repair	9 (6.6%)	lobular carcinoma, DCIS; ductal car ductal carcinoma in situ and lob		

Table 2. Breast tumors details based on pathology report in patients with recurrence



46 (33.3%)

**Figure 1.** *Total disease-free survival of the recurrence patients* 

X axis is duration of evaluation in weeks Y axis shows the survival rate of patients

Mastectomy

Table 3. Death, & Location of recurrent breast cancer

estrogen receptor, PR; progesterone receptor, HER2 human

epidermal growth factor receptor 2.

Site	
Local (Breast/Skin)	32 (27.6%)
Bone	20 (17.2%)
Visceral (lung/liver)	50 (43.1%)
Brain	14 (12.1%)
Death	54 (39.1%)

Analysis of Stage of tumor in patients showed that 35 (25.4%) patients had stage 4 of breast cancer while 4 (2.9%) had stage 3B of breast cancer and 12.3% had stage 2B. Sixty five percent of patients were Luminal A or B, 31 (22.5%) patients triple negative receptor and 17 (12.3%) patients were Her 2 enrich.

Based on site of metastasis 32 (27.6%) of patients had local cutaneous metastasis, 50 (43.1%) patients had visceral metastasis, 20 (17.2%)

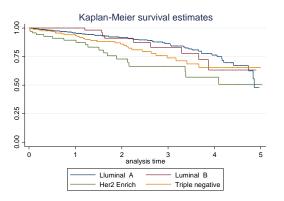
Patients with bone metastasis and 14 (12.1%) patients with brain metastasis According to the follow up of patients, mortality was 39.1% (54 cases) among recurrent patients and As the Graph 1a shows, the 1-, 3- and 5-year disease-free survival were 96.13%, 86.51% and 77.10% respectively. (Graph 1a)

Due to 4 types of recurrence that categorized, the log rank was calculated and there was a high significant difference in recurrence events after the surgery in 4 groups. Luminal B and HER2 Enriched were the subtypes of breast cancer with the lower and the highest recurrence probability after the surgery among all four subtypes, respectively. The equity of survival function was investigated using log-rank test (P = 0.0022). (Figure 3)

The 1-year survival of local, bone, visceral and brain metastasis in patients were 64.99%, 63%, 32.83%, and 21.57% respectively. Based on survival analysis of metastasis in 4 recurrence groups, there was significant difference among groups in the period of metastasis till death. (p=0.0022) (Figure 2)

Disease-free survival analysis was done and log rank test showed a significant difference in 4 groups based on recurrence type. (p=0.0110) (Figure 4).

The mean of surgery time to recurrence diagnosis was higher in skin/breast (local) metastasis in



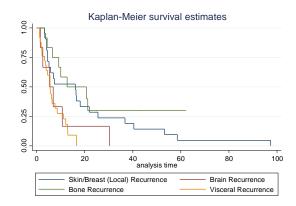
**Figure 2.** Survival probability in the first 5-year after surgery based on hormone receptor subtypes (4 subtypes), (Event: recurrence). X axis is indicative of years and Y axis is the survival rate

comparison of other groups. However, the recurrence time to death was lower in the visceral metastasis eventually. (Table 4)

The Duration of liver and lung metastasis from the first surgery was  $24.9\pm3.51$  and  $32.58\pm6.25$  months respectively (95%CI: 17.96,31.83 months for liver recurrence and 20.23,44.9 months for lung recurrence). Also, the time to death from liver and lung recurrence diagnosis were  $10.65\pm3.38$  and  $5.76\pm0.85$  months respectively (95%CI: 3.91,17.39 months for liver recurrence).

#### DISCUSSION

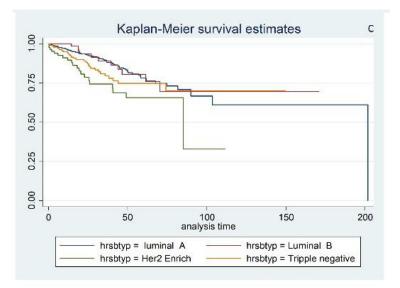
Several studies considered different variables as prognostic factors for breast cancer survival and recurrence. Gluck et al<sup>14</sup>, devita et al<sup>15</sup> and kuru et al<sup>16</sup> demonstrated that lymph node involvement, primary tumor size and stage of malignancy in addition to older age (>50 years), number of positive lymph nodes (>0), SBR grade (>1), negative HR status and treatment with adjuvant chemotherapy, estrogen, progesterone and HER2 receptors, pathology, invasion, calcification, grade, receptor type are inextricably intertwined with survival or recurrence of patients with breast cancer.<sup>17,18,19</sup>



**Figure 3.** Survival from metastasis until death in recurrence patients. X axis is indicative of weeks and Y axis is the survival rate

Table 4. Mean duration between surgery to recurrence and recurrence to death
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Group	Mean time (Months)		Standard error of mean (Months)		95% CI (low- High)	
	Surgery to Recurrence	Recurrence to Death	Surgery to Recurrence	Recurrence to Death	Surgery to Recurrence	Recurrence to Death
Skin/breast (Local)	31.571	21.444	4.477	5.252	(22.722-40.419)	(10.947-31.941)
Brain	22.872	9.538	6.222	4.354	(10.577-35.167)	(0.837-18.240)
Bone	26.150	11.008	5.561	2.424	(15.160-37.140)	(6.163-15.853)
Visceral	27.976	8.967	3.274	2.255	(21.505-34.446)	(4.460-13.475)



Our study confirms that prognosis and survival of patients can be different according to receptor subtypes, molecular subtypes and baseline characteristics, which is compatible with previous related studies.<sup>19</sup>

One of the main concerns in prognosis and survival of patients with breast cancer is DFI which is considered as an effective factor on survival of patients. The longer DFS the longer survival in patients with breast cancer even after recurrence.<sup>20</sup> Thus, disease free survival as a prognostic factor for recurrence of breast cancer could be evaluated through different aspects for instance, in our study we assessed disease free survival based on hormone receptors which showed that during first 5 years of evaluation and follow up Her 2 enrich receptor positive patients had the worst outcome and also they had low disease free survival and the Luminal B and triple negative breast cancer subtypes had greater disease free survival. the worst prognosis of HER 2 enrich receptor breast cancers could be correlated with the fact that most of the patients with Her 2 enrich receptor subtype who survived in our study received monoclonal antibodies and new found drugs but on the other hand vast majority of patients included in our trial have not been treated with Her 2 receptor drugs recently found, thus survival of triple negative patients was more than Her 2 enrich receptor subtype .This finding will be altered by the consumption of new drugs in our patients and moreover results will be similar as new released studies that consider best prognosis of breast cancer attributed to HER 2 enrich receptor subtypes. Survival of patients following to metastasis averagely lasts more than 24 months in our study which is associated with triple negative treatment and HER 2 enrich treatment.

In accordance with site of metastasis many studies evaluated 5 years interval of patient's survival and

**Figure 4.** Disease-free survival based on hormone receptor. Y axis shows survival and X axis shows duration (months)

several studies demonstrated that bone metastasis regardless of its prevalence has the best prognosis among all sites of metastasis.<sup>27,28,29</sup> In our study Follow up of patients in 3 different intervals 0-5,5-10 and 10-15 months from the diagnosis reveals that survival among patients with bone metastasis was exactly higher than others, whereas visceral metastasis had the higher mortality. Thus, Site of metastasis is another concerning factor which can play an important role in survival and prognosis of patients with breast cancer recurrence.<sup>30</sup>

Akbari et al <sup>29</sup> depicted that visceral metastasis has poor prognosis in comparison to local or other particular sites of metastasis. It also showed that bone metastasis had more mortality than loco-regional metastasis. In our study, visceral metastasis similarly has the worst prognosis and the lowest survival however there are different results in various articles performed on this subject which could possibly be related to the sample size and group of patients that have been analyzed<sup>26</sup> regarding bone metastasis; our trial demonstrated that bone metastasis has better prognosis almost among other subtypes and in fact it has best survival following to local metastasis which could show the contrast between our study and other studies. Sta et al<sup>20</sup> showed that factors mentioned above as prognostic factors or related factors to recurrent and survival of patients with breast cancer are also correlated with metastasis of breast cancer. It concludes that based on receptor type of different breast cancers "Visceral metastasis was found to be significantly dependent (p = 0.05) on 8 variables: age, menopausal, status, stage, primary tumor size, lymph node involvement, estrogen and progesterone receptor status and pattern. this study supports our findings regarding prognosis of patients with visceral metastasis which is considered as a poor prognostic category in patients with breast cancer. But on the other hand, we consider bone metastasis with better prognosis and survival in comparison to visceral and brain metastasis.

A study performed by Rosa mendosa<sup>24</sup> calculated the average survival of patients with metastatic breast cancer as 12 to 24 months and moreover late recurrence has been experienced more than early recurrence in patients. Interestingly we assessed Mean duration between surgery to recurrence and recurrence to death among patients and it demonstrated that breast cancers with brain metastasis had  $22.872 \pm 6.222$  months' duration between initial surgery and recurrence and also 9.538  $\pm$  4.354 months' duration between recurrence and death apart from that visceral metastasis duration between surgery and recurrence was  $27.976 \pm 3.274$ and the duration between recurrence and death was  $8.967 \pm 2.255$  so these two sites of metastasis are considered as the worst metastatic breast cancer. Skin or local metastasis significantly had better outcome and the duration between surgery and recurrence was  $31.571 \pm 4.477$  and the duration between recurrence and death was  $21.444 \pm 5.252$ .

Strength of this trial is the extended analysis on different aspects of breast cancer; disease free survival for each receptor subtype and Survival from metastasis till death in patients with recurrence breast cancer are precisely assessed. There are few studies which evaluate the same elements of breast cancers. Mean duration between surgery to recurrence and recurrence to death provided a great horizon through

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understanding of different manners of subtypes and sites of recurrence.

#### CONCLUSION

This study performed on breast cancer recurrence and its survival among Iranian population. According to the outcome based on receptor subtypes; totally after 5 years evaluation of each patient in our study it showed that Her2 enrich positive breast cancers had the worst survival and on the other hand triple negative receptor breast cancers showed the best survival while other studies demonstrated outcome completely opposite to our finding. this contrast is due to discovery of Herceptin drugs for Her 2 enriched receptor breast cancers which ameliorates their prognosis and survival, also drugs related to luminal A and luminal B which are a huge step to improve their survival and hormonal therapy could be associated with their better prognosis in comparison to triple negative receptor subtype. Since our patients from 17 years ago till recent years did not consume Herceptin drugs difference among our findings and other studies could be related to release of this category of drugs. Based on sites of metastasis bone and local metastasis among our patients showed the best survival while brain and visceral metastasis had the worst survival and prognosis.

CONFLICTS OF INTERST None.

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Outcome of Breast Cancer Patients with COVID-19 Infection: A Report from a Tertiary Cancer Center in India

Indhuja Muthiah Vaikundaraja<sup>a</sup>, Manikandan Dhanushkodi<sup>\*a</sup>, Venkatraman Radhakrishnan<sup>a</sup>, Jayachandran Perumal Kalaiyarasi<sup>a</sup>, Gangothri Selvarajan<sup>a</sup>, Siva Sree Kesana<sup>a</sup>, Carthikeyan<sup>a</sup>, Priya Iyer<sup>b</sup>, Balasubramanian Ananthi<sup>b</sup>, Arvind Krishnamurthy<sup>c</sup>, Velusamy Sridevi<sup>c</sup>, Tenali Gnana Sagar<sup>a</sup>

<sup>a</sup> Department of Medical Oncology, Cancer Institute (WIA), Chennai, India

<sup>b</sup> Department of Radiation Oncology, Cancer Institute (WIA), Chennai, India

<sup>c</sup> Department of Surgical Oncology, Cancer Institute (WIA), Chennai, India

ARTICLE INFO

#### ABSTRACT

Received:	<b>Background:</b> There is no data on the outcome of COVID-19 infection in patients with
07 May 2021	breast cancer from India. This study was done to assess the outcome of patients with breast
Revised:	cancer who had COVID-19 infection.
03 June 2021	
Accepted:	Methods: We analyzed patients with breast cancer who were diagnosed with COVID-19
06 June 2021	infection from May to September 2020 in the medical oncology department of a tertiary
00 Julie 2021	cancer center in India. Symptomatic patients (fever and influenza-like illness symptoms) or
	asymptomatic patients planned for systemic therapy were tested for COVID-19 by RT-PCR.
	<b>Results:</b> A total of 441 breast cancer patients received 1174 systemic therapies from May
	to September 2020. Among them, 36 patients who had COVID-19 infection were analyzed in
	detail. The majority (86%) were asymptomatic at presentation. The most common symptoms
	were fever followed by cough. Patients were either admitted to the hospital (53%) or kept in
	home quarantine (47%). Patients who received oxygen, non-invasive assisted ventilation
	(NIV), and mechanical ventilation (MV) were 8%, 3%, and 3% respectively. The median
	duration of hospitalization and home quarantine was 11 days and 19 days respectively. The
	recovery of patients with COVID-19 infection was 94%. The median duration to clearing
Keywords:	SARS-COV-2 by RT-PCR was 19 days. The total/all-cause mortality was 6% (n=2). The
Breast cancer,	mortality due to COVID-19 infection was 3% (n=1). Subsequently, 89% were restarted on
COVID-19 infection,	systemic therapy. The median delay in restarting systemic therapy was 23 days.
outcome	Conclusion: Systemic therapy can be safely administered during the ongoing COVID-
	19 pandemic. Further, follow-up of patients is warranted to assess the long-term impact of
	COVID-19 infection.
	ccess article distributed under the terms of the Creative Commons Attribution-Non-Commercial 4.0 International License which permits copy
and redistribution of the material in a	ny medium or format or adapt, remix, transform, and build upon the material for any purpose, except for commercial purposes.

INTRODUCTION

As per GLOBOCAN 2020, breast cancer is the most common cancer in Indian women (26.3%) with

\* Address for correspondence: Manikandan Dhanushkodi MD, DM, DNB Associate Professor, Medical Oncology, Address: Cancer Institute (WIA), Chennai 38, Sardar Patel Road, Chennai – 600036 Tel: +91 044 22350241 Email: dmani1982@gmail.com an age-standardized incidence rate of 25.8%.<sup>1</sup> India has the 2<sup>nd</sup> largest number of COVID-19 confirmed patients in the world next to the United States and currently is in the second wave of the pandemic. Although India ranks 3<sup>rd</sup> in the highest number of deaths due to COVID-19 infection, the number of deaths per million population is low (243).<sup>2</sup>

COVID-19 pandemic has significantly disrupted breast cancer management right from screening, diagnosis, and treatment.<sup>3-5</sup> The long-term impact of COVID-19 infection in patients with breast cancer is

unknown. Currently, there is no report on the outcome of breast cancer patients with COVID infection from India. This study was done to assess the demographics and the outcome of breast cancer patients who had COVID infection.

#### **METHODS**

We included patients with breast cancer (age >18 years) who were diagnosed with COVID-19 infection from May to September 2020 who presented to the medical oncology department of a tertiary cancer center in India. COVID-19 testing was performed by RT-PCR at the Indian Council of Medical Research (ICMR) approved diagnostic laboratories. COVID-19 testing was performed for symptomatic patients [fever and ILI (influenza-like illness) symptoms] or asymptomatic patients who were planned for injectable systemic therapy. Besides, the test was repeated every 3 weeks for patients receiving injectable systemic therapy (chemotherapy or targeted therapy). In addition to information from medical records, patients were also contacted by telephone for the acquisition of all data.

The patients were classified into 3 age groups that included 18 to 40 years, 41 to 60 years, and above 60 years. Patients diagnosed with COVID-19 were deferred systemic therapy and referred to a government-designated COVID-19 center for further management. Patients were restarted on systemic therapy if they were asymptomatic and further

COVID-19 tests were negative after 2 to 3 weeks. This study was approved by the Institutional Ethical Committee (IEC/2021/April 03).

#### RESULTS

A total of 441 breast cancer patients received 1174 systemic therapies from May to September 2020. Among them, 36 patients who had COVID-19 infection were analyzed in detail. The most common systemic therapy was dose dense Adriamycin cyclophosphamide/paclitaxel chemotherapy. All the patients were women, and the largest age group was 41 to 60 years (66%). The most common comorbid illness was diabetes mellitus (33%) followed by systemic hypertension (31%). None of the patients had a history of smoking. The most common stage was III (42%) followed by IV (33%). The majority of the patients (94%) were on systemic therapy at the time of COVID-19 positivity.

The most common symptoms were fever followed by cough. Among the 5 symptomatic patients, 2 had lung involvement (Table 1).

Patients were either admitted for observation or treatment in government-designated COVID hospitals (53%) or kept in home quarantine (47%). Patients who received oxygen, non-invasive assisted ventilation (NIV), and mechanical ventilation (MV) were 8%, 3%, and 3%, respectively. The median duration of hospitalization and home quarantine was 11 days and 19 days, respectively (Table 2).

Table 1. Baseline characteristics of breast cancer patients with COVID-19 infection (N=36).	

Variable	N(%)	
Age (years)		
18-40	6 (17)	
41-60	24 (66)	
> 60	6 (17)	
Comorbid illness		
Diabetes mellitus	12 (33)	
Systemic hypertension	11 (31)	
Hypothyroidism	5 (14)	
Coronary artery disease	3 (8)	
Bronchial asthma	2 (5)	
Psychiatric illness	2 (5)	
Place		
Urban	22 (64)	
Rural	13 (36)	
Stage		
Stage I	0	
Stage II	9 (25)	
Stage III	15 (42)	
Stage IV	12 (33)	



Variable	N(%)	
Treatment		
Dose dense Adiamycin Cyclophosphamide/ Paclitaxel (Curative)	22 (61)	
Paclitaxel (Palliative)	6 (17)	
Docetaxel cyclophosphamide	4 (11)	
Trastuzumab based therapy	2 (5)	
Zoledronic acid letrozole	1 (3)	
Not yet started on treatment	1 (3)	
At presentation		
Asymptomatic	31 (86)	
Symptomatic	5 (14)	
Symptoms/signs at presentation		
Fever	4 (80)	
Cough	2 (40)	
Shortness of breath	0	
Pulmonary involvement <sup>a</sup>	2 (40)	
Gastrointestinal involvement	1 (20)	

<sup>a</sup> Details available only for patients who had imaging of chest (chest X-ray or computed tomography of chest available).

Table 2. Treatment for COVID-19 infection.

Variable	N(%)
Place	
Hospitalised	19 (53)
Home quarantined	17 (47)
Median duration	
Hospitalisation	11 days (range: 3 to 17 days)
Home quarantine	19 days (range: 7 to 28 days)
Oxygen support	
Yes	3 (8)
No	33 (92)
Non-invasive ventilation	
Yes	1 (3)
No	35 (97)
Mechanical ventilation	
Yes	1 (3)
No	35 (97)

The recovery of patients with COVID-19 infection was 94%. The median duration to clearing SARS-COV-2 by RT-PCR was 19 days and mortality was 6% (n=2). The all-cause mortality due to COVID-19 infection was 3% (n=1). Subsequently, 89% of the patients were restarted on systemic therapy. The median delay in restarting systemic therapy was 23 days (range: 0 to 150 days). None of the patients progressed due to a delay in restarting systemic therapy. The details of the patients who died are mentioned below (Table 3).

**Patient 1**: A 43-year-old female with hypertension and hypothyroidism was diagnosed with stage IV breast carcinoma. She had fever at presentation and tested positive for COVID-19. She was referred and admitted to a government designated COVID hospital. Subsequently, she developed cough and shortness of breath for which she was initially treated with oxygen and NIV followed by MV. The patient died 13 days later. She had not received further systemic therapy.

**Patient 2:** The second patient was a 51-year-old female with diabetes, hypertension, and HbsAg positivity, with metastatic breast cancer, and prior treatment with anthracyclines, taxanes, and trastuzumab. After 1 year of disease-free survival, she had bone metastasis and was treated with and palliative radiation to the spine followed by gemcitabine carboplatin chemotherapy and zoledronic acid. After 6 cycles, she presented with



Variable	N (%)	
Recovered	34 (94)	
Died <sup>1</sup>	2 (6)	
Died due to COVID-19 infection	1 (3)	
Median time to COVID-19 negativity	19 days (range: 1 to 54 days)	
Restarted on systemic therapy		
Yes	32 (89)	
No <sup>b</sup>	4 (11)	
Median delay in restarting systemic therapy	23 days (range: 0 to 150 days)	

**Table 3.** Outcome and further treatment of Covid-19 positive breast cancer patients.

<sup>a</sup>One patient died due to progressive disease

<sup>b</sup>Two patients died, and two patients defaulted further treatment

progressive pleural effusion. Incidentally, she was found to be COVID-19 positive and was hospitalized for 1 week and treated symptomatically and later

#### DISCUSSION

This is the first report from India on the outcome of breast cancer patients with COVID-19 infection. The recovery rate was high (94%) and most patients (89%) were restarted on systemic therapy with a median delay of 23 days. The one patient who died of COVID-19 infection had metastatic breast cancer with a comorbid illness of hypertension and hypothyroidism and was symptomatic with fever and breathlessness.

Prior reports of COVID-19 infection in cancer patients have shown that advanced age, smoking, multiple comorbid illnesses, and those receiving chemotherapy have a higher risk of morbidity and mortality.<sup>6-9</sup> Breast cancer patients with dyslipidemia can have increased susceptibility and severity of COVID-19 infection.<sup>10</sup> It was hypothesized that COVID-19 infection in breast cancer patients could cause resistance to chemotherapy and tamoxifen could increase susceptibility to COVID-19 infection.<sup>11</sup>

COVID-19 infection-related anxiety and fear could lead to refusal of procedure/surgery in patients with breast cancer or those who are under evaluation of breast lump.<sup>12</sup> Suspension of screening for breast cancer due to the COVID-19 pandemic has led to breast cancer increase.<sup>13</sup> There was a larger decline in screening in women from underserved racial/ethnic groups and lower socioeconomic status.<sup>14</sup> A survey from Europe reported that significant modification of breast cancer treatment occurred during this pandemic.<sup>15</sup> COVID-19 vaccination can cause axillary lymphadenitis and false-positive uptake in PET (positron emission tomography) uptake.<sup>16</sup>

COVID-19 and cancer consortium registry study analyzed 846 patients with breast cancer and COVID-19 infection and reported that 48% were hospitalized and 9% died. Patients with older age, poor performance status, increased comorbidity burden, discharged and became COVID-19 negative. However, the patient had recurrent pleural effusion after 1 month and died due to progressive disease.

and presence of progressive cancer were associated with increased 30-day mortality risk.<sup>17</sup> A systemic review showed that the case fatality rate of patients with breast cancer (n=1296, 14.2%) was lower compared to patients with lung cancer (n=1135, 32.4%) with COVID infection.<sup>18</sup>

A report from France analyzed 59 breast cancer patients with COVID infection from March to April 2020 and found a mortality of 7% (n=4). All the patients who died had a significant non-cancer comorbid illness. Univariate analysis showed that hypertension and age > 70 years were associated with a high risk of intensive care unit admission and death.<sup>19</sup>

A report from Wuhan, China compared 3 groups of COVID-19 infected patients [breast cancer (n=35); non-breast cancer (n=81); and non-cancer (n=55). This study showed that there was no difference in disease severity and outcome between non-cancer COVID-positive patients and breast cancer patients with COVID infection. Also, as compared to other cancer patients with COVID infection, breast cancer patients with COVID-19 infections were mild and predominantly asymptomatic.<sup>20</sup>

A prior report from Cancer Institute (WIA), Chennai showed that the incidence of COVID positivity in asymptomatic patients who were planned for daycare systemic therapy was low (1.45%). Also, 45% were COVID-19 positive on repeat testing before subsequent cycles of systemic therapy.<sup>21</sup> This pandemic has led to many operational changes in National Clinical Trials Network Breast Cancer Trials like electronic consent for enrolment, telemedicine visits, and mail order pharmacy.<sup>4</sup> Breast cancer survivors with high spiritual well-being and psychological resilience are less likely to experience the fear of recurrence.<sup>22</sup>

The strength of the present study is that it is the first study reporting the outcome of breast cancer patients



with COVID-19 infection in India. The limitations of the study are that it is a single-center study with a small sample size and it lacks complete information on treatment for COVID-19 infection as the patients were treated elsewhere in a government-designated COVID-19 hospital. Despite not managing the COVID-19 patients in-house, all the patients returned to our institute for further cancer treatment. Thus, we could effectively determine the COVID-19 outcomes in our patient cohort.

#### CONCLUSION

Despite the small sample size of this study, our results showed that the mortality rate of breast cancer patients with COVID-19 is not much different from

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that of the normal population. According to our results, systemic therapy of breast cancer patients can be safely administered during the COVID-19 pandemic. Further follow-up of patients is warranted to assess the long-term impact of COVID-19 infection.

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CONFLICT OF INTEREST None.

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### Assessing Clinicopathological Features and Prognosis of Triple-Negative Breast Cancer Patients: A Single-Center Study in Turkey

Saliha Karagoz Eren\*a, Alaettin Arslan<sup>b</sup>, Ebru Akay<sup>c</sup>, Nail Özhan<sup>d</sup>, Yunus Dönder<sup>a</sup>

<sup>a</sup> Department of General Surgery, Kayseri City Training and Research Hospital, Kayseri, Turkey

<sup>b</sup> Department of Radiation Oncology, Kayseri City Training and Research Hospital, Kayseri, Turkey

<sup>c</sup> Department of Pathology, Kayseri City Training and Research Hospital, Kayseri, Turkey

<sup>d</sup> Department of Medical Oncology, Kayseri City Training and Research Hospital, Kayseri, Turkey

#### **ARTICLE INFO** ABSTRACT Background: Triple-negative breast cancer (TNBC) is defined as tumors without **Received:** 25 May 2021 estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor **Revised:** receptor 2 (HER2) expression. This cancer is associated with higher rates of recurrence risk 20 June 2021 when compared to other subtypes of breast cancers. In this study, we aimed to explore the Accepted: basic clinicopathological characteristics, prognosis, and recurrence patterns of TNBC 24 June 2021 patients. Methods: In the current study, forty-five TNBC female patients operated on for breast cancer in the General Surgery Clinic of Kayseri City Training and Research Hospital between 2016 and 2021 were included and retrospectively evaluated. **Results:** The percentage of TNBC was 12% of the 502 breast cancer patients who could access all three pieces of receptor information. The mean age of the patients was 58.9±15.2 years (27-90), and the mean BMI was $30.4\pm5.17$ (21.5-40.6). It was observed that the most common histological subtype was invasive ductal carcinoma, and at the time of diagnosis, 11 patients were stage 1 (24.4%), 31 patients were stage 2 (68.8%), 2 patients were stage 3 (4.4%), and 1 patient was stage 4 (2.2%). During the follow-up period, 11 patients (24,4%) developed metastasis and the most common sites were the brain and bones. The mean time from diagnosis to metastasis was 20.7±5.75 (12-29) months. The 3-year disease-free Keywords: survival was 62%, and the 3-year overall survival (OS) was 70%. Triple negative breast neoplasms, Conclusion: TNBCs are cancers with varying prevalence, poor prognosis, and prognosis, limited treatment alternatives. The prevalence of TNBC in our center was found to neoadjuvant therapy, be lower than the literature rates and consistent with the literature, the lymph node survival rate stage was related to poor OS and disease free survival (DFS). Copyright © 2021. This is an open-access article distributed under the terms of the Creative Commons Attribution-Non-Commercial 4.0 International License which permits copy

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### INTRODUCTION

Breast cancer has molecular subtypes based on the expression of hormone receptors and human epidermal growth factor receptor 2 (HER2) and has been shown

\* Address for correspondence: Saliha Karagöz Eren, MD Address:Department of General Surgery, Kayseri City Training and Research Hospital, Kayseri, Turkey Tel: +903523157700 Email: <u>skaragozeren@gmail.com</u> to have different clinicopathological features and prognoses.<sup>1</sup> Triple-negative breast cancer (TNBC) is defined as tumors without estrogen receptor (ER), progesterone receptor (PR), and HER2 expression.<sup>2</sup> TNBC is seen at a frequency of 15-20% of all breast cancers and is associated with increased local recurrence, distant metastasis, and poor prognosis in the first 3 to 5 years after diagnosis.<sup>3, 4</sup> TNBC-related risk factors have been identified, such as BRCA mutation, ethnic differences, young age, and body



#### Clinicopathological features of TNBC

mass index.<sup>5, 6</sup> In a large series conducted by the National Breast Cancer Registry Program of the Turkish Breast Diseases Associations, the incidence of TNBC in this country was found to be 8.1%, but studies from different regions reported different incidences such as 12% and 27% and different survival rates.<sup>7-9</sup> Today, breast cancer is a systemic disease, and individual treatment plans for the tumor are recommended. TNBC has no targeted treatment alternative; it can be seen in different incidences in different societies, treatment responses are also different, and it is said to be a heterogeneous disease in itself. This study aims to determine the prevalence of TNBC in our regional hospital where breast cancer patients are treated and to assess the associated risk factors and prognosis in our regional population.

#### **METHODS**

The study was conducted with the approval of the Clinical Research Non-interventional Ethics Committee of Kayseri City Training and Research Hospital (Protocol No: 2021/391). Forty-five of 523 patients diagnosed and operated on for breast cancer in the General Surgery Clinic of Kayseri City Training and Research Hospital between January 2016 and January 2021 and whose data could be accessed were included in the study. All patients had a histologically confirmed diagnosis of invasive breast cancer; initial breast cancer staging was determined according to the American Joint Committee on Cancer (AJCC). Primary tumor grade was evaluated depending on the Nottingham modification of Bloom-Richardson criteria. Baseline ER and PR status was determined by IHC staining, and if the percentage of positively stained cells was less than 1%, they were considered negative. Patients diagnosed with breast cancer in our hospital but whose treatment was continuing in another center were excluded from the study. Locoregional recurrence was defined as the involvement of ipsilateral axillary, internal mammarian. or supraclavicular lymph nodes and/or skin or subcutaneous tissue with or without ipsilateral breast parenchyma involvement. Disease-free survival (DFS) was defined as the time from the moment of diagnosis to the moment of detecting a local recurrence or metastasis. Overall survival (OS) was defined as the time elapsed from the time the patient was diagnosed to the last visit or death.

#### Statistical analysis

performed Statistical evaluations were on computers using the SPSS 24 statistics software. Descriptive given statistics were as mean±standard deviation or median with the interquartile range (IQR), minimum maximum [minmax] depending on the distribution of the continuous

variables, while categorical variables were summarized as numbers and percentages. The normality test of the numerical variables was controlled by the Kolmogorov-Smirnov test. Wilcoxon Signed Ranks test was used to compare dependent continuous variables. **Relationships** between the variables to DFS and OS were assessed by the Kaplan-Meier survival analysis. The level of significance was established at P < 0.05.

#### RESULTS

All three pieces of receptor information could be accessed in 502 out of the 523 patients who were operated on between 2016 and 2021, 45 (%8.6) of these patients were triple negative. The mean age of the patients was 58.9±15.2 years (27-90), and the mean BMI was 30.4±5.17 (21.5-40.6). The median follow-up time was 30.1 (21.5) months (6-60).

It was observed that the most common histological subtype was invasive ductal carcinoma (IDC), where two patients had medullary carcinoma, and 3 patients with IDC had medullary features. In one patient, an osteoclast-like giant cell carcinoma and an invasive ductal component were observed. At the time of diagnosis, 11 patients were stage 1 (24.4%), 31 patients were stage 2 (68.8%), 2 patients were stage 3 (4.4%), and 1 patient was stage 4 (2.2%). Mastectomy was the most common surgical procedure. Only 2 (4.4%) of the patients had grade 1 tumors. Ki 67 value was reached in 39 patients, and in 29 (74.4%) of these patients, Ki-67 was observed to be 30 and above. The tumors were 25 (55.6%) upper outer quadrant, 9 (20%) upper inner quadrant, 4 (8.9%) lower outer quadrant, and 3 (2.2%) lower inner quadrant. Tumor localization in the breast was 53.3% on the right side. The demographic data and tumor characteristics of the patients are summarized in table 1.

In total, two patients were not given adjuvant therapy due to advanced age and comorbidities, 10 patients (22.2%) received neoadjuvant chemotherapy (NACT), and all other patients received adjuvant chemotherapy. It was observed that 8 (80%) of the patients who received NACT were clinically positive for lymph nodes, and two patients with N0 had T2 tumors. One patient with N0 underwent breast-conserving surgery (BCS) after treatment, and the other patient underwent mastectomy and reconstruction with implants due to lack of response after treatment. In three patients (30%), axillary lymph node dissection (ALND) was performed without sentinel lymph node biopsy (SLNB) after treatment. It was observed that SLNB was applied to the remaining seven (70%) patients and in 3 of them, ALND was performed due to the detection of one metastatic lymph node. It was observed that 4 (50%) of the patients with clinically

(P=0.008). Overall, 4 (40%) patients who received

NACT had a complete pathological response (grade 5



positive lymph nodes had a complete axillary response, 3 (37.5%) had 1-3 LN positivity, and one (12.5%) had 4 LN positivity despite the treatment. It was observed that the median tumor diameter before treatment was 30 mm (3.25) [23-50] and after treatment  $14.5\pm16.0$  mm (0-50) and was statistically significantly regressed

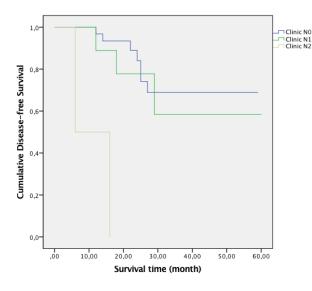
according to the Miller-Payne rating), 2 (20%) patients
had no change in tumor size, and 4 (40%) patients had
a partial response.

Table 1. Patients and tumours characteristics.

Variables	N(%)(Min-Max)
Age† (years)	
<50 years	14 (31.1)
$\geq$ 50 years	31 (68.9)
Menapausal Status†	
Pre-menopause	28 (62.2)
Post-menopause	17 (37.8)
Breast Density†	
А	8 (17.8)
В	17 (37.8)
С	15 (33.3)
D	5 (11.1)
BMI†	
<30	24 (53.3)
≥30	21 (46.7)
Site†	
Right	24 (53.3)
Left	21 (46.7)
Surgery†	
Mastectomy	31 (54.5)
BCS	14 (45.5)
ALND†	
Yes	24 (53.3)
No	21 (46.7)
ALN number¶	24±15.9 (4-38)
Metastatic ALN number‡	1(1.25) (1-5)
Histological Type†	
IDC	42 (93.3)
Noroendokrin Carsinom	1 (2.2)
Meduller Carcinoma	2 (4.4)
PNI†*	
Yes	7 (17.5)
No	33 (82.5)
LVI†*	
Yes	14 (35.0)
No	26 (65.0)
Grade†	
Ι	2 (4.4)
II	20 (44.4)
III	23 (51.1)
Clinical T Stage <sup>†</sup>	
1	12 (26.7)

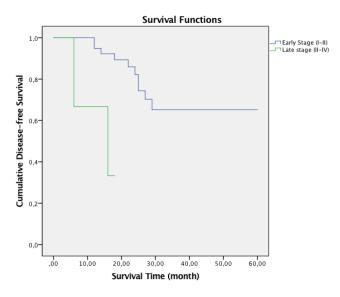
Variables	N(%)(Min-Max)	
2	29 (64.4)	
3	2 (4.4)	
4	2 (4.4)	
Clinical Stage†		
1	11 (24.4)	
2	25 (55.6)	
3	3 (17.8)	
4	1 (4.4)	
Ki-67‡*	50 (30) [10-90]	

<sup>¶:</sup> mean±standard deviation [minimum-maximum], ‡:median (interquartile range) [minimum-maximum]. ‡: n (%), \*: less than 45 patients, IDC: Invasive ductal carcinoma, PNI: Perineural invasion, LVI: Lymphovascular invasion, BCS: Breast-conserving surgery, ALND: Axillary lymph nodes dissection, ALN: Axillary lymph nodes, BMI: Body mass index



**Figure 1.** The association of cN stage with disease-free survival (Kaplan-Meier).

Metastasis developed in 11 of the patients (24.4%); it was observed that liver metastasis developed in the follow-up of a patient who had bone metastatic disease at the beginning and a locally advanced disease. It was observed that the most common metastatic sites were bone and brain metastasis, where three patients had brain and lung metastases, and one patient had bone and lung metastases. The mean time from diagnosis to metastasis was 20.7±5.75 (12-29) months. It was observed that 7 (15.6%) patients who died were all metastatic, 5 had brain metastases, one had lung and bone metastasis, and the other had bone and liver metastases. Two patients with isolated bone metastasis, one patient with mediastinal lymph node metastasis, and one patient with liver metastasis, were also observed. A patient with liver metastasis was treated with Cyclophosphamide-anthracycline + taxane. Still, the tumor did not regress, the patient



**Figure 2.** The association of the clinical-stage with disease-free survival (Kaplan-Meier).

developed extensive liver metastases in the 12th month, and the metastases disappeared under gemcitabine treatment and were disease-free in the 40<sup>th</sup> month of follow-up. It was observed that local recurrence was observed in only two patients. One of them was a patient with lung and bone metastases, and the other patient received NACT for clinical N1 disease. SLNB was applied to the second patient after the treatment, and 4 lymph nodes were sampled, and dissection was not performed because all four were negative. However, it was observed that 7 lymph nodes were dissected, and 2 metastatic lymph nodes were found in the patient who underwent axillary dissection due to axillary recurrence at the 12<sup>th</sup> month, and was disease-free in the 39th month of the followup. The treatment and metastasis regions of the patients are shown in table 2.

The 3-year DFS was 62% and the 3-year OS was 70%. The overall predicted DFS time is  $[45.2\pm3.5]$ 



Table 2. Treatment modalities and recurrence sites.

Variables	N (%)	
	10 (22.2)	
	33 (73.3)	
Cyclophosphamide-anthracycline	8 (17.8)	
Cyclophosphamide-anthracycline + taxane	35 (77.8)	
Radiotherapy	37 (82.2)	
Metastasis Sites		
Bone	3 (36.4)	
Brain	5 (45.5)	
Liver	2 (18.2)	
Lung	4 (36.4)	
Mediastinal Lymph Node	1 (9.1)	
Multiple	5 (45.5)	

months (38.4-52.0)], in the group with clinical N stage 2 [11.0 $\pm$ 5.0 months (1.2-20.8)], the DFS time predicted from N stage 1 [44.0 $\pm$  7.4 months (29.6-58.4)] months and N stage 0 [47.5 $\pm$  3.7 months (40.4-54.7)] group was significantly shorter (*P*=0.000) (Figure 1). The predicted DFS time in the clinical early stage (I-II) group [46.8 $\pm$ 3.5 months (40.0-53.6)], from the group with clinical late stage (III-IV) [13.3 $\pm$ 3.0 months (38.4-52.0)] was significantly longer (*P*=0.002) (Figure 2).

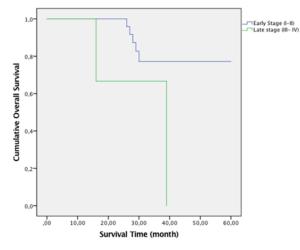
The overall predicted OS time is  $[50.9\pm 3.0 \text{ months} (45.1-56.7)]$ , in the clinical early stage I-II group 52.7±2.9 months (47.1-58.4), from the group with clinical late stage III- IV  $[31.3\pm 8.9 \text{ months} (14.0-48.7)]$  was significantly longer (*P*=0.008) (Figure 3). In the group with clinical N stage 2  $[27.5\pm 11.5 \text{ months} (5.0-50.0)]$ , the OS time predicted from N stage 1  $[54.7\pm 4.9 \text{ months} (45.1-64.2)]$  months and N stage 0  $[51.5\pm 3.3 \text{ months} (45.0-57.9)]$  group was significantly shorter (*P*=0.021) (Figure 4).

#### DISCUSSION

Breast cancer is the most common malignancy in women, and it is a highly heterogeneous disease. Epidemiological data show that TNBC occurs mostly in premenopausal young women below the age of 50, which accounts for approximately 10-20% of all breast cancer patients.<sup>10, 11</sup> In studies conducted in Turkey, this rate has been reported to be between 12 and 27%.<sup>7-9</sup> In our series, 62.2% of the patients were in the premenopausal period, but only 14% were 50 years and younger; TNBC prevalence was found to be lower, unlike the literature, standing at 8.6%.

In the literature, body weight and BMI have been defined as risks for TNBC.<sup>1212</sup> Young women in the premenopausal period showed a 5% increase in risk per 5 kg increase in body weight and a 16% increase in risk per 5 kg / m2 increase in BMI and are associated with a worse prognosis. In our series, it was found that 84% of the patients had a BMI of 25 and above.<sup>12, 13</sup> In the literature, the relationship betweenmammographic density and breast cancer has been investigated, showing that women with high mammographic density are more likely to develop breast cancer in their lifetime compared to women with low MD.<sup>14</sup> However, the study by Mema *et al.*<sup>15</sup> showed that women with entirely fatty breasts on mammography had increased odds of having TNBC compared to women with higher mammographic density. In another study, high mammographic density was associated with recurrence in patients treated for early-stage TNBC.<sup>16</sup> Premenopausal higher MD is associated with higher subsequent risk of ER-negative than ER-positive cancer, whereas postmenopausal higher MD is associated with similar risk of both ER subtypes. In addition, the combination of obesity and higher breast density in premenopausal women is also associated with a higher risk of ERnegative cancer.17, 18 A limitation of our study includes the fact that we recruited a small sample size, and given the limited number of patients, we were not able to perform an analysis regarding these factors or to make any comparison with other subtypes.

In general, data from the literature show that 9-32% of patients with TNBC are germline BRCA (gBRCA) mutation carriers.<sup>19</sup> In another study involving 802 TNBC patients with no family history of breast or ovarian cancer, the prevalence of gBRCA

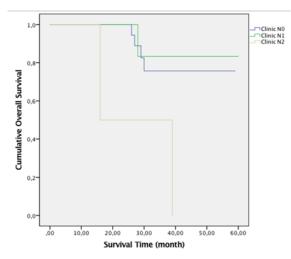


**Figure 3.** The association of the clinical-stage with overall survival (Kaplan-Meier).

mutations was 16 %.<sup>20</sup> In patients under the age of 40, this rate was reported to rise to 24 %.<sup>21</sup> A meta-analysis involving 46870 TNBC patients concluded that patients with the gBRCA1 mutation were 3 and 9 times more likely to have TNBC compared to gBRCA2 carriers and non-carriers, respectively.<sup>22</sup> Of the 30 patients whose family history could be accessed, only 3 (6.7%) patients had a family history of breast cancer, and only 8 patients had a germline mutation analysis. It was observed that only one patient had a BRCA2 mutation and had a family history of breast cancer. This patient underwent a skin-sparing mastectomy, and a prophylactic mastectomy for the contralateral breast. The National Comprehensive Cancer Network (NCCN) guidelines recommend the gBRCA test for all TNBC patients diagnosed at  $\leq 60$  years of age, regardless of their family history, and breast cancer patients diagnosed at any age with a strong family history.<sup>23</sup>

Ki-67 guides the planning of treatment for luminallike breast cancer, but its role for TNBC is unclear. In a study involving 800 early TNBCs, it was stated that it was an almost independent prognostic and predictive factor for both DFS and OS, and the cutt-of value for Ki-67 level in prognosis was found to be 30 %.<sup>24</sup> In our study, the Ki-67 level of 86.7% of the cases was known, and the total of 64.4% was 30 and above. However, in our study, no difference was found in survival according to Ki-67 levels.

TNBC is not sensitive to endocrine or molecular targeted therapy due to its specific molecular phenotype. Therefore, chemotherapy is the main systemic therapy, but the effectiveness of conventional postoperative adjuvant chemoradi-otherapy is poor. The remaining metastatic lesions will eventually lead to tumor recurrence. Approximately 46% of TNBC patients are known to develop distant metastases, have been shown to have weaker DFS and OS than other



**Figure 4.** The association of cN stage with disease-free survival (Kaplan-Meier).

breast cancer subtypes, and the mortality rate within the first 5 years after diagnosis is 40 %.<sup>4, 25</sup> Distant metastases usually occur 3 years after diagnosis, after which the median survival time is only 13.3 months, with a posto-perative recurrence rate of up to 25 %.<sup>26</sup> Unlike bone and visceral metastases in luminal-like tumors, distant metastases usually involve the brain and lungs. In our series, the median survival of 11 metastatic cases after metastasis was 10 months (9.3-23), and the most common metastatic region was brain and bone metastasis. Patients with isolated bone metastases had a survival advantage.

Although NACT is the standard treatment for locally advanced breast cancer, pathological complete response (pCR) rates for TNBC are associated with better DFS and OS.<sup>27</sup> In our series, it was seen that 80% of the patients who received NACT had clinical N1 disease, and the other two patients had T2 tumors (tumor diameter was over 3 cm in both cases), and NACT was planned due to tumor size/breast ratio. It was observed that 4 patients with pathological complete response did not show recurrence. Unlike the survival advantage in those with pathological complete responses, unresponsive cases are associated with worse outcomes.<sup>28, 29</sup> In our series, it was observed that one of the two patients who had no response after NACT had T2N0 disease, and metastases disappeared after the capecitabine treatment given due to liver metastases in the 12<sup>th</sup> month and was in the follow-up at the 5<sup>0th</sup> month. It was observed that the other patient had T2N1 disease and was receiving capecitabine due to bone metastasis in the 29<sup>th</sup> month of the follow-up and still receiving treatment. CREATE-X has recently shown a survival benefit in using capecitabine as adjuvant therapy for TNBC patients unable to achieve pCR.<sup>30</sup> In its 2021 guideline, the American Society of Clinical Oncology recommends NACT with an anthracycline and taxane-containing regimen for



patients with TNBC with the clinically node-positive disease as well as at least T1c disease.<sup>31</sup> One of the advantages of NACT treatment is to see the effectiveness of chemotherapy in breast cancer and to guide the use of adjuvant therapy. Despite the limited number of cases, NACT is beneficial in determining adjuvant therapy in metastatic cases. The limitations of this study are the limited number of patients and the short follow-up period. Another limitation is the low number of patients receiving NACT for this group, for which NACT is especially recommended due to its survival advantage. In our clinic, NACT is considered a priority in early-stage disease as recommended by current guidelines.

#### CONCLUSION

TNBCs are cancers with varying prevalence, poor prognosis, and limited treatment alternatives. The

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TNBC rates of the patients in our study were lower than the data from our country and consistent with the literature, and the lymph node stage was related to poor OS and DFS.

#### FINANCIAL DISCLOSURE

The authors declared that this study has received no financial support.

#### **CONFLICT OF INTEREST**

The authors have no conflicts of interest to declare.

#### ETHICAL CONSIDERATION

Ethical committee approval was obtained for this study.

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# Impact of Molecular Subtypes of Breast Cancer on Axillary Lymph Node Metastasis: A Tertiary Center Experience

Dharmendra Singh\*a, Soumen Mukherjeeb

<sup>a</sup> Department of Radiotherapy, All India Institute of Medical Sciences, Patna, Bihar, India

<sup>b</sup> Department of Radiotherapy, North Bengal Medical College, Darjeeling, West Bengal, India

#### **ARTICLE INFO** ABSTRACT Background: Axillary lymph node metastasis (ALNM) is one of the important **Received:** 30 May 2021 prognostic factors of breast cancer. The objective of this study was to assess the risk **Revised:** of ALNM in different molecular subtypes determined by estrogen receptor (ER), 26 June 2021 progesterone receptor (PR), and human epidermal growth factor receptor 2 Accepted: (her2neu) of breast cancer. 07 July 2021 Methods: This retrospective study was conducted on patients who had undergone upfront breast conserving surgery (BCS) or modified radical mastectomy (MRM). Patients were classified as HR (hormone receptor) +/ her2neu- (ER or PR positive and her2neu negative), HR+/her2neu+ (ER or PR positive and her2neu positive), HR-/her2neu- (ER, PR and her2neu negative or triple negative or basal type), and HR-/her2neu+ (ER or PR negative and her2neu positive). The association between clinicopathological variables and ALNM was evaluated in logistic regression analyses. **Results:** In this study, 476 patients met the inclusion criteria, and had 67.2% ALNM at diagnosis. ALNM was statistically significantly correlated with age $\leq 40$ years Keywords: (p=0.026), tumor grade (p=0.007), pathological tumor size (P<0.001), estrogen molecular subtypes, receptor (P=0.045), molecular subtypes (P=0.021), LVI (P<0.001), and PNI breast cancer, (P<0.001). Post Hoc test revealed that HR-/her2neu+ subtypes of breast cancer had the axillary lymph node, highest and HR+/her2neu- had the lowest risk of ALNM. metastasis Conclusion: ALNM may be predicted by molecular subtypes of breast cancer. The risk of ALNM is less in TNBC although it is clinically more aggressive. These findings may play an important role in gauging the individualized axillary management in breast cancer. Copyright © 2021. This is an open-access article distributed under the terms of the Creative Commons Attribution-Non-Commercial 4.0 International License which permits copy and redistribution of the material in any medium or format or adapt, remix, transform, and build upon the material for any purpose, except for commercial purposes.

# **INTRODUCTION**

Breast cancer ranks top in cancer incidence in 2020 surpassing the lung cancer, estimating 11.7% of all cancer cases.<sup>1</sup> The incidence of breast cancer in India is 13.5%, ranking first in cancer incidence and cancer

\* Address for correspondence: Dharmendra Singh, MD Address: Department of Radiotherapy, All India Institute of Medical Sciences, Patna, Bihar, India. PIN-801507 Tel: +91 9433325398 Email: <u>babu.dsingh.singh35@gmail.com</u> related death.<sup>2</sup> Breast cancer is one of the most heterogeneous diseases considering its origin, pathology, tumor biology, molecular subtypes, gene mutations, disease progression, therapeutic response, and clinical outcome.<sup>3</sup> Axillary lymph node metastasis is one of the important factors in determining staging, treatment, and outcome in breast cancer patients.<sup>4</sup> Several factors including age, tumor location, tumor size, tumor grade, and lymphovascular invasion (LVI) predict the axillary lymph nodes metastasis (ALNM).<sup>5</sup> Breast cancers are classified into different molecular subtypes based on the expression of estrogen receptor

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(ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (Her2neu) on immunohistochemical analyses. These subtypes may be used to guide the treatment, predict the response and outcome of breast cancer.<sup>6, 7</sup> However, the role of breast cancer molecular subtypes (BCMS) in predicting the axillary lymph node metastasis is not well established.<sup>8, 9</sup> In this study, we retrospectively analyzed clinicopathological data to predict the risk of axillary lymph node metastasis according to breast cancer molecular subtypes.

#### METHODS

A retrospective single institutional observational study was conducted at the department of radiotherapy, Institute of Post Graduate Medical Education and Research (IPGME&R), Kolkata. All cases of registered nonmetastatic breast carcinoma from January 2013 to September 2018 were retrieved from medical records files and analyzed. The patients included were females who had histopathologically confirmed diagnosis of unilateral breast carcinoma and underwent either breast conservative surgery (BCS) or modified radical mastectomy (MRM) with axillary lymph node dissection (ALND). Patients treated with neoadjuvant chemotherapy or recurrent breast carcinomas were excluded from the study. Four hundred and seventy six (476) patients meeting the inclusion criteria were included in this study. Clinicopathological features including age at presentation, age group  $\leq 40$  years<sup>10, 11</sup>, post-menopausal, pathological tumor size (pT), pathological lymph node (pN), molecular subtypes of nonmetastatic breast carcinoma were studied with respect to axillary lymph node metastasis. Tumors with immunohistochemistry (IHC) of estrogen receptor (ER), progesterone receptor (PR) having expression  $\geq 1\%$ were considered positive. Immunohistochemistry (IHC) for her2neu was done on formalin-fixed paraffinembedded sections by polymer horseradish peroxide technique. A score of +3 for human epidermal growth factor receptor 2 (her2neu) was considered as her2neu positive, a score of 0 or +1 was considered as her2neu negative while a score of +2 was considered as equivocal. A histopathological specimen having an IHC score of +2 for her2neu was considered for fluorescence in situ hybridization (FISH) study to find out whether the tissue specimen was her2neu negative or positive. FISH negative for her2neu was considered as her2neu negative and FISH positive for her2neu was considered as her2neu positive. The IHC result of Ki-67 was not available for all patients; therefore, molecular subtypes were not classified as per criteria provided at St. Gallen International Breast Cancer Conference.<sup>12</sup>

In this study, patients were classified as HR (hormone receptor)+/her2neu- (ER or PR positive and her2neu negative), HR+/her2neu+ (ER or PR positive

and her2neu positive), HR-/her2neu- (ER, PR and her2neu negative or triple negative or basal type), and HR-/her2neu+ (ER or PR negative and her2neu positive). The histological grades of tumor were determined using modified Scarff-Bloom Richardson scale.<sup>13</sup> All the patients were staged according to American Joint Committee on Cancer (AJCC TNM), seventh edition.<sup>14</sup> The patients received their adjuvant treatment including systemic chemotherapy, radiotherapy, hormonal therapy, and trastuzumab according to stage, risk factors, hormonal receptor (HR), and her2neu status. Those patients who had undergone BCS were advised for whole breast radiotherapy and lumpectomy cavity boosts were given according to their indication.<sup>15</sup> Following completion of the treatment, the patients were followed up once every month for the first three months, every three months for one year, every six months for the next five years. The information was entered into predesigned proforma (data capture sheet) followed by analysis of different clinicopathological characteristics and their correlations.

#### Ethical approval

The Ethics Committee of Institute of Post Graduate Medical Education and Research, Kolkata waived ethical approval in view of retrospective nature of the study and all the procedures being performed were part of the routine care.

# Statistical analysis

The Statistical Package for Social Sciences (IBM SPSS for Windows, version 25.0) was used for statistical analysis. Descriptive statistics were used to characterize the study population using frequencies, mean, and median. Continuous variables were analyzed using the student's t-test. Univariate analysis of factors associated with axillary lymph node metastasis was conducted using logistic regression analysis and factors found to be significant were included in multivariate logistic regression analysis to find out the independent factors associated with axillary lymph node metastasis. Analysis of variance (ANOVA) was used to compare the means of the different groups with regard to a variable. A P<0.05 was considered statistically significant in all performed analyses.

#### RESULTS

In this study, 476 nonmetastatic breast cancer patients were registered at our institute from January 2013 to September 2018 and underwent upfront BCS or MRM. The median age at diagnosis was 46 years. Axillary lymph nodes showed 67.2% nodes positive and 32.8% nodes negative for metastasis. The median number of retrieved axillary lymph nodes in the pathological specimen was 10 (1-51) and the median number of metastatic axillary lymph nodes was 2 (0–32). Estrogen and progesterone receptors were found



positive in 46, and 32.8%, respectively. IHC results for her2neu showed a score of 1 (294), a score of 2 (23), and a score of 3 (159) in 61.8, 4.8, and 33.4% of the patients. The tissue specimen of patients showing an IHC score of 2 for her2neu underwent FISH study (23), which showed 52.1% (12) of them were FISH positive and 47.8% (11) FISH negative for her2neu gene amplification. During the median follow-up of 38 months (14 - 59), 25% showed recurrence. The different clinicopathological features are depicted in Table 1. Association between molecular subtypes, axillary lymph node metastasis, and tumor size is depicted in Table 2.

Table 1. Clinicopathological characteristics of patients

Variables		Ν	%
	Pain	59	12.4
	Lump	279	58.6
	Lump with pain	80	16.8
	Nipple discharge	10	2.1
	Lump with ulcer	48	10.1
	Right	225	47.3
	Left	251	52.7
	Cribriform	24	5.0
	ILC	5	1.1
	Medullary	12	2.5
	NOS	435	91.4
	Grade I	9	1.9
	Grade II	210	44.1
	Grade III	257	54.0
	BCS	54	11.3
	MRM	422	88.7
	pT1	24	5.0
	pT2	193	40.5
	рТ3	173	36.3
	pT4	86	18.1
	pN0	156	32.8
	pN1	143	30.0
	pN2	144	30.3
	pN3	33	6.9
	Stage IA	18	3.8
	Stage IIA	74	15.5
	Stage IIB	107	22.5
	Stage IIIA	170	35.7
	Stage IIIB	72	15.1
	Stage IIIC	35	7.4
	Negative	80	16.8
	Positive	396	83.2
	Negative	199	41.8
	Positive	277	58.2
	Negative	156	32.8
	Positive	320	67.2
	HR+/her2neu-	150	31.5
	HR+/her2neu+	91	19.1
	HR-/her2neu-	155	32.6
	HR-/her2neu+	80	16.8
	Yes	177	37.2
	No	299	62.8



Axillary lymph node involvement is statistically significantly associated with age  $\leq 40$  years ( $\chi^{2}$ -4.925; P=0.026), tumor grade ( $\chi^{2}$ -9.846; P=0.007), pathological tumor size ( $\chi^{2}$ -24.645; P<0.001), estrogen receptor ( $\chi^{2}$ -4.015; P=0.045), molecular subtypes ( $\chi^{2}$ -9.711; P=0.021), LVI ( $\chi^{2}$ -26.686; P<0.001), and PNI ( $\chi^{2}$ -37.136; P<0.001). Axillary lymph node involvement is statistically significantly not associated with her2neu status ( $\chi^{2}$ -2.972; P=0.226), progesterone receptor ( $\chi^{2}$ -0.196; P = 0.658), and post-menopausal status ( $\chi^{2}$ -0.058; P=0.809).

The univariate logistic regression analysis showed that the age group  $\leq 40$  years (Odds ratio-0.604; P=0.027), tumor grade (Odds ratio-1.498; 0.026), tumor size (Odds ratio-1.724; P<0.001), LVI (Odds ratio-3.518; P<0.001), PNI (Odds ratio-3.371; P<0.001), and molecular subtypes (Odds ratio-1.286;

*P*=0.006) are statistically significantly associated with axillary lymph node metastasis (Table 3). The multivariate logistic regression analysis of factors associated with axillary lymph node involvement showed that tumor size (Odds ratio-1.460; *P*=0.005), LVI (Odds ratio-2.261; *P*=0.004), PNI (Odds ratio-2.592; *P*<0.001), and molecular subtypes (Odds ratio-1.442; *P*<0.001) are the independent factors affecting the axillary lymph nodes metastasis (Table 4).

The results of analysis of variance (ANOVA) with post hoc test of least significant differences (LSD) between axillary nodal metastasis and molecular subtypes of breast cancer are presented in Table 5 and Figure 1. It is clear from Figure 3 that HR-/her2neu+ subtypes of breast cancer had the highest risk of axillary lymph node involvement and HR+/her2neuhad the lowest risk of axillary lymph node involvement.

Table 2. Association between tumor size, axillary lymph node metastasis and molecular subtypes of breast cancer.

	Molecular subtypes							
	HR+/he	er2neu-	HR+/her2neu+		HR-/he	HR-/her2neu-	HR-/her2neu+	2neu+
	Count	N %	Count	N %	Count	N %	Count	N %
pT1	8	1.7	3	0.6	9	1.9	4	0.8
pT2	56	11.8	40	8.4	67	14.1	30	6.3
pT3	55	11.6	30	6.3	55	11.6	33	6.9
pT4	31	6.5	18	3.8	24	5.0	13	2.7
pN0	63	13.2	26	5.5	48	10.1	19	4.0
pN1	35	7.4	31	6.5	48	10.1	29	6.1
pN2	37	7.8	27	5.7	54	11.3	26	5.5
pN3	15	3.2	7	1.5	5	1.1	6	1.3

Table 3. Univariate logistic regression analysis of factors associated with axillary lymph node metastasis.

Characteristics	Odds ratio	95% Confidence Interval	Р
Age $\leq 40$ years*	0.604	0.386 - 0.945	0.027
Post-menopausal#	1.049	0.711 - 1.547	0.809
Tumor grade <sup>@</sup>	1.498	1.050 - 2.137	0.026
Tumor size <sup>\$</sup>	1.724	1.346 - 2.207	< 0.001
LVI**	3.518	2.145 - 5.770	< 0.001
PNI <sup>##</sup>	3.371	2.262 - 5.023	< 0.001
Molecular subtypes <sup>\$\$</sup>	1.286	1.076 - 1.538	0.006

\*age ≤ 40 vs > 40 years; <sup>#</sup> Yes vs No; <sup>@</sup> grade I vs Grade II and III; <sup>\$</sup> T1 vs T2, T3, T4; \*\* Positive vs Negative; <sup>##</sup> Positive vs Negative; <sup>\$</sup>HR+/her2neu- vs HR+/her2neu+, HR-/her2neu+, HR-/her2neu+

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Characteristics	Odds ratio	95% Confidence Interval	Р
Age $\leq 40 \text{ yrs}^*$	0.662	0.407 - 1.074	0.95
Tumor grade <sup>@</sup>	1.280	0.865 – 1.893	0.218
Tumor size <sup>\$</sup>	1.460	1.120 - 1.903	0.005
LVI**	2.261	1.308 – 3.909	0.004
PNI <sup>##</sup>	2.592	1.676 - 4.010	< 0.001
Molecular subtypes <sup>\$\$</sup>	1.442	1.181 – 1.759	< 0.001

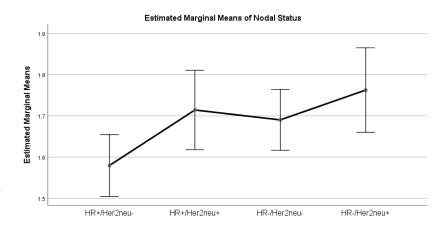
Table 4. Multivariate logistic regression analysis of factors associated with axillary lymph node metastasis.

\*age ≤ 40 vs > 40 years; <sup>@</sup> grade I vs Grade II and III; <sup>\$</sup> T1 vs T2, T3, T4; \*\* Positive vs Negative; <sup>##</sup> Positive vs Negative; <sup>\$\$</sup>HR+/her2neu-vs HR+/her2neu+, HR-/her2neu+, HR-/her2neu+

**Table 5.** Analysis of variance showing post hoc test with least significant differences (LSD) between axillary lymph node metastasis and molecular subtypes of breast cancer.

(I) Molecular	(J) molecular	Mean	D	95% Co Inter	onfidence val
subtypes	subtypes	Difference (I-J)	P –	Lower Bound	Upper Bound
	HR+/her2neu+	-0.13*	0.031	-0.26	-0.01
	HR-/her2neu-	-0.11*	0.040	-0.22	-0.01
	HR-/her2neu+	$-0.18^{*}$	0.005	-0.31	-0.06
	HR+/her2neu-	0.13*	0.031	0.01	0.26
	HR-/her2neu-	0.02	0.698	-0.10	0.15
	HR-/her2neu+	-0.05	0.500	-0.19	0.09
	HR+/her2neu-	$0.11^{*}$	0.040	0.01	0.22
	HR+/her2neu+	-0.02	0.698	-0.15	0.10
	HR-/her2neu+	-0.07	0.262	-0.20	0.05
	HR+/her2neu-	$0.18^{*}$	0.005	0.06	0.31
	HR+/her2neu+	0.05	0.500	-0.09	0.19
	HR-/her2neu-	0.07	0.262	-0.05	0.20

\*The mean difference is significant at the 0.05 level



**Figure 1.** Axillary lymph node metastasis according to molecular subtypes of breast cancer.

#### DISCUSSION

This study aimed to assess the impact of molecular subtypes of breast cancer on axillary lymph nodal involvement. The median age at presentation in this study was 46 years, which was similar to other Indian studies, which reported median age at diagnosis between 45 and 49 years.<sup>11, 16-18</sup> The median age at diagnosis of our patients is similar to that for Korean and Taiwanese population but lower than that for



western population.<sup>19, 20</sup> Majority of the patients (58.2%) were postmenopausal. Raina et al. reported in their study an equal distribution of pre- and postmenopausal women, 49% vs. 48%, respectively.<sup>21</sup> Pathologically pT1, pT2 and pT3 were observed in 5.1%, 40.5%, and 36.3% patients, respectively. A similar finding was observed by Kumar et al. in their study on 56 patients with breast cancer and reported pT1, pT2, and pT3 were 14.2%, 55.4%, and 30.4%, respectively.<sup>22</sup> Nene et al. in reported almost 60% patients having a tumor size of 5 cm or less.<sup>23</sup> A study of 186 patients by Harish et al. reported almost 30% of patients presented with pT3.16 These studies corroborate the findings of our study. In this study, we found stage IA in 3.8%, stage IIA in 15.5%, stage IIB in 22.5%, stage IIIA in 35.7%, stage IIIB in 15.1%, and stage IIIC in 7.4% of the patients. A large Indian multicentric study by Doval et al. reported stage I in 11.8%, stage IIA in 40.9%, stage IIB in 25.9%, stage IIIA in 12.6%, and stage IIIC in 8.8% of the participants in their study.<sup>24</sup> In this study, 67.2% of the patients had axillary lymph node metastasis. Gogoi et al. reported 80% of axillary lymph node metastasis.<sup>25</sup> A large Indian study reported about 48% of patients with pathologically involved axillary lymph nodes.<sup>24</sup> A larger percentage of patients in our cohort presented with tumors of size > 5cm and axillary lymph node positivity compared to the participants in other Indian or Western studies, possibly due to inclusion of patients who had undergone upfront surgical intervention in this study.<sup>26</sup> This may also reflect the late presentation of the disease in association with the natural course of tumor biology, treatment seeking behavior of patients towards cancer or lack of public awareness.

Axillary lymph node metastasis was associated with age, grade of the tumor, tumor size, LVI, PNI, and molecular subtypes as reported by most of the studies in the literature, a finding which is also supported by findings of this study.<sup>16, 21, 23-25</sup> The distribution of molecular subtypes of breast cancer in this study was HR+/her2neu- in 31.5%, HR+/her2neu+ in 19.1%, HR-/her2neu- (TNBC) in 32.6%, and HR-/her2neu+ in 16.8% of their subjects, respectively. Indian studies HR+/her2neuin 19.5 55.2%, reported \_ HR+/her2neu+ in 21.3 - 30%, TNBC in 14.7 - 38.2%, and HR-/her2neu+ in 17.7 - 21%.25, 27-29 A large retrospective study including 1945 patients by Dawood et al. reported Luminal A in 65.8%, Luminal B in 14.3%, TNBC in 10.4%, Her2neu enriched in 4.9% of their participants, respectively.<sup>30</sup> Studies from China, Korea and Malaysia reported Luminal A in 34 - 53.1%, Luminal B in 21.7- 59.2%, TNBC in 13.6 - 20%, and her2enriched in 9 - 27.2% of their participants.<sup>31-33</sup>

HR-/her2neu+ and HR+/her2neu- had the highest and lowest risk of axillary lymph node metastasis found in our study. Vaidyanathan et al. in their study on 368 patients reported her2neu over-expression was more significantly associated with axillary lymph node metastasis compared to hormone receptor positive cases.<sup>34</sup> There is paucity of data on molecular subtypes and risk of axillary lymph node metastasis. There are very few Indian studies directly analyzing the risk of lymph node metastasis with molecular subtypes of breast cancer. Si et al. reported HR-/her2neu- and HR+/her2neu+ having the lowest and highest risk of axillary lymph node metastasis.<sup>35</sup> He et al. reported in their study on more than 3000 patients that HR-/her2neu- had the lowest risk of axillary lymph node metastasis compared to other molecular subtypes of breast cancer.<sup>36</sup> Rossing et al. also reported that TNBC or basal like tumor had the least risk of axillary lymph node metastasis.<sup>37</sup> Several studies suggest the association between molecular subtypes and axillary lymph node metastasis<sup>38-42</sup>, while Jones et al. and Gangi et al. reported no association between molecular subtypes and axillary lymph node metastasis.43, 44 There is also an interesting finding in the Korean study, which reported higher risk of axillary lymph node metastasis in patients with TNBC.<sup>44</sup> However, the role of molecular subtypes of breast cancer in predicting the axillary lymph node metastasis is controversial due to heterogeneous findings in the literature.<sup>45, 46</sup>

There are some limitations in our study. It is a single center retrospective study. The classification of molecular subtypes was not done according to criteria laid down at St. Gallen International Breast Cancer Conference.

# CONCLUSION

In conclusion, our results show that axillary lymph node metastasis may be predicted by molecular subtypes of breast cancer. HR-/her2neu+ subtypes of breast cancer had the highest and HR+/her2neu- had the lowest risk of axillary lymph node metastasis. The risk of axillary lymph node metastasis is less in TNBC although it is clinically more aggressive. These finding may play an important role in gauging the individualized axillary management in breast cancer. However, a larger study needs to confirm our findings.

# **CONFLICT OF INTEREST**

The authors declare that they have no conflicts of interest.

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# Comparison of CEA and CA15-3 Markers with Serotonin, Ceruloplasmin and Copper in Breast Cancer Recurrence after Chemotherapy

Sahar Rezaei<sup>a</sup>, Hemen Moradi-Sardareh<sup>b</sup>, Mohammad-Hasan Khadem Ansari<sup>1a</sup>, Fatemeh Kheradmand<sup>\*c</sup>

<sup>a</sup> Department of Clinical Biochemistry, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran

<sup>b</sup> Department of Clinical Biochemistry, Asadabad School of Medical Science, Asadabad, Iran

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<sup>c</sup> Department of Clinical Biochemistry, Faculty of Medicine, Cellular and Molecular and Solid Tumor Research Centers, Urmia University of Medical sciences, Urmia, Iran

ARTICLE INFO	ABSTRACT
Received: 15 April 2021 Revised: 06 July 2021 Accepted: 13 July 2021	<ul> <li>Background: The serotonin, copper, and ceruloplasmin markers are altered in various cancers, including breast cancer. It has been reported that these markers have the potential to be used in the study of cancer recurrence. The purpose of this study was to compare the levels of serotonin, copper and ceruloplasmin besides the routine breast cancer markers such as CEA and CA15-3 in the blood sample of patients with invasive ductal breast cancer, before and after chemotherapy.</li> <li>Methods: This study was performed on 30 patients with breast cancer. Blood samples were taken from the patients before and after chemotherapy. Necessary data including age, tumor grade and status of Her-2, ER, PR receptors were obtained from patient records. Serotonin, CEA and CA15-3 levels were measured by ELISA method. Ceruloplasmin and copper were measured by nephelometry and colorimetric methods, respectively.</li> <li>Results: Results showed a decrease in serotonin, ceruloplasmin, copper, CEA</li> </ul>
Keywords: Serotonin,	and CA15-3 after treatment but only the levels of serotonin and ceruloplasmin showed a steady decrease. No significant relationship was observed between tumor grade and ER-PR, Her-2 receptors.
ceruloplasmin,	<b>Conclusion:</b> This study showed that chemotherapy resulted in steady decline in
copper,	serotonin and ceruloplasmin levels but this decrease was not steady in levels of
CEA,	CA15-3 and CEA. Therefore, if our results are confirmed by further research, they
CA15-3,	can be considered as a viable alternative to routine markers in cancer recurrence after
breast cancer	chemotherapy.
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# **INTRODUCTION**

Breast cancer is by far the most frequently diagnosed cancer and cause of death among women. According to statistics from 2021 in the United States,

\* Address for correspondence:

Address: Department of Biochemistry, Cellular and Molecular, and Solid Tumor Research Center, Urmia University of Medical Sciences, Urmia, Iran. Email: fkheradmand@yahoo.com it is estimated that breast cancer with 284,200 new cases is ranked first in new cases of cancer.<sup>1</sup> Currently, the most common pathological factors used to assess a patient's condition include tumor size, lymph node status, tumor grade, estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor-2 (HER-2) status.<sup>2, 3</sup>

Serum tumor markers have attracted increasing attention for their use in the screening and monitoring of different types of cancer.<sup>4</sup> Among several tumor markers, CEA and CA15-3 are the two most widely

Fatemeh Kheradmand, MD, PhD,

used markers for diagnosis and follow up in the treatment and recurrence of breast cancer. Numerous studies have been performed to quantitatively evaluate the serum levels of these two tumor markers, which have identified a wide range of cut-off values for predicting the prognosis of poor survival in breast cancer.<sup>5-8</sup> Beyond these routine markers, introducing new markers may help early diagnosis and monitoring the treatment of patients with breast cancer.

Serotonin (Ser) (5-hydroxytryptamine; 5HT) is a monoamine neurotransmitter which mediates a wide range of physiological actions in the human body. Serotonin is implicated in psychiatric and neurological disorders and also plays a fundamental role in tumor growth, differentiation and gene expression. It acts as a growth factor for several types of tumor and non-tumor cells.9 Increased tryptophan metabolism via the Ser pathways could, therefore, be linked to malignant progression in breast cancer.<sup>10, 11</sup> Fröbe et al. studied plasma free serotonin (HT5) as a new tumor marker in breast cancer patients and reported that serotonin levels were increased among those who had recurrence after treatment.<sup>12</sup> In addition to its known functions, serotonin is known as a mitogenic agent for a wide range of natural cells such as vascular smooth muscle cells, muscle cells, lung fibroblasts, renal mesenchymal cells, liver cells, etc.9 A recent study confirmed the plasma serotonin as a predictor for recurrence and poor prognosis in colorectal cancer patients.13

Ceruloplasmin (CP) is an acute phase protein which is normally synthesized in the liver.<sup>14</sup> Serum Copper (Cu) and CP have been reported to be useful markers of disease activity in patients with Hodgkin's disease, Non-Hodgkin's lymphoma, acute leukemia, gastrointestinal tract cancer, lung cancer and breast cancer.<sup>15, 16</sup> CP is suggested to have a role in cancer since it is involved in angiogenesis and neovascularization. Previous studies reported that CP mRNA exists in human colon and breast cancer cell lines.<sup>17</sup> The aim of the current study was to investigate the potential of Ser, CP and Cu beyond the CA15-3 and CEA in breast cancer recurrence after chemotherapy.

#### Methods

#### Patients

Among patients with invasive ductal carcinoma who underwent surgery at Omid Hospital of Urmia, Iran (May 2019 - May 2020), and those who referred to this hospital for chemotherapy, 30 individuals were randomly selected. The patients with severe systemic or cardiovascular disease, other malignant tumors, hematologic diseases, liver and kidney dysfunction, infection, pregnancy, smoking, and taking NSAIDs were excluded from the study. All participants were aware of their participation and had willingly signed a consent form. The study protocol was approved by the Medical Ethics Committee of Urmia University of Medical Sciences.

#### Sampling

Blood samples (6 ml) were collected from each patient at 3 different times (before chemotherapy, before the 4th cycle of chemotherapy, and before the 6th cycle of chemotherapy) and centrifuged after clotting. Serum was harvested and stored at -70°C until used.

#### Chemotherapy program

Chemotherapy was performed in a course of 6 cycles for each cycle of 21 days. Adriamycin and Cyclophosphamide were used for all patients in the first three cycles of chemotherapy and Taxotere and Taxol were used in the second three cycles of chemotherapy. In addition, Herceptin was prescribed to HER2 positive patients.

#### Assessment of serum serotonin

The serum serotonin was determined by serotonin competitive ELISA kit (abcam; cat No. ab133053) following the manufacturer's instructions. Absorbance values were observed at 405 nm. The results were expressed as nanograms per milliliter.

#### Assessment of serum ceruloplasmin

The immunonephelometry method was used to measure the serum ceruloplasmin by NEPHSTAR Ceruloplasmin (CER) Kit (Cat No. DK018) according to the manufacturer's protocol.

# Assessment of serum copper

The serum copper was measured by colorimetric assay kit (Sigma-Aldrich Co, Cat No. MAK127), according to the manufacturer's instructions. The method utilizes a chromogen that forms a colored complex specifically with copper ions. The intensity of the color was measured colorimetrically at 359 nm. The range of linear detection was 7  $\mu$ g/dL (1.0  $\mu$ M) to 300  $\mu$ g/dL (47  $\mu$ M).

# Clinical pathology outcomes

Patients' clinical information including the expression patterns of Estrogen Receptor (ER), Progesterone Receptor (PR), HER-2, stages of tumor, and age were obtained from the patients' files and the results of their pathology tests.

#### Assessment of serum CA15-3 marker

The measurement of serum CA15-3 was been done by DIAMETRA kit (REF: DKO055). Absorbance (E) was read at 450 nm against Blank.

# Assessment of serum CEA marker

The CEA levels in patients' serum were determined quantitatively using CEA EIA Kit (Padtan Elm Co, Iran) according to the standard protocol of manufacture.

	Pre- chemotherapy	Post- chemotherapy (3 cycle)	Post- chemotherapy (5 cycle)	F
CA 15-3	14.813±0.990	12.514 ±0.789 <sup>a</sup>	13.008 ±0.794	14.03
CEA	1.182 ±0.111	0.948 ±0.094 <sup>a</sup>	0.921 ±0.113	5.24
Cu	116.161 ±3.292	$108.460 \pm 2.952^{a}$	106.486 ±2.903 <sup>a, b</sup>	17.06
CP	$34.628 \pm 1.09$	31.266 ±0.939 <sup>a</sup>	$29.666 \pm 0.858^{a, b}$	62.36
Ser	131.477 ±7.153	$113.059 \pm 5.889^{a}$	106.164 ±5.906 <sup>a, b</sup>	41.73

a: compared with pre-chemotherapy, b: compared with 3 cycles of chemotherapy; p<0.01. Cu: Copper, CP: Ceruloplasmin, Ser: Serotonin

#### Statistical Analyses

All analyses were performed by Statistical Package for the Social Sciences (SPSS, V.23). The normality distribution of data was determined by Kolmogorov-Smirnov test. In normal distribution, paired T- test and repeated measures ANOVA test were used to examine the changes of serum levels of these factors. The P<0.05 was considered statistically significant.

#### Results

Demographic and clinical characteristics of the patients

All patients in this study were female with a mean age of 52.03 years (ranging from 32 to 86 years). According to the clinical information, most of the patients were at stage II (63.3%) and only 4 patients were at stage III (13.3%). Furthermore, it was observed that most patients were ER and PR positive (73.3% and 70%, respectively) but HER-2 negative (56.7%).

Changes in study markers during chemotherapy

As can be seen from Table 1, the serum levels of Ser, CP, and Cu levels were steadily decreasing significantly after each cycle of chemotherapy. Levels of serum CA15-3 and CEA significantly decresed only after 3 cycles of chemotherapy and serum CA15-3 levels increased insignificantly after 5 cycles of chemotherapy.

According to the results, the alternations of these markers seemed to be associated with the time points of sampling. The reduction efficacy of chemotherapy for CP, Ser, Cu, CA15-3, and *CEA* were 68.3%, 59%, 37%, 32.6%, and 15.3%, respectively.

According to our data, HER-2 showed a nonsignificant inverse relationship with the average level of all factors except Cu (R: 0.051, *P* value 0.791). Also, Ser, CA15-3 and CEA showed a non-significant inverse correlation with tumor grade (R: -0.087, *P*:0.649; R: -0.039, *P*:0.837; and R: -0.160, *P*:0.397, respectively).

The results showed that there was no significant difference in the levels of Cu, CA15-3, CEA, CP and Ser between patients with HER-2, ER, and PR receptor positive and negative before and after chemotherapy (data not shown). The results also showed that there was no significant difference in the levels of Cu, CA15-3, CEA, CP and Ser in patients with different stages of breast cancer (stages I, II, and III) (data not shown).

#### DISCUSSION

The purpose of the present study was to evaluate the status of routine markers (CEA and CA 15-3) in breast cancer patients during chemotherapy and compare them with CU, CP and Ser as possible markers in monitoring chemotherapy.

There are many different treatments for cancer, nanoparticles<sup>18</sup>, herbal medicines<sup>19</sup>, including chemical drugs, etc. Each treatment shows different effects in specific patients and the same results are not achieved in all cases. Therefore, it is important to follow up and evaluate cancer recurrence with reliable markers. CEA and CA15-3 as tumor markers are widely used in the diagnosis and monitoring of breast cancer. Yijie Fu reported that CEA and CA15-3 increased only in malignant tumors and their values did not change in benign tumors.<sup>20</sup> Several studies demonstrated that these markers do not have enough sensitivity and specificity.

According to the results reported by Agrawal et al.<sup>21</sup>, ductal breast cancer is associated with increased serum CEA and normal levels of CA15-3. However, Duffy et al., in a study measuring CA15-3 to evaluate the treatment process in patients with breast cancer, considered the use of CA15-3 marker appropriate only in advanced breast cancer.<sup>22</sup> In a review study, Mirabelli et al. showed CA15-3 was not suitable to use as a routine marker due to lack of sensitivity and specificity in the early stages of the disease.<sup>23</sup> They also showed that CA15-3 marker was a more specific marker than CEA, and noted the CEA marker was unreliable due to its low sensitivity and lack of specificity in distinguishing benign patients from healthy individuals. CA15-3 also had low specificity and sensitivity in STAGE 1 and 2 of cancer.<sup>23</sup>

In the present study, by comparing the changes of CEA to CA15-3 before and after chemotherapy, a greater decrease in CEA values was observed. CA15-3 levels appear to be affected by surgery and CEA levels appear to be affected by chemotherapy. The findings of the current study are consistent with those



of Shooshtary *et al.* who reported the treatment process (surgery and chemotherapy) reduced the serum level of CA15-3 more than the serum level of CEA.<sup>24</sup>

In the current study, serotonin levels decreased following chemotherapy. There are several possible explanations for this result. The most likely reason is the induction of monoamine oxidase enzyme expression. This finding corroborates the results reported by Gordon *et al.* who suggested that chemotherapy induces the expression of monoamine oxidase enzyme.<sup>25</sup> In the present study, serotonin was reduced as a biomarker for monitoring the treatment process, but this reduction cannot be attributed to the effectiveness of chemotherapy drugs.

Frobe *et al.* found that in patients who responded to the initial treatment but had a recurrence of the disease, the plasma serotonin levels were significantly higher than in the control group, but that the level of CA15-3 remained in the normal range.<sup>12</sup>

A review study by Siddiqui found that serotonin is a growth factor for a variety of tumor and non-tumor cells.<sup>9</sup> This study, referring to the role of serotonin in carcinogenesis and tumor growth, can explain why serotonin increased in patients in our study. Xia Y *et al.* introduced preoperative plasma serotonin elevation as a functional prognostic biomarker for recurrence of colorectal cancer.<sup>13</sup>

In the present study, serum ceruloplasmin and copper levels were significantly reduced during chemotherapy. This finding is in agreement with findings reported by Ohanlon *et al.* who showed the acute phase response can be activated in a variety of

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malignancies.<sup>26</sup> Shenkin also noted an increase in copper controlled by interleukin-1, interleukin-6, and TNF, which could be a reason for high levels of copper before starting chemotherapy.<sup>27</sup>

However, the findings of the current study do not support some previous research such as Schapira who showed that ceruloplasmin levels in metastatic breast cancer patients increased again after an initial decrease after chemotherapy.<sup>28</sup> Due to these contradictory results, further studies in this regard seem necessary. However, our results are broadly consistent with earlier results regarding copper and ceruloplasmin such as the study conducted by Vaidya on breast cancer patients.<sup>29</sup>

#### CONCLUSION

In conclusion, the present study was designed to determine the effect of chemotherapy on Serotonin, Ceruloplasmin, copper, CEA and CA15-3 as biomarkers of breast cancer. One of the most significant findings to emerge from this study is that Serotonin showed a steady decrease compared to other markers during chemotherapy, but further studies are needed to evaluate serotonin as a marker in monitoring breast cancer recurrence after chemotherapy.

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# **CONFLICTS OF INTEREST**

The authors declare that there is no conflict of interests regarding the publication of this paper.

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# Management of Early Breast Cancer at an Australian Cancer Centre During the Early Phase of COVID-19 Pandemic

Elissa J Zhang<sup>a,b</sup>, Kirsty Stuart<sup>a,c,e</sup>, Rina Hui<sup>a,c,e</sup>, Rhiannon Mellor<sup>e</sup>, Wei Wang<sup>a,e</sup>, Verity Ahern<sup>a,c,e</sup>, Farid Meybodi<sup>a,c</sup>, James French<sup>a,c</sup>, Elisabeth Elder<sup>a,c</sup>, Meagan E Brennan<sup>a,c,d</sup>

<sup>a</sup> Westmead Breast Cancer Institute, Westmead Hospital, Westmead, NSW, Australia

<sup>b</sup> Faculty of Medicine, University of New South Wales, Kensington, NSW, Australia

<sup>c</sup> Westmead Clinical School, Faculty of Medicine and Health, University of Sydney, Westmead NSW, Australia

<sup>d</sup> School of Medicine Sydney, University of Notre Dame Australia, Darlinghurst, NSW, Australia

<sup>e</sup> Crown Princess Mary Cancer Centre, Westmead Hospital, Westmead, NSW, Australia

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#### ABSTRACT

Background: This study aimed to prospectively record changes to treatment for **Received:** 25 May 2021 early breast cancer patients during the first wave of the COVID-19 pandemic in **Revised:** Australia. The purpose was to assess the impact on breast cancer outcomes and to 20 June 2021 determine the need for any mitigative actions. Accepted: Methods: The study was conducted in the breast cancer unit of a tertiary referral 24 June 2021 hospital. Patients with early (non-metastatic) breast malignancy discussed in multidisciplinary team meetings between March and June 2020 were included. Patients were newly diagnosed, post-operative or post-neoadjuvant chemotherapy. Standard treatment was defined by Westmead Breast Cancer Institute protocols and any variations related to the pandemic were recorded. Results: In the study, 145 patients were included (median age 59 years). Pandemic-related changes to management were noted in 13 of 145 (9.0%) patients. Four patients experienced a delay to cancer treatments, four were not offered reconstructive/ symmetrisation surgical procedures, three had altered radiotherapy protocols and two patients were not offered enrolment to a clinical trial. These impacts affected the groups presenting with new cancers (n=7/86, 8.1%), postoperative cases (n=4/25, 16.0%) and post-neoadjuvant chemotherapy cases Keywords: presenting for surgical planning (n=2/34, 5.9%). Breast cancer, Conclusion: Most patients (91.0%) received standard treatment during the first Coronavirus, wave of the pandemic. The minor variations from institutional protocols observed in surgery, this study are unlikely to affect local control or survival in this patient cohort, but radiotherapy, close follow-up is required. Quality of life may have been affected for four patients chemotherapy

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who had downgraded or delayed reconstructive procedures.

\* Address for correspondence: Elissa Zhang, MD

Address: Westmead Breast Cancer Institute, Westmead Hospital, Block F/189 Cnr, Hawkesbury & Darcy Rd, Westmead NSW 2145, Australia Email: <u>elissa.zhang@health.nsw.gov.au</u>

# **INTRODUCTION**

During the early phase of the COVID-19 pandemic in Australia (March to June 2020), restrictions were placed on the treatment of early breast cancer following public health recommendations to contain the spread of the novel coronavirus.<sup>1</sup> In the state of New South Wales (NSW), the national population screening program



BreastScreen closed between late March and early May, and elective surgery was suspended between 25 March and 1 July of 2020.<sup>2</sup> Professional bodies in Australia and world-wide released guidelines for cancer treatment due to the pandemic (Table 1).<sup>3-6</sup> The type and extent of breast cancer operations were minimized to reduce hospital admission days and risk of complications. Surgery for low grade DCIS, re-excision of involved margins, complex oncoplastic procedures, contralateral risk-reducing or symmetrising procedures and most breast reconstruction were recommended against.<sup>1, 4</sup> Radiotherapy and chemotherapy restrictions mandated considering the risks and benefits of treatment to minimize the numbers of patients attending hospital and numbers of immunosuppressed patients in the community. This included staged plans to reduce radiotherapy if staff illness reduced capacity, using hypofractionation, omitting boost treatments, and omitting treatment for DCIS and invasive cancer in older women.<sup>7</sup> Systemic treatment recommendations included the use of G-CSF with chemotherapy, neoadjuvant or adjuvant endocrine therapy without chemotherapy for patients with low risk ER-positive/HER2-negative breast cancer, trastuzumab and paclitaxel without anthracycline-based chemotherapy for node negative small size HER2-positive cancer, delay of routine follow-up echocardiograms, and bone mineral density scans.<sup>8-10</sup> Many consultations during treatment and follow-up were moved to telehealth.

Recommending body	Recommendation
NSW Health- Government (Health Directive) <sup>1</sup>	<u>Elective surgery during COVID-19</u> Surgery restricted to Category 1 (most urgent) only. No non-urgent surgery. Implemented 25/3/2020; staged return to normal from 17/4/2020. (Relevance for breast service: No breast reconstruction, risk reducing surgery, complex oncoplastic procedures or contralateral procedures during this time.)
Breast Surgeons of Australia and New Zealand (Guidelines) <sup>4</sup>	<u>Guidelines from BreastSurgANZ Council relating to the COVID-19 pandemic</u> Surgery should be restricted to Category 1 cases. Defer surgery for low and intermediate grade DCIS. Limit complexity of surgery- consider deferral of immediate breast reconstruction and contralateral risk reducing mastectomy. Reconsider need and delivery of chemotherapy. Consider neoadjuvant endocrine therapy when surgery is delayed. Rigorous MDT* discussion and documentation are required.
Australian Society of Plastic Surgeons and Breast Surgeons of Australia and New Zealand (Position statement) <sup>3</sup>	<u>Position statement regarding breast reconstruction during the COVID-19 pandemic</u> In general, breast reconstruction should be delayed. Delayed breast reconstruction and planned secondary or revision breast reconstruction should be postponed. Immediate autologous flap reconstruction for breast reconstruction should be delayed where possible. Immediate tissue expander or direct to implant reconstruction can be evaluated on a case-by- case basis.
Royal Australian and New Zealand College of Radiologists (Guidance) <sup>7</sup>	<u>Principles for Radiation Oncology Practices</u> In summary, limiting transmission where possible, segregation of teams, segregation of well patients from those with or with suspected COVID-19, maximising communication between staff members, and for radiotherapy protocols consideration of shorter fractionation, delay in commencement of treatment, very low risk disease deferment.
Peter MacCallum Cancer Centre, Victoria, Australia (Department of Radiation Oncology, Clinical Response Plan) <sup>5</sup>	<u>COVID-19 Clinical Response Plan for Radiation Oncology</u> Advising patients should stay away if unwell with COVID-19 symptoms, staff quarantine after exposure, reduction of transmission on hard surfaces, hand sanitisation, reduction in visitor access, increased telehealth facilities, segregation of teams, working from home where possible, avoidance of non-essential contact. The plan also discusses service impact levels correlating with proportion of working staff available.

Recommending body	Recommendation
	Practical Considerations for Treating Patients With Cancer in the COVID-19 Pandemic
	In summary, strongly consider the use of primary prophylactic G-CSF with second- and third-
Medical Oncology Group	generation adjuvant regimens, identify patients for no requirement of chemotherapy or less
of Australia <sup>9</sup>	intensive regimens where possible, e.g. neoadjuvant and adjuvant endocrine therapy without
	chemotherapy for low risk ER positive/HER2 negative cancer, paclitaxel with trastuzumab
	without AC for small size, node negative, HER2 positive cancer and conversion to telehealth
	consultations where possible.

From May 2020, there was a staged easing of restrictions in public hospitals in NSW after a six-week period of 'lockdown'. Social distancing rules, mask wearing and limitations on hospital visitors remained until March 2021. Overall NSW experienced a low COVID-19 caseload, peaking at 213 cases in a single day in late March 2020 (Figure 1), with a cumulative total of 56 deaths.<sup>11, 12</sup>

Nationally subsidised breast cancer related imaging including mammogram, 3D-tomosynthesis and MRI decreased by 37% from March to April 2020, before fully returning to pre-COVID service numbers in June 2020.<sup>13</sup> Mastectomies, breast lesion excisions and axillary lymph node procedures in public hospitals across Australia remained stable in March to April before decreasing by 33% in May, reflecting the flow-on effects of reductions in imaging and subsequent cancer diagnoses.<sup>13</sup>

Impacts on early breast cancer treatments during this period may have resulted in sub-optimal treatment, placing them at risk of recurrence or other complications. For example, if a patient did not have an involved margin re-excised, or had altered radiotherapy and chemothereapy regimes, there might be a higher risk of local or distant relapse. Quality of life might be adversely affected if oncoplastic procedures or breast reconstructions were not offered. Understanding alterations to management will help to plan future care and surveillance for patients treated during this time.

The aim of this study was to prospectively document and analyze any changes to treatment for early (non-metastatic) breast cancer during the first wave of the COVID-19 pandemic in NSW, compared to evidence-based protocols.

# Methods

This study was conducted at the breast cancer unit of a tertiary referral hospital (the NSW Westmead Hospital Breast Cancer Institute) between March 15 to June 15, 2020 during the most severe restrictions on hospital treatment. Inclusion criteria were: female patients who were either newly diagnosed DCIS or non-metastatic invasive cancer, post-operative, or finishing neo-adjuvant chemotherapy (NACT), who were being discussed in MDT meetings held three times a week during the study period. Patients with benign lesions or recurrent/metastatic breast cancer were excluded. Patient demographic and cancer data were collected. Each case was allocated a category of "new cancer", "post-op cancer" and "post-NACT", corresponding to the reason for their first MDT discussion during the study period. Therefore, new cancer cases referred before the study period were classified as 'post-operative' or 'post-neoadjuvant' when re-discussed at MDT meeting. Management recommended by the MDT was recorded and any changes from local protocols ('ideal' management) caused by COVID-19 restrictions were documented.

Data were collected in an Excel spreadsheet and analysed in IBM SPSS v26. Cases where the actual (MDT-recommended) management differed from the 'ideal' management were identified, examined in detail and were flagged for recall for closer follow-up post COVID-19 restrictions easing.

# Ethical Approval

The study was registered with the Australian New Zealand Clinical Trials Registry (ACTRN126-20000869976). Approval was obtained from the Western Sydney Local Health District Human Research Ethics Committee (Project ID 2020/PID00900).

# Results

There were 344 new referrals to the institute (86 new malignancies; the remainder atypical or benign). This compares to 456 cases (109 new malignancies) for the same three-month period in 2019, a 24.6% reduction in total new referrals and a 21.1% reduction in new malignancy referrals.

Overall, 145 eligible cases of early breast cancer (DCIS or invasive cancer) were discussed across the MDT meetings. The demographics of the study population are shown in Table 2. All patients were female. Median age was 59 years.

All patients had core biopsies of the breast performed prior to referral or organised through the institute. The majority of patients (n=123, 84.8%) had invasive cancer, and 22 (15.2%) had DCIS on biopsy results.



Variables			<u>N (%)</u>
Age (years)	Mean	57.7 (SD 11.8)	-
Age groups (years)	30-39		11 (7.6)
	40-49		23 (15.9)
	50-59		46 (31.7)
	60-69		40 (27.6)
	70-79		20 (13.8)
	80+		5 (3.4)
Cancer Demographics		Core Biopsy (n=145) N (%)	Surgical Histology (n=127) N (%)
DCIS		22 (15.2)	15 (11.8)
Grade	Low	2 (9.1)	0
	Intermediate	11 (50.0)	10 (66.7)
	High	7 (31.8)	5 (33.3)
	Missing	2 (9.1)	
Invasive cancer		123 (84.8)	109 (86.5)
Histological Type	No specific type	88 (71.5)	77 (70.6)
	Infiltrating lobular carcinoma	18 (14.6)	18 (16.5)
	Invasive other	13 (10.6)	8 (7.3)
	Atypical **	3 (2.4)	-
	pCR	NA	6 (5.5)
	Missing	1 (0.8)	-
Histological Grade	1	17 (13.8)	23 (21.1)
	2	65 (52.8)	53 (48.6)
	3	25 (20.3)	24 (22.0)
	Missing	16 (13.0)	9 (8.3)
Receptor status	ER positive	97 (78.9)	90 (82.6)
	HER2 positive	18 (14.6)	11 (10.1)
	Triple negative	13 (10.6)	7 (6.4)

#### Table 2. Participant and cancer demographics.

\*Including invasive tubular, invasive mucinous, invasive papillary, invasive carcinoma

\*\* Atypical including atypical apocrine, atypical hyperplasia na=not applicable

Details of presentation (reason for discussion by the MDT) are shown in Table 3. Of the sample, 86 (59.3%) were new cancer cases, 25 (17.2%) were post-operative cancer cases and 34 (23.4%) were cases returning for surgical planning after neoadjuvant chemotherapy. Of the 86 new cancer referrals, up-front surgery was recommended in 69 (80.2%) cases and neoadjuvant therapies in 17 (19.8%) cases.

#### COVID-related changes to treatment

Changes to management related to COVID-19 were recorded in 13 of 145 (9.0%) patients. Of these, four patients experienced a delay to cancer treatments, four were not offered their ideal reconstructive or symmetrisation procedures, three had altered radiotherapy protocols and two were not recruited to eligible clinical trials. These impacts mostly affected new cancer patients (n=7, 54%), followed by post-operative (n=4, 31%), then post-NACT (n=2,15%). (Table 4).

# Changes to surgery

Six patients experienced COVID-related changes to surgical management:

• Two patients with intermediate grade DCIS less than 20mm in size had their surgery deferred. They showed no microinvasion on surgical 6

histopathology and were both treated with breast conservation surgery when restrictions were lifted. Patient C had surgery delayed by three months, and Patient E by only ten days.

• Patients B, F and L were denied immediate contralateral symmetrising breast reduction, breast

reconstruction for low grade DCIS, and contralateral prophylactic mastectomy, respectively. Delayed surgical procedures were planned. One patient requested autologous reconstruction, which was changed to implantbased reconstruction.

<b>Reason and Recommendations</b>	N (%)	
Group 1: New cancer	86 (59	0.3)
Upfront surgery	69 (80.2)	
<ul> <li><u>Breast</u></li> <li>Wide local excision (WLE) +/- local flap</li> </ul>	53 (76.8)	
<ul> <li>WLE + contralateral symmetrisation</li> </ul>	1 (1.4)	
- Simple mastectomy	9 (13.0)	
- Mastectomy and immediate reconstruction	4 (5.8)	
- Excision of atypical lesions	2 (2.9)	
Axilla		
- Sentinel lymph node biopsy alone	54 (78.3)	
- Axillary lymph node dissection	3 (4.3)	
- No axillary surgery (DCIS)	12 (17.4)	
Neoadjuvant therapy	17 (19.8)	
- Chemo/targeted	13 (76.5)	
- Endocrine only	4 (23.5)	
COVID-related changes	7 (8.1)	See Table 4; Cases A-G
Group 2: Post-operative Cancer	25 (17.2)	
Further breast surgery	6 (24.0)	
Further axillary surgery	3 (12.0)	
COVID-related change	4 (16.0)	See Table 4; Cases H-K
Group 3: Post NACT surgical planning	34 (23.4)	
Breast		
- WLE	22 (64.7)	
- Simple mastectomy	7 (20.6)	
- Mastectomy and immediate reconstruction	5 (14.7)	
Axilla		
- Sentinel lymph node biopsy alone	14 (41.2)	
- Axillary lymph node dissection	12 (35.3)	
- Targeted axillary dissection	8 (23.5)	
COVID-related change	2 (5.9)	See Table 4; Cases L-M

3

Case	Age	Category	Cancer information	Treatment Changed	Details
A	71	New cancer	pT1cN0M0 Inv NST G1 ER+PR+HER2-	Trial	Not recruited for clinical trial due to trial recruitment shut down for COVID (EXPERT candidate) *
В	57	New cancer	pT2N0M0 Inv NST G3 Triple negative; WLE and SNB with adjuvant radiotherapy and chemotherapy	Surgery/NACT	Would have considered NACT but surgery was recommended to avoid multiple trips to hospital with immunosuppression. Would have elected immediate contralateral reduction but was delayed due to COVID policy
С	58	New cancer	DCIS	Surgery	to avoid non-urgent surgery with potential complications. DCIS surgery deferred due to COVID. Followed up after 3 months –WLE performed at that time- restrictions lifted. Radiotherapy not initiated
D	71	New cancer	PT1cN0M0 Inv mucinous G1 ER+PR+HER2-	Radiotherapy	immediately post op - delayed 3 months. Commenced on endocrine therapy in interim. Operation in March.
E	67	New cancer	DCIS	Surgery	Radiotherapy completed in Au DCIS surgery cancelled, but only delayed by 10 days- restrictions lifted.
F	47	New cancer	DCIS	Surgery	No reconstruction offered post mastectomy (low grade DCIS) Referred to plastic surgery team for delayed reconstruction.
G	54	New cancer	pTicN0M0 Inv NST G2 ER+PR+HER2neg	Trial	Not recruited for clinical trial due to trial recruitment shut down for COVID (EXPERT candidate) *
Н	61	Post op cancer	pT2N0M0 Inv NST G3 ER-PR-HER2pos	Surgery	Not offered free flap despite patient preference. Had implant-based reconstruction.
Ι	52	Post op cancer	DCIS 12mm intermediate grade	Radiotherapy	Boost to tumour bed omitted.
J	59	Post op cancer	DCIS 16mm intermediate grade	Radiotherapy	Boost to tumour bed omitted.
K	61	Post op cancer	pTicN0M0 Inv mucinous G2 ER+PR+HER2equiv	Radiotherapy	Radiotherapy delayed until 4 months post operatively. Commenced on Endocrine therapy in interim.

Case	Age	Category	Cancer information	Treatment Changed	Details
L	35	Post NACT	pT1bN0M0 Inv NST G3 Triple positive	Surgery	Requested bilateral mastecomy but only offered unilateral.
М	56	Post NACT	IDC triple negative, node positive, RCB- II	Radiotherapy	Treated with hypofractionated radiotherapy rather than conventional fractionated post- mastectomy radiotherapy

\*The EXPERT Trial is a randomised phase III trial of adjuvant radiation therapy versus observation following breast conserving surgery and endocrine therapy in patients with molecularly characterised luminal A early breast cancer.<sup>14</sup>

NACT=neoadjuvant chemotherapy

# Changes to chemotherapy

Of 145 patients, 104 (71.7%) involved a medical oncologist in their breast cancer management. Of the 104 patients, 30 (28.8%) completed neoadjuvant systemic treatment and proceeded to surgery, 19 (18.3%) commenced neoadjuvant or adjuvant chemotherapy +/- anti-HER2 treatment, 17 (16.3%) proceeded to adjuvant endocrine therapy, 5 (4.8%) did not receive any systemic treatment and 33 (31.7%) had their medical oncology consultations after the study period.

One patient (B) experienced COVID-19 related changes to chemotherapy:

Neoadjuvant chemotherapy was ideally recommended due to her grade 3 triple negative cancer with high proliferative index of 70%. Ultimately up-front surgery was recommended to avoid multiple hospital visits and immunosuppression. She promptly underwent wide local excision and sentinel node biopsy (11 days after MDT discussion), followed by adjuvant chemotherapy (47 days after surgery) and radiotherapy.

There were no pandemic related changes to adjuvant chemotherapy regimens or dosing. However, prophylactic addition of pegfilgrastim to doxorubicin/cyclophosphamide (AC) chemotherapy was implemented in March 2020. None of the 16 patients who started AC experienced febrile neutropenia during the study period, compared to an average of two patients per month prior to this period.

The time intervals between MDT discussion and Medical Oncology consultation (nine days for neoadjuvant chemotherapy and 12 days for adjuvant chemotherapy) and subsequent first cycle of chemotherapy (12 days) were comparable to outside this time period without delay.

Patients on clinical trials continued treatment as per protocol without interruption, but telehealth safety monitoring was instigated during the study period. There was no new recruitment to clinical trials during this period.

# Changes to radiotherapy

There were 124 of 145 women (85.5%) recommended to receive radiotherapy. Of these, 99 (79.8%) were consented for radiotherapy during the study period, 19 (15.3%) were awaiting consent at the end of the study period, two (1.6%) declined radiotherapy, one (0.8%) was deemed unsuitable due to prior radiotherapy, and three (2.4%) had their treatment at a different centre. COVID-related radiotherapy consent alterations only occurred in the first two months of the first wave of the pandemic. During this period, ultra-hypofractionation regimens such as the FAST Forward trial were not implemented in this centre.<sup>15</sup>

Five patients experienced a change to radiotherapy due to COVID-19.

- Two patients (D, K) had a delay to treatment start. Both were over 60 years of age with either a grade 1 or 2 tumour and were commenced on adjuvant endocrine therapy starting radiotherapy three months later.
- Three patients had changes to their radiotherapy protocols:
  - Initially, a change in prescription to shorten the course of radiotherapy was recommended in five patients. However, between consent and initiation of treatment (2-5 weeks), two prescriptions were reverted to standard treatment. This involved one patient where a boost was to be omitted but was delivered, and one patient who was consented for a hypofractionated protocol but changed to conventional fractionation for whole breast and comprehensive nodal irradiation during the radiotherapy planning process as COVID-19 cases in NSW declined.
  - Two patients had a boost to the surgical cavity omitted to shorten treatment course duration
  - One patient received hypofractionated chest wall radiotherapy rather than conventional fractionated post-mastectomy radiotherapy post-NACT.



#### DISCUSSION

These results show that in the context of low pandemic burden, a minority of early breast cancer patients (9.0%) received altered management, with the majority still receiving ideal treatment regimens. These findings provide reassurance that minimal patients may experience future recurrence as a result of 'less than ideal' management during the pandemic. The modest changes recorded are likely influenced by factors including reduced new presentations, institutional mitigative strategies and comparatively low COVID-19 transmisison. These observations can provide insights in minimising compromises to oncological care for other institutions with improving COVID-19 rates and for future pandemics.

The changes to surgery, radiotherapy, and chemotherapy treatments were unlikely to impact significantly on risk of recurrence. The patient who was treated with up-front surgery rather than neoadjuvant chemotherapy is unlikely to have adverse effects from the change in sequencing. For other chemotherapy patients, the use of prophylactic G-CSF reduced the incidence of febrile neutropenia in neoadjuvant or adjuvant treatments and resulted in hospitalisations reducing substantially. Approximately 20% of patients may experience adverse events with bone pain, which can often be controlled with analgesia. Primary prophylaxis using pegylated G-CSF with AC may become routine treatment for the post-COVID-19 era.

Radiotherapy changes include two DCIS cases treated with whole-breast radiation with omission of a boost to the tumour bed. The benefit of a boost to the tumour bed for patients with non-low risk DCIS was not known until the publication of the TROG DCIS trial results in December 2020.<sup>16</sup> One case of low-grade mucinous cancer experienced an intentional three-month delay to her radiotherapy treatment and commenced endocrine therapy in the interim. The likelihood of this significantly increasing risk of recurrence is considered very small.

Two patients were not referred for an eligible trial study during MDT discussions. Recommendation for trial therapy was not primarily discussed during these meetings and possibly many more patients referred for eligible trials at other points of management were not captured by this study. This has impacted the recruitment and progression of clinical trials suspended during this period.

Institutions abroad facing higher COVID-19 prevalence implemented stricter precautions to minimize the risk of infecting vulnerable cancer patients and experienced higher levels of resource competition.<sup>17-19</sup> Strategies to cope included "hub and spoke" models, where a central facility coordinates care that is carried out in designated "COVID-19

free" satellite locations.<sup>17-19</sup> Similarly in this study, most new referrals recommended for upfront surgery underwent their operations at COVID-free facilities within the same public hospital district or at locally affiliated private hospitals. This allowed the institution to focus on COVID management, protect surgical cancer patients from viral exposure, and utilize available resources in the private sector, which experienced a massive reduction in work-load during the suspension of elective surgeries. Breast cancer specific services and personnel remained largely operational during the study period, and were not significantly impacted by resource limitations. This was facilitated by a comparatively low burden of local COVID-19 cases in NSW. No patients in the study cohort were found to be COVID-19 positive although there may have been a low infection risk from isolated events of community transmission to healthcare staff who tested positive during this period.

Telemedicine is also being increasingly utilized for breast cancer management and follow-up appointments. Long terms consequences may still emerge such as potentially missing recurrences and early signs of complications due to the omission of clinical examination. Alternatively, it may encourage saving of healthcare resources and increase patient convenience.<sup>20</sup>

The full impact of COVID-19 on early breast cancer may take years to understand as delayed presentations manifest. This study was conducted over the three-month period of most severe lockdowns and restrictions in NSW and showed a 25% reduction in new cancer referrals during the study period. This correlates to when the national screening program was closing down.<sup>2</sup> Furthermore, national data demonstrated a decrease of 30-50% in Medicare benefits (Australian universal healthcare scheme) claimed for breast cancer diagnostic and treatment services in April/May 2020, with some recovery in later months.<sup>13</sup> Significant reductions in new breast cancer referrals observed during lockdown period in Australia are reflected in other countries such as the UK where urgent new cancer referrals dropped by up to 80% in early 2020, attributed to disrupted screening programs, reduced primary care presentations, and more cautious health-seeking behaviours.<sup>21, 22</sup> Diagnostic delays may be associated with a backlog of patients who could present with more advanced disease requiring more complex treatment courses involving systemic therapies, upgraded surgical procedures. and poorer outcomes.<sup>21, 23</sup> The national BreastScreen program has already observed a surge of 12000 screening mammograms in July to September 2020 following re-opening, compared to the same period in 2018.<sup>24</sup> Modelling of the impact of diagnostic delays for

patients presenting in a 12 month period under different COVID-19 scenarios estimates an increased number of deaths due to breast cancer by 7.9-9.6% in the ensuing 5 year period.<sup>22</sup>

These flow-on effects affect not only new patients at diagnosis and primary management but cause delays to "non-urgent" treatments, such as reconstructive procedures. After non-urgent surgeries were suspended between March and July 2020 in the NHS, there is now a backlog of over 1500 patients awaiting post mastectomy breast reconstructions.<sup>25</sup>

This study has some strengths and limitations. The strengths include the prospective documentation of 'ideal' treatment plans and COVID-related changes through robust data collection by the MDT. This was limited by excluding post-operative patients who were already receiving adjuvant treatment during the study period as they had treatment plans decided pre-COVID. This study also did not measure the psychological impact of treatment changes, which may be significant. Several patients were unable to have their preferred plans for breast reconstruction even though the importance of choice for these treatments on psychological well-being has been documented.<sup>26</sup> Further research is needed to explore the impacts on quality of life by changes to treatment related to COVID-19, and the psychological impacts of a cancer diagnosis at this time of heightened overall distress in the community. A further limitation is that patient-reported outcomes were not collected. The study did not continue beyond mid-2020 to see if there was a 'catch-up' in referrals, as would be expected according to national data.<sup>13</sup> Also, follow-up data is not available at this stage, and this will be required to evaluate the impact of changes to treatment.

Much of the research on COVID-19 impacts on breast cancer care comprises observational, single centre data, to which this paper contributes perspectives from a low pandemic burden institution. For healthcare systems integrating pandemic-related strategies into mid and long-term practice, larger multicentre studies and systematic reviews are required to establish evidence-based protocols. As

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vaccination programs roll out, countries entering recovery status may take precedent from countries with low pandemic burden when accounting for surging capacity in routine screening and treatment. Similarly, countries with rising cases related to easing lockdowns and spread of new COVID strains may more quickly adopt proven guidelines to protect cancer patients from infection while preserving ideal oncological outcomes. Approximately 12 months from the lifting of the initial lock-down, Sydney experienced a second wave of COVID-19 caused by the Delta variant, so the experience obtained from the first wave was put into practice again.

#### CONCLUSION

Despite the temporary cessation of breast cancer screening, restrictions on surgery and modified radiotherapy and chemotherapy recommendations, 91.0% of patients at our institution received standard care for their breast cancer during the first wave of the COVID-19 pandemic in NSW. This was possible due to the relatively small COVID-19 incidence in Australia, combined with partnerships between public and private hospitals to facilitate the provision of urgent cancer surgery. Review of the cases experiencing a change to treatment shows that the modifications are unlikely to be of oncological significance. It is possible, however, that the restricted access to breast reconstruction and contralateral surgery options may have quality of life implications which will be addressed in the follow-up of these

#### **CONFLICT OF INTEREST**

RH has participated in advisory boards for AstraZeneca, Bristol Myers Squibb, Eli Lilly, Merck, Merck Sharp and Dohme, Novartis, Oncosec, Pfizer, Roche and Seagen and has received speaker honoraria from Merck Sharp and Dohme, Novartis and Roche. EE has participated in advisory board for Merck Sharp and Dohme. There are no conflicts of interests to declare for the other authors. There were no funding sources to declare. All authors confirm that they had full access to all the data in the study.

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# Serum Inflammation Biomarkers and Micronutrient Levels in Nigerian Breast Cancer Patients with Different Hormonal Immunohistochemistry Status

Ganiyu Olatunbosun Arinola\*<sup>a</sup>, Fabian Victory Edem<sup>a</sup>, Abayomi Benjamin Odetunde<sup>b</sup>, Christopher Olusola Olopade<sup>c</sup>, Olufunmilayo Ibironke Olopade<sup>d</sup>

<sup>a</sup> Department of Immunology, College of Medicine, University of Ibadan, Ibadan, Nigeria

<sup>b</sup> Institute of Medical Research and Training (IAMRAT), College of Medicine, University of Ibadan, Ibadan, Nigeria

<sup>c</sup> Department of Medicine and the Center for Global Health, University of Chicago, Chicago, USA

<sup>d</sup> Center for Clinical Cancer Genetics and the Center for Global Health, University of Chicago, Chicago, USA

ARTICLE INFO	ABSTRACT
Received: 02 June 2021 Revised: 25 July 2021 Accepted: 26 July 2021	<b>Background</b> : The importance and relevance of serum inflammation biomarkers and DNA methylation-dependent micronutrients in breast tumorigenesis is gaining wider acceptance. However, the association of serum inflammation biomarkers and micronutrient status with expression of estrogen receptors (ER), progesterone receptors (PR) and human epidermal growth factor receptor-2 (HER-2) by the tumor has not been investigated in Nigerian breast cancer patients. The objective of this study was to determine the levels of serum biomarkers of inflammation [Homocysteine, Nitric Oxide (NO), Hydrogen peroxide (H <sub>2</sub> O <sub>2</sub> ), Myeloperoxidase (MPO), Tumor necrosis factor alpha (TNF- $\alpha$ ), Interleukins 6 and 8 (IL-6 and IL-8)] and DNA methylation-dependent micronutrients [Zinc (Zn), Folic acid, Vitamin B6 and B12] in breast cancer patients with different hormone receptors (ER, PR and HER-2). <b>Methods</b> : One hundred and fifteen women (80 with breast cancer and 35 controls) were randomly recruited for this study. Serum levels of homocysteine, folic acid, vitamins B6, vitamin B12, TNF- $\alpha$ , IL-6 and IL-8) were analyzed using ELISA, while the levels of NO, MPO, H <sub>2</sub> O <sub>2</sub> and Zn were determined using spectrophotometer in patients with breast cancer and control subjects without breast cancer as well as breast cancer patients with ER, PR and HER-2 expression were determined. <b>Results:</b> The results showed that mean serum levels of IL-6 (p=0.002), IL-8 (p=0.018) and H <sub>2</sub> O <sub>2</sub> (p=0.000) were significantly increased while TNF- $\alpha$ (p=0.014) and NO levels (p=0.044) were significantly decreased in breast cancer patients compared to healthy controls. However, there were no statistically significant differences in the levels of Zn, homocysteine, Vitamin B6, Vitamin B12 and MDO
Cytokines,	MPO in breast cancer patients and controls. Furthermore, the levels of serum inflammatory biomarkers
	and methylation-dependent micronutrients were similar in breast cancer patients with HER-2, ER and
DNA-methylation,	PR expression.
Inflammation,	Conclusion: Systemic inflammation exists in breast cancer patients but the inflammation
Micronutrients,	biomarkers and methylation-dependent micronutrients did not differ among breast cancer patients with PR, ER and HER-2 expression.

Copyright © 2021. This is an open-access article distributed under the terms of the <u>Creative Commons Attribution-Non-Commercial 4.0</u> International License which permits copy and redistribution of the material in any medium or format or adapt, remix, transform, and build upon the material for any purpose, except for commercial purposes.

# INTRODUCTION

Globally, breast cancer is the most commonly diagnosed cancer in women<sup>1</sup> and while progress has

\* Address for correspondence: Ganiyu Olatunbosun Arinola, Ph.D Department of Immunology, College of Medicine, University of Ibadan, Nigeria Tel: +234 80 2345 1520, +234 80 9812 1520 Email: drogarinola64@gmail.com been made over the last decades in understanding the biology of breast cancer, the mechanisms for growth and progression of breast cancer with different hormonal phenotypes and therapeutic resistance are still not fully understood.<sup>2,3</sup> Inflammation is an important factor in carcinogenesis; hence, the use of non-steroidal anti-inflammatory drugs such as aspirin in cancer prevention adjuvant therapy<sup>4,5</sup> and micronutrients as supplements was suggested.<sup>6</sup> There is evidence that DNA methylation and its associated micronutrients influence incidence of cancers through regulation of inflammatory genes and that the tumor cells produce proinflammatory factors that encourage chronic inflammation and tumor growth.<sup>7.8</sup>

Studies associating circulating homocysteine with overall breast cancer risk are limited and inconsistent.<sup>9</sup> Homocysteine is associated with oxidative damage and metabolic disorders which may lead to carcinogenesis. It is transsulfurated to cysteine or remethylated to methionine using cystathionine  $\beta$ -synthase with vitamin B6 and methionine synthase with vitamin B12 plus folate, respectively.<sup>10</sup> In vitro studies have shown that homocysteine levels were positively associated with proliferation rates of cells in several tumors, including breast cancer.<sup>11,12</sup> Similar associations have been observed with oxidative damage to cells apart from breast cancer cells.<sup>13,14</sup> One case-control study reported a positive association between homocysteine levels and breast cancer risk<sup>15</sup>, whereas another cohort study did not observe such association.9

Folate, vitamin B6, Zn and vitamin B12 are important in cancer prevention by upholding DNA integrity and regulation of gene expression.<sup>16,17</sup> Observational studies have suggested an inverse association between high intake or blood levels of folate, vitamin B6, and vitamin B12 and increased risk of cancer, particularly colorectal and breast cancers.<sup>18-21</sup> Zinc activates inflammasome<sup>22</sup>, induces IL-1 $\beta$  secretion by macrophages<sup>23</sup>, reduces IL-6 and TNF- $\alpha$  in human monocytes<sup>24</sup>, and neutralizes generation of reactive oxygen species (ROS). Two case-control studies demonstrated statistically significant inverse associations between serum Zn exposure and breast cancer risk.<sup>25,26</sup> However, two nested case-control studies reported no association between zinc in benign breast tissue and breast cancer risk.<sup>27,28</sup> Another case-control study also found no difference in plasma level of zinc between breast cancer cases and controls.<sup>29</sup> Thus, interlinkage of Zn, vitamin B6, B12, folate and homocysteine metabolism seems essential for carcinogenesis but the results are inconsistent.

The importance of steroid hormone receptors to the biology of breast cancer was recognized when human breast cancers were shown to be dependent on estrogen and/or progesterone through their receptors for growth.<sup>30</sup> Breast cancer cells may or may not have estrogen receptor (ER), progesterone receptor (PR), and Human Epidermal Growth Factor Receptor 2 (HER-2). These receptor types have been found to determine breast cancer aggressiveness, treatment and prognosis<sup>31,32</sup> but the basis of this is not yet fully elucidated.

Metastasis involves cellular elements that secrete humoral products, which modulate the behavior of

tumor cells in the micro-environment.<sup>33,34</sup> Hence, the presence of hypoxia and abundance of cytokines (Colony Stimulating Factor-1, TNF-α, IFN-γ and MIF in the tumor determines the macrophage subtypes (M1 and M2)<sup>35,36</sup> which affect the clinical course of the tumor. M1 macrophages are 'primed' by the cytokine IFN- $\gamma$  for activation either by TNF- $\alpha$  or (and more importantly) by activation of toll-like receptors<sup>37</sup>. Activated M1 macrophages secrete proinflammatory cytokines such as interferons and interleukins (IL-12, IL-23)<sup>36</sup>, generate toxic oxygen species and activate inducible NO synthase (iNOS) gene to produce nitric oxide (NO). Nitric oxide is an intermediate reactive oxygen species and prolonged exposure to NO results in DNA damage that is linked to cancer development.<sup>37</sup> M2 macrophages are activated by cytokines or by immune complexes to induce T-helper 2 immune responses.

Overall, micronutrients and other inflammation factors play important roles in tumor progression. Therefore, knowledge of functions and levels of biomarkers of inflammation, and micronutrients involved in tumorigenesis could provide better understanding of tumor development, prevention and immunotherapy. This study explored the relationship between pro-inflammation factors (IL-6, IL-8, TNF- $\alpha$ , NO, MPO and H<sub>2</sub>O<sub>2</sub>) and DNA methylation-dependent micronutrients (Zn, folate, vitamin B6, B12 and homocysteine) in breast cancer patients compared with controls in different breast cancer hormone subtypes in Nigerian women.

These markers (pro-inflammation factors and DNA methylation-dependent micronutrients) were evaluated in the participants because they were directly linked with breast cancer immune-pathology and progression.

# METHODS

#### Subjects

Approval for the study was obtained from the ethics committee of UI/UCH, Ibadan. After obtaining informed consent, eighty women with breast cancer who attended the University Teaching Hospital Ibadan, Oyo State, in Nigeria were recruited between March 2016 and June 2019. The mean age of women was 57.9±11.1 years. Breast cancer patients who received any therapy prior to diagnosis (surgery/radiotherapy/chemotherapy) were excluded. Patients who presented with other malignancies, advanced organ failure or active infection were also excluded. The diagnosis of breast cancer was confirmed by histopathological and immunohistochemistry examination of the tumor tissue samples. Thirty-five healthy age-matched female consented volunteers (55.9±9.0 years) who had no history or clinical evidence of breast problem or cancer drawn from the Hospital and University communities were selected as healthy controls. Confounding factors such as age of menarche, ethnicity, age of first birth and parity were not taken into consideration during the study. However, smokers, hormone and alcohol users, and obese participants were excluded. All participants on compulsory medications or on food supplements were also excluded. Blood samples collected from breast cancer patients before treatment and in the healthy controls were allowed to clot, centrifuged at 8000 rpm for 10 minutes, serum separated and stored at  $-80^{\circ}$ C until analyzed.

#### Serum IL-6, IL-8 and TNF-α assays

The procedure followed the manufacturer's (ABCAM USA) instructions as previously described.<sup>38</sup> All reagents were brought to room temperature (18-25ºC) prior to use. Up to 100µL of each standard and sample was added into appropriate wells. The wells were covered and incubated for 150 minutes at room temperature. The solutions were discarded and washed 4 times, by filling each well with 1X wash solution (300µL) using a multi-channel pipette. Supernatant was completely removed at each step. After the last wash, any remaining wash buffer was removed by decanting and the plate was blotted against clean paper towels. Then,  $100\mu$ L of 1X biotinylated TNF- $\alpha$ detection antibody was added to each well and incubated for 60 minutes at room temperature with gentle shaking. The solution was decanted and the wash was repeated. Then, 100µL of 1X HRP-Streptavidin solution was added to each well. The plate was incubated for 45 minutes at room temperature with gentle shaking. The solution was decanted and the wells were washed. Then, 100µL of TMB one-step substrate reagent was added to each well and incubated for 30 minutes at room temperature in the dark with gentle shaking, and 50µL of stop solution was added to each well. The wells were read at 450 nm. A standard curve was constructed for each method using the respective standard and used for the determination of unknown respective serum sample concentrations of IL-6, IL-8 or TNF-α.

#### Serum nitric oxide (NO) determination

Nitric oxide concentration was determined using Griess reagent (Sulpanilamide and N-1napthyethylenediamine dihydrochloride) as previously described.<sup>38</sup> The assay was based on a reaction that utilized sulpanilamide and N-1napthyethylenediamine dihydrochloride (NED) under acidic (phosphoric acid) conditions. Nitrite forms colored chromophore with reagent, with an absorbance spectrum maximum at 540nm. The production of nitrite was quantified by comparing the

result with absorbance of standard solutions of sodium nitrite.

#### Serum hydrogen peroxide determination

Hydrogen peroxide concentration was determined as previously carried out. The assay was based on peroxide-mediated oxidation of Fe2+, followed by the reaction of Fe3+ with Xylenol orange to form Fe3+-Xylenol orange complex with an absorbance maximum of 560nm. Plasma  $H_2O_2$  was determined by comparing absorbance with standard solutions of  $H_2O_2$ .

# Serum myeloperoxidase (MPO) activity determination

MPO activity was determined as previously carried out.<sup>39</sup> The rate of decomposition of  $H_2O_2$  by peroxidase, with guaiacol as hydrogen donor, produced tetraguaiacol which was measured at 436nm and at 25°C.

#### Vitamin determinations

Vitamins B6, B12, folic acid and homocysteine were determined using Enzyme Linked Immunosorbent assay (ELISA) method as previously carried out.<sup>38,39</sup>

#### Statistical analysis

Data obtained were analyzed using SPSS version 20, which are presented in the Tables as Means and Standard error of means. The data were evaluated using Student *t*-test for the patient group relative to the control group (Table 1) while Analysis of Variance (ANOVA) was used to compare data between the three groups of breast cancer patients. Student *t*-test was used to compare data between two groups of breast cancer patients in Tables 2, 3, Figures 1, 2, 3 and 4.  $P \le 0.05$  was taken as significant.

#### RESULTS

The mean values and statistical comparison of TNF-a, IL-6, IL-8, MPO, NO and H<sub>2</sub>O<sub>2</sub> in breast cancer patients and the control group are presented in Table 1. As the results show, the mean values of IL-6 (p=0.002), IL-8 (p=0.018) and H<sub>2</sub>O<sub>2</sub> (p=0.000) were significantly increased while TNF- $\alpha$  (p=0.014) and NO (p=0.044) were significantly decreased when breast cancer patients were compared with apparently healthy controls. However, there were no statistically significant differences in the levels of MPO, Zn, homocysyteine, vitamin B6 and vitamin B12 when breast cancer patients were compared with controls (Table 1). Further, there were no statistically significant differences in the values of inflammation factors (Table 2) and micronutrients (Table 3) when breast cancer patients with different immunohisto -



chemical hormonal status were compared or when breast cancer patients with double positives, single positive or no hormonal receptors were compared (Figures 1 and 2). There were no statistically significant differences in the values of inflammation factors and micronutrients when breast cancer patients with different receptors were compared (Figures 3 and 4).

Table 1. Comparison (Mean±SEM)	of inflammation biomarkers and micronutrients in breast cancer	patients and controls.

Inflammation biomarkers	BCa (n=80)	Controls (n=35)	t-values	Р
$H_2O_2(\mu mol/L)$	37.98±1.64	18.18±1.27	9.521	0.000*
IL-6 (pg/mL)	31.72±6.10	11.54±0.37	3.305	0.002*
IL-8 (pg/mL)	29.65±2.48	23.33±0.77	2.438	0.018*
TNF-α (pg/mL)	$7.96 \pm 1.24$	19.49±5.95	2.507	0.014*
NO (µmol/L)	25.32±2.48	32.58±2.54	2.046	0.044*
MPO (U/mL)	1.15±0.20	1.29±0.02	0.695	0.489
Micronutrients				
Zn (µg/dL)	102.66±1.23	104.49±2.00	0.780	0.439
Hcy (µmol/L)	12.69±0.54	12.14±0.81	0.560	0.570
Folate (µg/L)	348.51±14.76	333.39±22.14	0.568	0.572
Vit B6 (ng/mL)	15.86±0.67	15.17±1.01	0.570	0.578
Vit B12 (ng/L)	419.12±17.29	405.73±26.95	0.416	0.677
*C:: f:+ D <0.05				

\*Significant at P<0.05

BCa= Breast cancer, ER= Estrogen receptor, PR= Progesterone receptor, HER-2= Human Epidermal Growth Factor Receptor 2, Hcy= Homocysteine, NO= Nitric Oxide, H<sub>2</sub>O<sub>2</sub>=Hydrogen peroxide, MPO= Myeloperoxidase, TNF- $\alpha$ = Tumor necrosis factor alpha, IL-6= Interleukin 6, Zn= Zinc, IL-8= Interleukin 8

**Table 2.** Comparison (Mean±SEM) of inflammation biomarkers in breast cancer patients with different immunohistochemical hormonal levels.

	$H_2O_2$	IL-6	IL-8	TNF-α	NO	MPO
	(µmol/L)	(pg/mL)	(pg/mL)	(pg/mL)	(µmol/L)	(U/mL)
ER+ve(n=17)	38.6±10.4	26.6±9.4	28.1±2.1	8.1±1.5	24.8±14.9	1.0±0.3
PR+ve (n=16)	35.8±14.0	24.2±8.5	27.3±3.7	8.7±2.3	31.9±32.2	$0.8\pm0.4$
HER-2+ve (n=16)	33.0±14.4	17.8±6.4	32.8±18.8	7.9±0.9	18.2±11.3	1.0±0.4
t, p-a	0.415, 0.686	0.497, 0.629	0.498, 0.628	-0.564, 0.584	-0.527, 0.609	0.851, 0.413
t, p-b	0.807, 0.437	1.947, 0.078	-0.669, 0.517	0.424, 0.680	0.879, 0.398	0.017, 0.987
t, p-c	0.335, 0.744	1.475, 0.171	-0.713, 0.492	0.883, 0.398	0.982, 0.349	-0.769, 0.460

a: ER+ve compared withPR+ve

b: ER+ve compared with HER-2+ve

c: PR+ve compared with HER-2+ve

 $\begin{array}{l} \mbox{ER=Estrogen receptor, PR=Progesterone receptor, HER-2=Human Epidermal Growth Factor Receptor 2, NO=Nitric Oxide, H2O_2=Hydrogen peroxide, MPO=Myeloperoxidase, TNF-\alpha=Tumor necrosis factor alpha, IL-6=Interleukin 6, IL-8=Interleukin 8 \\ \end{array}$ 

**Table 3.** Comparison (Mean±SEM) of micronutrients in breast cancer patients with different immunohistochemical hormonal levels.

	Zn (µg/dL)	Hcy(µmol/L)	Folate (µg/L)	Vit B6 (ng/mL)	Vit B12 (ng/L)
ER+ve (n=17)	102.3±9.5	11.3±4.2	310.3±115.1	14.1±5.2	377.6±140.1
PR+ve (n=16)	$101.7 \pm 7.6$	12.4±3.7	339.8±102.1	15.5±4.6	413.5±124.2
HER-2+ve (n=16)	$100.3 \pm 8.2$	12.5±5.2	343.5±143.1	15.6±6.5	418.1±174.1
t, p-a	0.131, 0.898	-0.485, 0.637	-0.485, 0.637	-0.485, 0.637	-0.485, 0.637
t, p-b	0.390, 0.704	-0.465, 0.651	-0.465, 0.651	-0.485, 0.651	-0.485, 0.651
t, p-c	0.287, 0.780	-0.052, 0.960	-0.052, 0.960	-0.052, 0.960	-0.052, 0.960

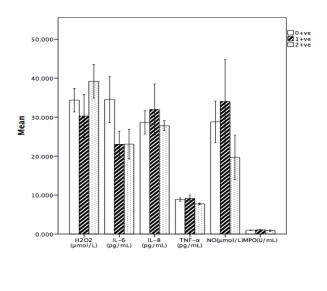
a: ER+ve compared withPR+ve

b: ER+ve compared with HER-2+ve

c: PR+ve compared with HER-2+ve

ER=Estrogen receptor, PR=Progesterone receptor, HER-2=Human Epidermal Growth Factor Receptor 2, Hcy=Homocysteine, Zn=Zinc, Vit B6=Vitamin B6, Vit B12=Vitamin B12

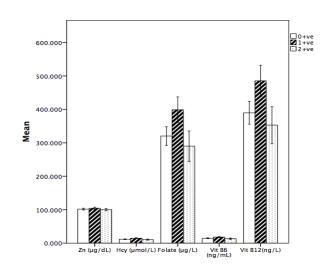




patients with double positives (2+ve), single positive (1+ve) or no (0+ve) hormonal receptor. NO=Nitric Oxide, H<sub>2</sub>O<sub>2</sub>=Hydrogen peroxide, MPO=

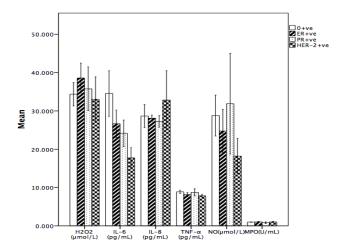
Figure 1. Inflammation biomarkers in breast cancer

Myeloperoxidase, TNF- $\alpha$ =Tumor necrosis factor alpha, IL-6=Interleukin 6, IL-8=Interleukin 8



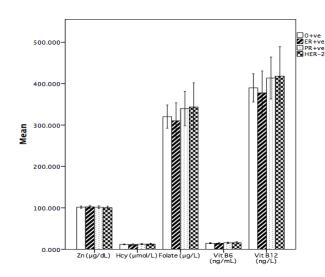
**Figure 2.** Micronutrients in breast cancer patients having double positives (2+ve), single positive (1+ve) or no (0+ve) hormonal receptor.

Hcy=Homocysteine, Zn=Zinc, Vit B6=Vitamin B6, Vit B12=Vitamin B12



**Figure 3.** Inflammation factors in breast cancer patients having HER-2+ve, ER+ve, PR+ve with no (0+ve) hormonal receptor.

ER=Estrogen receptor, PR=Progesterone receptor, HER-2=Human Epidermal Growth Factor Receptor 2, Hcy=Homocysteine, NO=Nitric Oxide,  $H_2O_2$ =Hydrogen peroxide, MPO=Myeloperoxidase, TNF- $\alpha$ =Tumor necrosis factor alpha, IL-6=Interleukin 6, IL-8=Interleukin 8



# DISCUSSION

Globally, breast cancer constitutes a large public health burden among females and is often associated with inflammation. Studies elsewhere proposed the use of non-steroidal anti-inflammatory drugs such as aspirin in cancer prevention and as adjuvant therapies<sup>4,5</sup> or use of micronutrients in reducing cancer progression.<sup>6</sup> Other studies found inverse correlation between high intake or blood level of folate, vitamin B6, and vitamin B12 and risk of breast cancer<sup>18–21,40</sup> while the studies associating circulating homocysteine with breast cancer risk are limited and with inconsistent result.<sup>9</sup> However, the bases of earlier propositions linking serum inflammation factors or micronutrient status with breast cancer hormonal types have not been completely established. This study provides additional insight into the complex role of systemic inflammation and micronutrients involved in DNA methylation pathway in breast cancer etiology and progression.

The present study observed that serum levels of  $H_2O_2$ , IL-6 and IL-8, which drive inflammation were elevated in women with breast cancer relative to control subjects. Therefore, control of inflammatory process might have a complementary role in the management of breast cancer patients. Esquivel-Velázquez et al. earlier reported that over-production of certain pro-inflammatory cytokines in breast cancer patients correlated with poor prognosis.<sup>41</sup> Serum IL-6 and IL-8 have been implicated in the initiation and progression of ductal carcinoma.42-47 Our finding of increased IL-8 in breast cancer patients support previous reports that IL-8 is a chemokine that has tumor-promoting role and cancer predictive potential.44,45 Angiogenesis is an important step in metastasis in which IL-8 plays a critical role.48 Increased serum IL-8 in our breast cancer patients supports its functions in angiogenicity, neovascularization and increased propensity for metastasis as earlier studies suggested.<sup>44,45,48</sup> Derin et

**Figure 4.** Micronutrients in breast cancer patients having HER-2+ve, ER+ve, PR+ve with no (0+ve) hormonal receptor.

ER=Estrogen receptor, PR=Progesterone receptor, HER-2=Human Epidermal Growth Factor Receptor 2, Hcy=Homocysteine, Zn=Zinc, Vit B6=Vitamin B6, Vit B12=Vitamin B12

*al.* reported high expression of IL-8 receptors on all breast cancer cells; thus, increased IL-8 in the breast cancer patients might be in response to over-expressed IL-8 receptors on breast cancer cells which accelerate angiogenesis.<sup>49</sup> Thus, our data support the use of IL-8 antagonist as a plausible complementary option in the management of breast cancer patients.<sup>48,49</sup>

Numerous cytokines, such as IL-6, IL-8 and TNF- $\alpha$  have been implicated in the initiation and progression of ductal carcinoma.<sup>42–49</sup> IL-6 is a pro-inflammatory cytokine that has multiple functions such as regulation of immune functions and hematopoiesis, inhibition of apoptosis of cancer cells and stimulation of tumor angiogenesis.<sup>24</sup> Serum IL-6 levels were increased in breast cancer patients which correlated with tumor stage and patient survival.<sup>12–16</sup> Therefore, IL-6 has a tumor-promoting role with predictive cancer potential as earlier research pointed out.<sup>44</sup>

Granulocytes produce  $H_2O_2$  via metabolic processes as an immunological response to foreign invaders. Hydrogen peroxide is catabolized to produce hypochlorous acid and hydroxyl radical. These highly reactive oxygen species have been reported to be effective in the killing of intracellular bacteria.<sup>6</sup> Hydrogen peroxide at high concentrations membrane damage, increases causes lactate dehydrogenase leakage, membrane permeability or cell necrosis.<sup>50</sup> A change in membrane permeability disturbs structural integrity, which could lead to the increased entry of toxins into cells and cause cell death at a later stage. Also, hydrogen peroxide has been reported to inactivate superoxide dismutase, which is an antioxidant.<sup>51</sup> Therefore, increased blood levels of H<sub>2</sub>O<sub>2</sub> might be responsible for gradual local tissue destruction or attempt to destroy invading micro-organisms in breast cancer patients.

Apart from its role in intracellular killing of pathogens and vascular smooth muscle relaxation,



Nitric oxide (NO) modulates gene expression via the agency of transcription factors, especially Nuclear Factor- $\kappa$ B (NF- $\kappa$ B), which regulates transcription of pro-inflammatory cytokines (IL-1β, TNF-α, IL-6 and IL-8) and enzyme (COX-2).<sup>52</sup> In this present study, serum concentration of NO in breast cancer patients was significantly decreased when compared with controls. This might be one of the mechanisms to reduce NO-induced inflammatory processes in breast cancer patients. Also, NO directly oxidizes DNA, resulting in mutagenic changes and damage to DNA repair proteins.<sup>53</sup> NO is produced by conversion of arginine to citrulline using inducible nitric oxide synthase in phagocytes. Therefore, we speculated that low arginine level might account for decreased NO levels in breast cancer patients, but this requires further investigation.

Breast cancer is distinguished by different molecular subtypes, risk factors, clinical behaviors, and responses to treatment and steroid hormone receptors such as progesterone receptor (PR), estrogen receptor (ER) and human epidermal growth factor receptor 2 (HER-2) on tumors have been shown to play important roles in cancer progression and prognosis.<sup>54,55</sup> Whether there are relationships between the wide spectrum of serum inflammation factors, micronutrients and immunohistochemistry status of breast cancer patients has not been fully addressed.

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Furthermore, expressions of ER, PR and HER-2 in breast carcinoma patients were found to correlate with prognosis.<sup>56–59</sup> This necessitates linking proinflammatory biomarkers with ER, PR or HER-2 expressions in Nigerian breast cancer patients.

#### CONCLUSION

In the present study, there were no differences in the levels of serum inflammation biomarkers and micronutrient levels in Nigerian breast cancer patients relative to ER, PR or HER-2 expression. The authors of the present study speculated that cytokine production and micronutrient status were not affected by ER, PR or HER-2 expression. However, recruitment of larger participants will further elucidate this conjecture. A key limitation of this study was the small number of breast cancer patients with different hormonal immunohistochemistry statuses.

#### CONFLICT OF INTEREST None.

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# Pure Mucinous Breast Carcinoma with Micropapillary Pattern in a 32-Year-Old Female

Nahid Sadighi<sup>a</sup>, Razieh Shahnazari\*<sup>b</sup>, Fariba Asadi<sup>a</sup>

<sup>a</sup> Department of Radiology, Tehran University of Medical Sciences, Tehran, Iran

<sup>b</sup> Department of Radiology, Iran University of Medical Sciences, Tehran, Iran

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#### ABSTRACT

Received: 07 March 2021 Revised: 01 July 2021 Accepted: 06 July 2021

Keywords: Breast, Mucinous Carcinoma, pure type, mixed type **Background:** Mucinous carcinoma (MC) is a rare breast malignancy with a large extracellular mucin secretion. It has a good prognosis in comparison to other breast malignancies.

**Case presentation:** We report a 32 years old female with multiple hard palpable masses in the left breast with rapid growth in 6 months. She was mentally retarded with lower limb varicose veins associated with mucocutaneous lesions on the face. She underwent ultrasound examination of the breast, thyroid and lower extremity veins. Core needle biopsy and fine needle aspiration from left breast lesions and right thyroid nodules showed MC and follicular lesion with hurtle cell change, respectively. Suspected metastasis in the left rib and calvarium in the subsequent bone scan survey and brain magnetic resonance imaging was reported. She underwent radical mastectomy and right hemi-thyroidectomy.

**Conclusion:** Pure mucinous carcinoma with micropapillary pattern (MUMPC) is a new histology variant of Pure Mucinous Carcinoma (PMC) that shows favorable prognosis with less aggression and occurs in older patients. However, PMC in our patient occurred at a young age with greater aggression.

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# INTRODUCTION

Mucinous carcinoma, also called Colloid carcinoma<sup>1</sup>, is an uncommon type of breast carcinoma classified as pure and mixed subtypes. It occurs mostly in older patients (7% in older versus 1% in younger patients).<sup>1-3</sup>

The pure subtype is more likely to be well circumscribed, lobulated, and soft according to the percentage of mucin in tumor tissue composition. In contrast, the mixed type is larger and more palpable upon examination, is ill-defined, and is associated with

\* Address for correspondence: Razieh Shahnazari, MD Assistant Professor of Radiology Address: Department of Radiology, Iran University of Medical Sciences, Tehran, Iran Tel: +989133298736 Email: <u>Rsh.medicen1999@gmail.com</u> microlobulation, necrosis and microcalcification in the ultrasound. The pure type occurs at younger age with good prognosis in comparison to mixed type.<sup>1-4</sup> Lymph node involvement in the pure type is about 2 to 14%, compared to approximately 46 to 64% in the mixed type.<sup>2</sup> The five-year survival rate in the pure type is near 90% versus 60% in the mixed type<sup>1</sup>. MUMPC is a new histology variant of PMC with tumor cells forming a micropapillary architecture that accounts for about 12-35% of all PMC.<sup>5</sup> We studied a 32 year old female suffering from left breast MUMPC with unusual manifestation as calcification and cystic changes as well as suspected bone metastasis.

# **CASE PRESENTATION**

A 32-year-old female with mental retardation was referred to the breast radiology department in Imam Khomeini Hospital with complaints of multiple firm



lumps in her left breast over the preceding 6 months with progressive distribution from one quadrant to involvement of the whole breast. She was mentally retarded and tall (190 cm) with multiple pigmentations



on the face and bilateral lower limbs varicose and twisted veins with skin discoloration from several years ago (Figure 1). Her familial history was unremarkable.

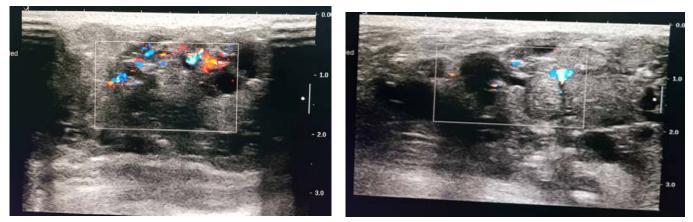




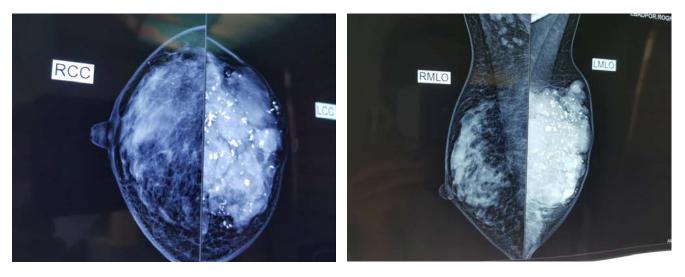
**Figure 1.** A 32-year-old female with hard palpable mass in the left breast (a) with bilateral varicose veins in lower limbs (b) and freckles on the face (c).

The ultrasonography (US) showed multiple varying size hypoechoic solid and cystic masses (4 cm to 10.5 cm) showing angular, micro-lobulated and spiculated margins and acoustic shadowing with duct extension, branch pattern with thick echogenic halo

associated with macrocalcification from areolar margin to the far zone of the whole left breast. Some cystic and mucocele-like masses had vascularity in color doppler ultrasound. Lymph node involvement was not detected in the US (Figure 2).



**Figure 2.** Supersonic ultrasonography images depict solid cystic, irregular and hypoechoic vascular mass (a) with duct extension and twinkling artifact due to calcification (b).



**Figure 3**. Craniocaudal(a) and mediolateral oblique(b) views of mammography show multiple hyperdense irregular shapes and margin lesions with coarse macrocalcification and a few coarse heterogenous microcalcifications.

Mammographic findings were multiple hyperdense irregular shape with micro-lobulated margin with coarse macrocalcification and a few coarse heterogenous microcalcifications with diffuse distribution (Figure 3). Subsequently, core needle biopsy showed MC with morphologic feature as well as IHC survey. IHC results were strongly positive for estrogen receptor (ER), weakly positive for progesterone receptor (PR), and negative for Herceptin receptor 2(HER-2); the ki67 was positive in 20 percent of cells.

Whole body bone scans showed suspected bone metastasis in the anterior arc of left 6<sup>th</sup> rib. In Brain MRI, suspected calvarium metastasis was detected. Due to the non-cooperation of the patient and the dissatisfaction of her family, further evaluation to confirm bone metastasis was not performed.

During the examination of the patient's breast, we noticed that the patient's thyroid was prominent, so we also did a thyroid ultrasonography. Two solid-cystic nodules with comet-tail artifact in the right lobe were seen that underwent FNA. pathologic evaluation showed follicular lesion with hurtle cell change. The patient underwent radical left mastectomy and left axillary lymph nodes dissection with right hemithyroidectomy. Surgical pathology reports showed MUMPC in the left breast. Macroscopic features of the tumor were tan-whitish color and gelatinous cut surface. It was multi-cystic with an area of solid component with the size of 10.5\*8\*4 centimeter at all quadrants of the left breast. Overall, the histologic grade was determined to be 1 with glandular differentiation score: 2, nuclear pleomorphism score: 2, mitotic score: 1.

Lymphovascular invasion was reported. Axillary nodal involvement and dermal lymphovascular invasion or necrosis were not detected. Pathologic stage was pT3pN0. Thyroid surgical pathology report was multinodular thyroid goiter and lymphocytic thyroiditis in the right thyroid lobe.

#### DISCUSSION

Breast cancer is probably the most common tumor in patients with intellectual disability (ID). The mean age of intellectually disabled women with breast cancer is lower than in the general population with breast cancer. Tumor size, lymph node involvement, and distant metastasis are more common in ID patients than in the general population. The detection of breast cancer in patients with ID is delayed due to the difficulty of clinical examination and communication problems and the lack of pain diagnosis by caregivers.<sup>6-</sup> <sup>8</sup> All of the above were the cause of our patient's advanced breast cancer. Due to the concurrence of breast cancer and thyroid abnormality, mucocutaneous lesions, vascular and mental retardation in the patient, we suspected Cowden syndrome. The clinical criteria for the diagnosis of Cowden syndrome are one of the following: three major criteria or two major criteria and three minor criteria.<sup>9</sup> Our patient had one definitive major criterion (breast cancer). Mucocutaneous lesions are part of the major criteria but her family did not allow skin biopsy. Mental retardation and multinodular goiter and vascular anomalies of the patient constituted minor criteria. We needed one major criterion for definitive Cowden syndrome diagnosis. Due to the dissatisfaction of the patient's family, evaluation of gastrointestinal tract for colon cancer and hamartoma was not performed. Our patient was younger than the average age of pure type patients with suspected bone metastasis. Cystic changes and calcification and distant metastasis in the pure type are unusual,<sup>2-5, 10</sup> but we detected these rare manifestations of the pure type in our patient. Differential diagnosis of PMC is invasive micropapillary carcinoma (IMPC) with associated mucin production. If MUMPC presents pathological

characteristics suggestive of PMC, biologically it is called IMPC. PMC and MUMPC have favorable prognosis, unlike IMPC. MUMPC has an IMPC-like form in the clinic with lymphovascular and lymph node invasion in pathology.<sup>5</sup> The association between Cowden syndrome and bone lesions has not been described in the literature. Therefore, we related the patient's bone lesions to breast cancer. Bone is the most common site for breast cancer metastasis but the frequency of the bone metastasis in MC was not well known and a few case reports have been published.<sup>11</sup>, <sup>12</sup> Finally, our patient who suffered from MUMPC, possible Cowden syndrome, and suspected bone metastasis showed unusual manifestations of PMC.

#### CONCLUSION

Pure MUMPC is a new subtype of PMC with good manifestation and prognosis. It occurs mostly in older

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patients. although, PMC in our patient occurred at a young age with unusual behavior.

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# **CONFLICT OF INTEREST**

The three authors have contributed sufficiently to the project to be included as authors. To the best of our knowledge, no conflict of interest, financial or other, exists.

# ETHICAL CONSIDERATION

A written consent was signed by the patient.

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# Dermatofibrosarcoma Protuberans and Adjuvant Radiotherapy: A Case Report with Uncertain Surgical Margins in The Breast

Alaettin Arslan\*a, Saliha Karagoz Eren<sup>b</sup>, Serdal Sadet Ozcan<sup>c</sup>, Ebru Akay<sup>c</sup>, Mustafa Ozdemir<sup>d</sup>

<sup>a</sup> Department of Radiation Oncology, Kayseri City Hospital, Kayseri, Turkey

<sup>b</sup> Department General Surgery, Kayseri City Hospital, Kayseri, Turkey

ABSTRACT

<sup>c</sup> Department of Pathology, Kayseri City Hospital, Kayseri, Turkey

<sup>d</sup> Department of Radiology, Kayseri City Hospital, Kayseri, Turkey

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Keywords: Dermatofibrosarcoma protuberans, uncertain surgical margins, adjuvant radiotherapy **Background:** Dermatofibrosarcoma Protuberans (DFSP) is a rare, locally aggressive superficial soft tissue tumor that can occur in many parts of the body. Surgical resection with a wide margin of safety is the main treatment modality of this rare tumor of the breast. According to the postoperative pathology report, the patient can be followed up or adjuvant radiotherapy (RT) can be added.

**Case presentation:** A 22-year-old woman presented with a mass filling the lower inner quadrant of her right breast. Tru-cut biopsy revealed a mesenchymal tumor, but excision was recommended for definitive diagnosis. A right breast quadrantectomy was performed. The result came as DFSP. Tumor diameter was 10x9x6.5 cm and the tumor was positive in most of the surgical margins. The patient underwent re-resection and a residual tumor with a diameter of 0.2 cm was detected at a distance of 3.3 cm from the surgical margin. Although the surgical margins were negative, the distance of the posterior surgical margin, in particular, could not be assured. Because of the uncertainty of surgical margins, 60 Gy RT was planned.

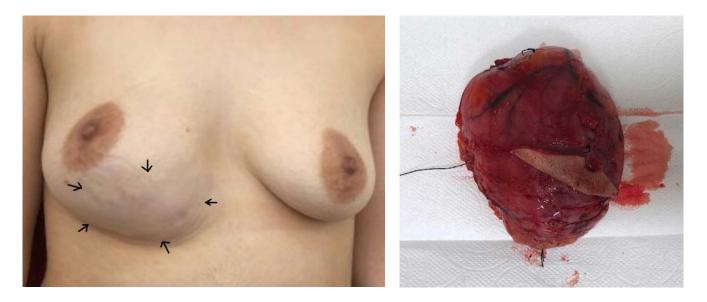
**Conclusion:** The localization of DFSP in the breast is extremely rare and surgery is the primary treatment. RT should be added as an adjuvant when safe surgical margins cannot be obtained.

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#### INTRODUCTION

Dermatofibrosarcoma Protuberans (DFSP) is a rare soft tissue tumor. It may occur in any region, but is mostly seen in the lower neck, upper chest and shoulder girdle regions.<sup>1</sup>The incidence of breast involvement is very low.<sup>2</sup> Rare cases of the male breast have also been reported.<sup>3</sup> Most studies have shown that

\* Address for correspondence: Alaettin Arslan, MD Address:Department of Radiation Oncology, Kayseri City Hospital, Kayseri, Turkey Tel: +903523157700 Email: alaettin.arslan@gmail.com both sexes are affected equally. It is a low-to-moderate grade superficial tumor originating mainly from the dermis. As in other soft tissue tumors, surgical resection is the primary and only potentially curative treatment in DFSP. The local recurrence rate varies between 1.6% and 50% depending on the type of surgery used.<sup>4</sup> Extremely low local recurrence rates were achieved with Mohs surgery with cure results of up to 98.5%.<sup>5</sup> Mohs surgery is internationally recognized as the preferred treatment for DFSP. In the National Comprehensive Cancer Network (NCCN) guidelines, Mohs surgery is listed first and wide local excision with a margin of 2-4 cm is recommended.<sup>6, 7</sup> Local recurrence usually occurs within three years,



**Figure 1**. a) Preoperative appearance. On physical examination, the mass image in the lower inner quadrant of the right breast, indicated by arrows. b) Postoperative tumor appearance. Tumor diameter was 10x9x6.5 cm.

depending on the status of surgical margins and the degree of tumor.<sup>8</sup> Although it is a locally aggressive tumor, distant metastasis is rarely seen and usually develops as a result of local recurrence. Adjuvant radiotherapy (RT) is still controversial. In the case of a positive surgical margin or macroscopic residue, RT between 50 and 66 Gy is recommended.<sup>6</sup> The approach differs in patients whose surgical margins are negative. The main reason for this is that DFSP requires wide local excision. However, the addition of adjuvant RT to large local excision has been reported to increase survival.<sup>9, 10</sup>

In this study, we present the methods of distinguishing a case diagnosed with DFSP from other similar pathologies and the principles of surgery and RT in its treatment.

#### **CASE PRESENTATION**

A 22-year-old woman presented with a mass filling the lower inner quadrant of her right breast (Figure 1a). In breast ultrasonography (USG), an encapsulated, well-circumscribed, oval-shaped mass measuring 7.5x3 cm was detected. On Doppler USG, a hypoechoic appearance was observed with significant blooding. MRI examination did not reveal any malignancy. Tru-cut biopsy revealed a mesenchymal tumor (juvenile fibroadenoma or phyllodes tumor), but excision was recommended for definitive diagnosis. A right breast quadrantectomy was performed. The result came as DFSP. Tumor diameter was 10x9x6.5 cm (Figure 1b). In the hematoxylin-eosin (H&E) sections, spindle cell proliferation without capsule, starting from the middle of the dermis, was observed. Skin appendages were observed within the spindle neoplasm (Figure 2). In immunohistochemical studies

(IHC), tumor cells showed diffuse positive staining with vimentin and CD34, supporting DFSP (Figure 3). Ki 67 proliferation index was 15%. In order to differentiate this tumor from other mesenchymal neoplasms, CD31, ALK, H-Caldesmon, SMA, CD117 and STAT 6 negative were found (Figure 4). ER, PR, desmin, S100, bcl2, pankeratin negativity helped to differentiate the tumor from the metaplastic carcinoma and melanoma (Figure 5). Honey-comb like infiltration of tumor cells into adipose tissue, preservation of skin appendages, and in immunohistochemical studies, CD34 positivity, ER, PR negativity, absence of pankeratin and epithelial component in any area are helpful in distinguishing the tumor from phyllodes tumor and supporting DFSP. Tumor was positive in most of the surgical margins. In this case, the patient consulted with RT and re-resection was recommended. The patient underwent re-resection and a residual tumor with a diameter of 0.2 cm was detected. The safe surgical margins were not assured regarding pathology report after re-resection. The presence of diffuse fibrocystic changes and fibroadenoma in the surrounding breast tissue as well as the tumor in the reresection material caused difficulty in determining the tumor margins. The pathology was reevaluated and the surgical margin was found to be 3.3 cm. The surgeon was also interviewed and the distance of surgical margins was accepted as 'indeterminate'. In surgical exploration, it was observed that the posterior surgical margin rested on the chest wall and the surgical margin could not be 3.3 cm at this distance. The posterior surgical margin was found not to be more than 1-1.5 cm. PET-CT was performed and several LNs were detected in the right axillary fossa. There was no metastasis in tru-cut axillary LN biopsy. Adjuvant

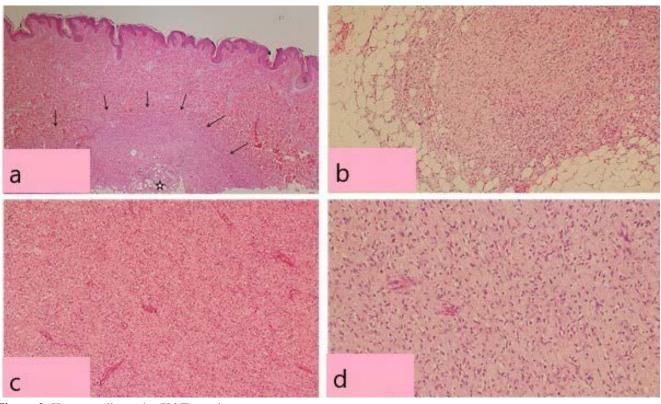


Figure 2. Hematoxylin-eosin (H&E) sections.

- a) HEx40. Multi-layered flat epithelial cover at the top, capsule-free spindle cell proliferation (arrow) starting from the middle of the dermis and proceeding deeper. One of the features of the lesion is the presence of preserved adnexal structures (asterisk).
- b) HEx100. Picture of tumoral cells infiltrating between the fat cells in the honeycomb style, whose borders cannot be clearly separated.
- c) HEx100. Tumor tissue, which is composed of spindle cells and does not contain epithelial components, is observed.
- d) HEx200. Close-up view of spindle cells of the tumor.

radiotherapy was considered to reduce the risk of local recurrence due to the uncertainty of surgical margins and 60 Gy was planned.

CT was taken for RT planning. The patient was initially operated on with a preliminary diagnosis of phyllodes tumor. Therefore, no clips were placed in the operation. Gross tumor volume (GTV) was contoured with reference to the incision scar. The recommended 60 Gy was given in two phases. Clinic target volume (CTV) 50 was formed by giving a 3 cm margin to the GTV and CTV 60 was formed by giving a 2 cm margin to the GTV. Natural barriers and critical organs were removed from CTV. Planning target volume (PTV) 50 and 60 were determined by a 0.5 cm margin for respiratory movements and set-up errors (Figure 6). The remaining right breast, left breast, both lungs, spinal cord, and liver were contoured after the operation to identify critical organ doses. Intensitymodulated RT (IMRT) technique was used with a 4fields plan. A 0.5 cm bolus was used to increase the skin dose. The dose homogeneity was targeted to be between 95% and 107% in PTV (Figure 7). Because of the uncertainty of surgical margins, 60 Gy RT was planned. On the RT planning, it was determined in the dose-volume histogram (DVH) where the organs at risk (OARs) dose limits were not exceeded (Figure 8). The doses received by OARs were found as follows: Right breast Dmean 20 Gy, left breast Dmean 1.5 Gy, heart Dmean 0.53 Gy, right lung (Dmean 1.7 Gy, V10 3.17%, and V20 1.7%), liver Dmean 7.48 Gy, and spinal cord Dmax 1.28 Gy.

Our patient has come to the controls regularly after the treatment. No pathology was found in the breast MRI taken at the last visit, and follow-up continues in the 24th month without the disease.

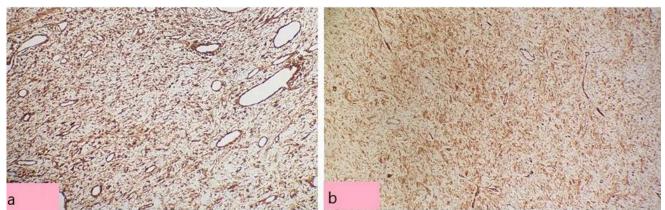
#### DISCUSSION

Treatment of DFSP requires a multidisciplinary approach. Radiological examinations are of great importance in the diagnosis stage. Ultrasonography and mammography are important tools for differentiating DFSP from primary breast lesion.<sup>11</sup> MRI may help define the depth of infiltration of the tumor.<sup>12</sup> The necessary surgical operation should be



performed with wide safety limits as described in the literatüre.<sup>3, 6, 9, 10, 13</sup> In case of insufficient surgical margins or residues, re-resection should be performed if the anatomical region makes it possible.<sup>3, 9</sup> There

are studies in the literature where 60 Gy RT was applied despite negative margin.<sup>14, 15</sup> After wound healing, RT should be applied if it is considered.<sup>10</sup>



**Figure 3.** Staining with Vimentin and CD34. Diffuse positive staining was achieved in tumor cells with a) Vimentin (x100), b) CD34 (x40).

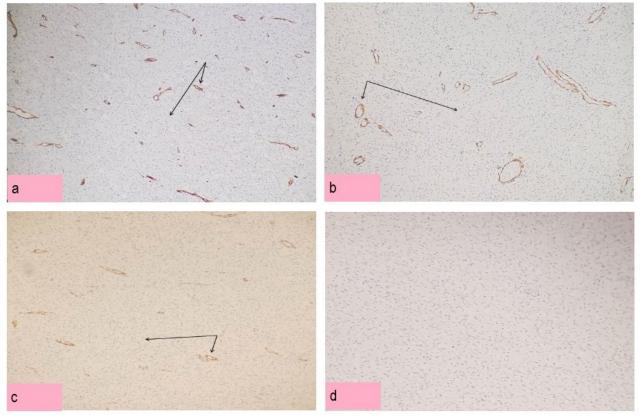


Figure 4. Staining used to distinguish it from other mesenchymal neoplasms.

- a) CD31x40. While positive staining was observed in the vessel walls (short arrow) with CD31, negative staining (long arrow) was obtained in tumor cells.
- b) SMAx40. While positive staining was observed in the vessel walls (short arrow) with SMA, negative staining (long arrow) was obtained in tumor cells.
- c) H-Caldesmonx40. While positive staining was observed in the vessel walls (short arrow) with H-caldesmon, negative staining (long arrow) was obtained in tumor cells.
- d) Cd117x40. Negative staining was obtained in tumor cells with CD117.

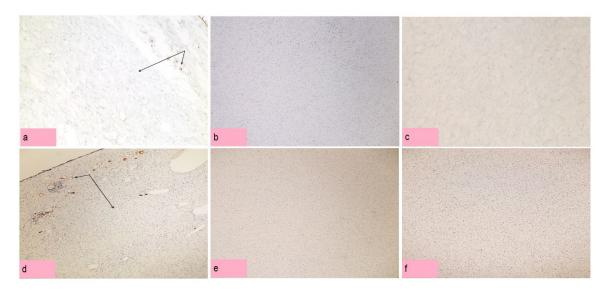
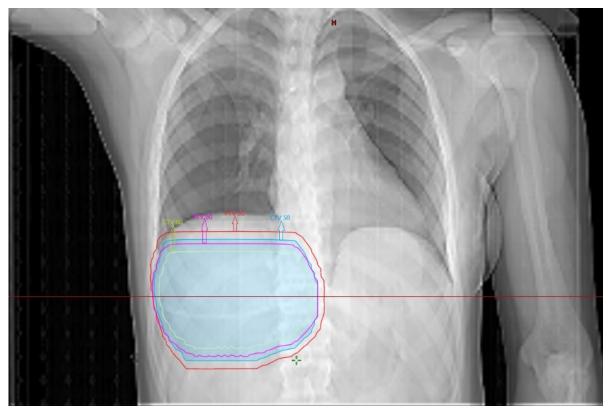
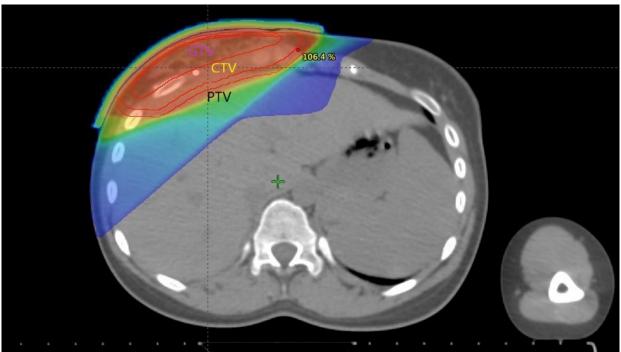


Figure 5. Staining used to distinguish it from the metaplastic carcinoma and melanoma.

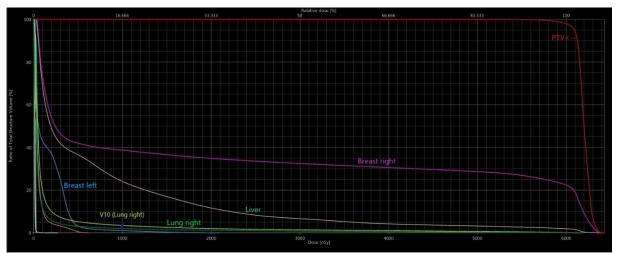
- a) ERx40. While positive staining was observed in several glandular epithelium trapped in the lesion periphery with estrogen (short arrow), negative staining (long arrow) was obtained in tumor cells.
- b) PRx40. Negative staining was obtained in tumor cells with progesterone.
- c) Desminx40. Negative staining was achieved in tumor cells with Desmin.
- d) S100x40. While positive staining was observed in several adiposides stuck with S100 (short arrow), negative staining (long arrow) was obtained in tumor cells.
- e) Bcl2x40. Negative staining was achieved in tumor cells with Bcl2.
- f) Pankeratinx40. Negative staining was obtained in tumor cells with pankeratin.



**Fig. 6.** Two-dimensional view of  $CTV_{50-60}$  and  $PTV_{50-60}$ . For the PTV 50-60, CTV 50-60 is given a 0.5 cm safety margin. CTV: Clinic target volume, PTV: Planning target volume.



**Figure 7.** PTV dose distribution. The dose homogeneity was targeted to be between 95% and 107% in PTV. GTV: Gross tumor volume (tumor bed), CTV: Clinic target volume, PTV: Planning target volume.



**Fig. 8.** Dose-volume histogram and V10 right lung volume. The graph shows the curves of PTV 60 and OARs. In addition, the volume of the right lung receiving 10 Gy dose was marked on the curve (3,17%). Of the unmarked curves, the turquoise-colored curve shows the left lung, the orange-colored curve shows the heart, the dark green-colored curve shows the total lung.

Research has been done on DFSP located in different parts of the body. In the study of Zhou *et al.*, 80 DFSP patients were evaluated retrospectively.<sup>7</sup> The rate of local recurrence was significantly lower in patients who underwent wide local excision (>3 cm) than the local excision (less than 3 cm) group. In addition, no recurrence was observed in 10 patients who underwent Mohs surgery. In a retrospective study of 14 patients by Chan *et al.*, all patients underwent extensive local excision with a 3 cm safety margin and then all patients received adjuvant RT (45-50 Gy) after wound healing.<sup>16</sup> After a mean follow-up of 30 months (18-68 months), no

recurrence was observed. This study demonstrates that the addition of RT to wide local excision has the effect of reducing local recurrence. Castle *et al.* in a study of 53 patients, patients underwent surgery followed by RT. Seven patients were treated with preoperative RT (50-50.4 Gy) and 46 patients with postoperative RT (60-66 Gy). Of the 46 patients receiving postoperative radiation, 3 had gross disease, 14 positive margins, 26 negative margins, and 3 uncertain margin status. Five-year and 10-year overall survival (OS) was 98%, while disease-free survival (DFS) was 98% and 93%, respectively. They concluded that DFSP is a disease with excellent local

control after conservative surgery and RT. They also emphasized that adjuvant RT should be considered when it causes significant morbidity for patients with large or recurrent tumors or for interventions at large surgical margins.<sup>14</sup>

Breast localized DFSP studies are much rarer. When PubMed is scanned, approximately 60 studies are published and almost all of them are case-based studies. Yihua Wang et al. studied 6 patients diagnosed with DFSP located in the breast. Five of the patients were women. Wide local excision was performed in 5 patients and mastectomy was performed in 1 patient. Tumor diameters in all patients ranged from 1-3 cm. The patients were operated on with a margin of 2-4 cm and postoperative RT was applied to 2 patients.<sup>17</sup> In the case presented by Salim Al-Rahbi et al., firstly, an excisional biopsy was performed and wide local excision was performed after positive surgical margins. Although negative surgical margins were obtained, postoperative RT was applied to the case.<sup>18</sup> In a case report by Amr Muhammed et al., DFSP located in the lower quadrant of the right breast was operated on with wide local excision, but the margin of safety remained less than 1.5 cm. Therefore, postoperative 60 Gy RT was applied to the patient. CTV was created by giving a 3 cm margin to the tumor bed, and PTV was created by giving a 1 cm margin to the CTV.<sup>19</sup>

Although the cases of DFSP in the breast are limited, the first priority issue is the removal of the tumor with wide local excision, as understood from the published cases. Although different information has been presented, surgical margins of 2-4 cm should be obtained. Adjuvant RT should be administered according to adverse risk factors that will reduce local control, such as tumor diameter Ki 67 value, and positive or near surgical margins after surgery. In fact,

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Chen *et al* reported that adjuvant RT should be applied regardless of surgical margins in a general DFSP meta-analysis study involving 167 patients.<sup>16</sup> Adjuvant RT was reported to provide 98% and 93% local control for 5 and 10 years, respectively.<sup>14</sup>

Based on the literature, we decided to use adjuvant RT: The size of the initial tumor, the surgical margin of approximately 3 cm despite the second operation, suspicion of the posterior surgical margin and because the patient was young. 60 Gy was preferred as the RT dose based on the studies. Our decision was reasonable as the anatomic location of the tumor was not suitable for a larger (>4 cm surgical margin) resection. Our patient fully complied with RT treatment. She continues her regular controls and no recurrence or metastasis has been observed.

#### CONCLUSION

In conclusion, DFSP is a rare but important disease in terms of both surgery and follow up, due to high local recurrence. Therefore, operation with wide safety margins should be the first choice and safe surgical margins should be obtained. Adjuvant RT should be decided by considering pathology, age of the patient and re-resection problems according to the location of the tumor.

#### ETHICAL CONSIDERATIONS

Written informed consent was obtained from the patient who participated in this study.

#### FINANCIAL DISCLOSURE

The authors declare that this study has received no financial support.

#### **CONFLICTS OF INTEREST**

The authors have no conflicts of interest to declare.

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# Spindle Cell Carcinoma of the Breast: A Case Report Representing Diagnostics, Therapeutics, Intervention, and Outcome

Mohammad Naser Athamnah<sup>a</sup>, Shatha M. Al-Barrak<sup>b</sup>, Nimah A. Rabai<sup>a</sup>, Tea Natelauri<sup>c</sup>, Hussein S O Al Azzam<sup>a</sup>, Mohammed Alorjani<sup>d</sup>, Omar M. Al-Rusan<sup>d</sup>, Amer Abu-Shanab<sup>\*e</sup>

<sup>a</sup> Department of General Surgery, Princess Basma Teaching Hospital, Irbid, Jordan

<sup>b</sup> Department of Acute Medicine, Ninewells Hospital, NHS Tayside, Scotland

<sup>c</sup> Clinical Oncology Department, EVEX Hospitals, Georgia.

<sup>d</sup> Department of Pathology and Microbiology, Faculty of Medicine, Jordan University of Science and Technology, Irbid, Jordan

<sup>e</sup> Office of Scientific Affairs and Research, King Hussein Cancer Center, Amman, Jordan

ARTICLE INFO	ABSTRACT
Received: 31 May 2021 Revised: 02 July 2021 Accepted: 07 July 2021	<b>Background:</b> Spindle cell carcinoma (SpCC) is an unusual form of squamous cell carcinoma (SCC) and can sometimes present in the breast. Owing to the rarity of breast SpCC, few case studies are available nowadays and proper evidence is scarce. <b>Case presentation:</b> We herein report a 60-year-old female patient, who was referred to the surgery services after presenting with a right breast ulcerated mass. On physical examination, a 7x7 cm mass was found along with a 3 cm ulcer on the top of it. Both mammography and ultrasound showed a dense mass, and tru-cut and skin punch biopsies confirmed neoplastic spindle cells within the lesion. The patient underwent a right total mastectomy with sentinel lymph node biopsies with no further chemotherapy or radiotherapy.
Keywords: Spindle-cell carcinoma,	<b>Conclusion:</b> Owing to the heterogeneity of SpCC, there is no exact treatment protocol for this type of cancer, and mastectomy or conservative surgery can be
breast cancer, Soft tissue sarcoma	performed in certain groups of patients depending on tumor size, stage, and lymph node involvement. Fortunately, promising medical and biological therapies might be of use in the near future.

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#### INTRODUCTION

Spindle cell carcinoma (SpCC) is an unusual form of squamous cell carcinoma (SCC) where part or all of the tumor resembles a sarcoma.<sup>1</sup> SpCC can grow in different anatomical locations of the human body such as the parotid gland, and is most commonly developed

\* Address for correspondence:

Address: Office of Scientific Affairs and Research Institute/University/Hospital: King Hussein Cancer Center, Queen Rania St., Amman, 11941, Jordan Tel: +962789504850

E-mail: amer\_abushanab23@yahoo.com

from epithelial cells.<sup>2</sup> Despite the controversy around SpCC, it is regarded as epithelial cancer.<sup>3</sup> SpCC of the breast is extremely rare, accounting for only 0.1% of all breast cancers.<sup>3, 4</sup> Owing to the rarity of breast SpCC, a few case studies are available nowadays and proper evidence is scarce. Therefore, determining the suitable diagnostic and therapeutic methods for breast SpCC is challenging. In order to gain more insight into management methods and generate guidelines for breast SpCC, a detailed review of newly diagnosed cases is required. This would be highly beneficial in deciding on the appropriate treatment for patients with newly diagnosed breast SpCC. Here, we present a case

Amer Abu-Shanab, MD



report of a female patient with right-sided SpCC of the breast resembling inflammatory carcinoma.

# **CASE PRESENTATION**

A 60-year-old woman, married, nulliparous, nonsmoker with no history of using any contraceptives was studied. Past medical history includes hypertension, which is controlled with once-daily 5mg tablets of bisoprolol (Bicor). The past surgical history is positive for an open appendectomy 30 years ago. She had no previous exposure to radiation and no family history of breast cancer.

She presented to the clinic with a two-week history of right breast mass which then got ulcerated. Her condition started as she sustained an accidental trauma involving the right side of the chest being hit by an edge of a door. Initially, she had mild pain along with bruising and swelling of the area. Two days later, she noticed an increment in the size of the swelling. After a trial of scrubbing the swelling with a body exfoliating cloth, the mass opened to reveal an ulcer with a foulsmelling yellowish discharge (pus). Consequently, the mass started to shrink in size, unlike the size of the ulcer which started to increase. The patient denies any associated symptoms throughout, such as fever, rigor, or generalized weakness.

Physical examination revealed a right-sided breast ulcerating mass (Figure 1). The mass was hard, immobile, around 7x7 cm in size, located in the upper outer quadrant. The ulcer was round with a diameter of 3 cm as shown in Figure 1. The base of the ulcer was yellowish-white in color, and it was surrounded by an elevated firm rim of erythematous thick skin. Moreover, a yellowish foul-smelling pus discharge mixed with bloody was noticed. Thus, the patient was labeled to have an infected ulcerated breast mass. The rest of the examination, including other lesions on the same/contralateral breast, nipple changes/discharge and palpable axillary lymph nodes, was unremarkable.



Figure 1. Gross presentation of the breast ulcer



Given the aforementioned information in addition to the physical examination findings of the patient and her breast, it was essential to rule out inflammatory breast cancer.

# Diagnostics

Arrangements were made for the patient to get further investigations including imaging studies and tru-cut and skin punch biopsies. Her blood tests including full blood count (FBC) and biochemistry showed unremarkable results. On mammography, a large dense lesion measuring approximately 7x7 cm with focal calcifications was found in the upper outer quadrant of the right breast in addition to thickening of the skin which suggests malignancy. Mammography of the left breast and bilateral axillary lymph nodes was unremarkable. The BI-RADS score for these mammographic findings was 4<sup>5</sup>. Upon further evaluations with an ultrasound scan of the right axillary area, there were no enlarged lymph nodes. Both tru-cut and skin punch biopsies showed a cellular spindle cell neoplastic lesion. Whole body CT-scan showed a right breast mass (7x6.5 cm, welldefined with soft tissue density); right renal simple cyst (2.3x2.3 cm); and fat density of the spleen (4 mm). CT-scan result showed no evidence of distant metastatic disease.

# Therapeutic Intervention

As soon as the results of the biopsies were received from the histopathology department, the patient was admitted to the surgical ward and underwent a right total mastectomy with sentinel lymph node biopsy (SLNB). The operation was smooth and uneventful. Although methylene blue dye was used to recognize the sentinel lymph node, it could not be detected. Therefore, an axillary sampling was performed instead. Tissues removed during the surgery were sent to the histopathology lab which confirmed the diagnosis of spindle cell carcinoma.

# Outcome and Follow-up

Postoperatively, the patient was vitally stable and reported no complications. She was discharged on day one post-operatively. A draining tube was left in place from postoperative day zero and removed on postoperative day two. Thereafter, she had a smooth recovery at home. On the first follow-up visit to the clinic (post-operative day four), she was doing well, and the wound looked healthy and clean. On the second follow-up visit (post-operative day eight), the wound was absolutely fine, and the stitches were removed. Further management with chemoradiotherapy was offered and discussed with the patient. However, she refused due to the uncertainty of chemo-radiotherapy benefits for this type of tumor.

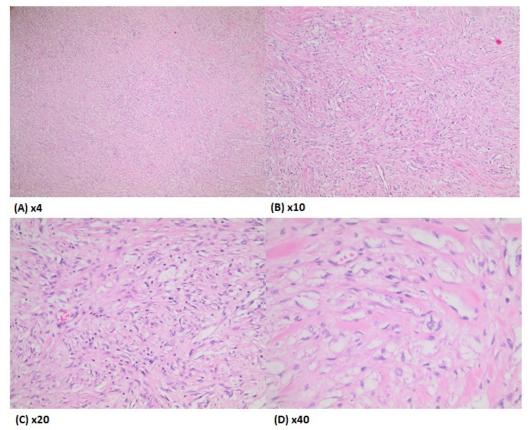
# DISCUSSION

In the past, the exact definition of SpCC was not clear due to the lack of information.<sup>4</sup> However, nowadays it is classified by the WHO (World health organization) as a metaplastic type of carcinoma. According to SpCC characteristics and depending on the morphological variety ranging from reactive to malignant lesions, it can be further classified into either benign or malignant tumors.<sup>2</sup> Additionally, the 5-year survival rate ranges between 28-68%.<sup>6</sup> The average age of diagnosis of SpCC is 54 years, although our patient was diagnosed at the age of 60 years.<sup>3</sup>

The case series of breast SpCC reported by Khan *et al.* showed that, macroscopically it could present as an ovoid or round-shaped high-density lesion.<sup>4</sup> In another study, most patients of SpCC had round-shaped lesions accompanied by decreased elasticity on physical examination.<sup>3</sup> Mammographically, breast SpCC is usually defined as BI-RADS 4 or BI-RADS 5 with no calcifications.<sup>4</sup> Our patient had a right-sided ovoid lesion, presented with signs of inflammation and discharge, and the lesion was categorized as BI-RADS 4 with local calcifications. These findings suggest that SpCC might have a wider spectrum of mammographic characteristics.

Histomorphologically, SpCC growths are divided into two groups: biphasic - a mixture of carcinomatous and sarcomatous components, or monophasic – largely composed of spindle cells.<sup>7</sup> By using immunohistochemistry, specific epithelial and mesenchymal markers can be detected, such as vimentin; cytokeratin (AE1/AE3); cytokeratin 14 (CK14); increased expression of prognostic markers p63 and Ki-67; and reduced expression of prognostic markers ck5/6 and CD10.8 In our case, tissue samples from tru-cut and skin punch biopsies were mainly composed of cellular spindle cells in a highly vascular hyalinized stroma and accompanied by an area of acellular hyaline myxoid degeneration (Figure 2). Hence, our patient was diagnosed with a right breast cellular spindle cell neoplastic lesion. Upon immunohistochemical evaluation, the tissue sample was reactive for basal markers CK5/6, p63, vimentin and SMA (Figure 3). It was negative for ER, PR, and HER2/neu (Figure 4), as well as Pan-CK, B-catenin, desmin, and CD34 (Figure 5).





**Figure 2.** Hematoxylin and eosin (H&E) stain. Microscopic examination revealed mild to moderately atypical spindle cells arranged in an interwoven pattern with elongated cytoplasm, mild nuclear pleomorphism was apparent.

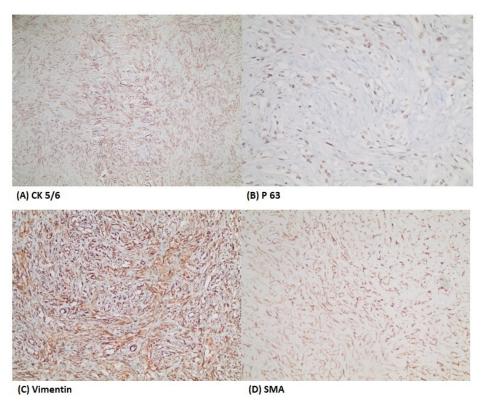


Figure 3. Immunohistochemistry for CK5/6, P63, Vimentin and SMA highlighting the spindle tumor cells.

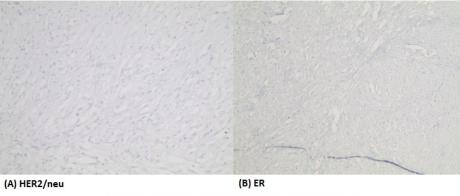
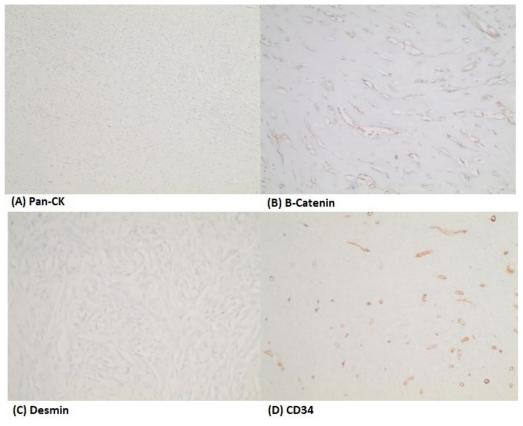




Figure 4. Immunohistochemistry for ER, PR and HER2/neu. The tumor cells are negative for those markers



**Figure 5.** Immunohistochemistry for Pan-CK, B-Catenin, Desmin and CD34. The tumor cells are negative for all these markers as seen.

It should be noted that SpCC is less likely to metastasize to regional lymph nodes; however, it has

a high rate of local recurrence.<sup>9</sup> In Maemura *et al.*'s study  $^3$ , only two out of 12 patients had confirmed



nodal involvement. Similarly, our patient clinically showed no involvement of axillary lymph nodes. Evidence suggests that tumor size and grade are regarded as more crucial prognostic factors than lymph node involvement.<sup>10</sup> Carter et al. suggested that tumor size is a strong prognostic factor.<sup>10</sup> This goes in line with an earlier study by Bauer et al. where the tumor size of more than 2 cm was associated with higher risks than with smaller tumors.<sup>11</sup> Results by Song *et al.* suggest that tumor size of > 5 cm, Ki-67 of > 14%, and nodal involvement are indicators of poor prognosis.<sup>12</sup> Not only the size of the tumor but also biomarkers, such as p53 and p63, can potentially indicate poor prognosis.<sup>13</sup> According to this evidence, our patient was regarded as a high-risk patient due to the big size of the lesion (7x7 cm) and the reactivity to some markers, mainly p63.

In terms of lymph node dissection, axillary lymph node dissection (ALND) is regarded as a conflicting issue. Podetta et al. in a study on fibromatosis-like spindle-cell metaplastic carcinoma (FLSpCC) found that the need for ALND depends on the type of cancer.<sup>14</sup> Given the fact that FLSpCC has a higher local recurrence rate than regional lymph node would be involvement, ALND unnecessary. Alternatively, wide local excision or mastectomy with negative margins can be performed in patients with FLSpCC.<sup>14</sup> Despite the low risk of lymph node involvement, some authors have stated that axillary ultrasound (AUS), fine needle aspiration (FNA), and sentinel lymph node biopsy are quite reasonable for accurate nodal status assessment and treatment.<sup>15</sup> Our patient had no palpable axillary lymph nodes; hence, AUS and SLNB were performed for exact staging. Intraoperatively, none of the axillary lymphatics was stained by methylene blue. Therefore, we decided to proceed with axillary sampling. Although six axillary lymph nodes were sampled, none of them were found to be carcinogenic in the final pathology report.

Due to the rarity of breast SpCC, the exact treatment plan is not yet established. Historically, mastectomy has been considered the mainstay treatment for patients with big-sized SpCC tumors.<sup>11</sup> Nevertheless, provided that SpCC does not tend to metastasize to lymph nodes, it is still unclear whether it is mandatory to perform axillary surgery. Mastectomy versus conservative surgery is also a matter of debate.<sup>16</sup> While gold-standard treatment for SpCC was total mastectomy<sup>11</sup>, the 10-year survival rate was higher following total mastectomy than conservative surgery, 82.5% vs 61.5%, respectively.<sup>16</sup> Therefore, nowadays, mastectomy is being used for patients with large SpCC tumors.<sup>17</sup> Moreover, conservative surgery can be performed in patients with early-stage SpCC, while radical mastectomy is highly recommended for late-stage disease<sup>16</sup>, which highlights the importance of staging in these cases. By taking the aforementioned information into consideration (i.e., the importance of tumor size and stage), the presented case underwent right-sided total mastectomy with sentinel lymph node dissection. She had a big-sized SpCC, and the pathological stage was reported to be pT4N0M0, G2.

According to Moten *et al.*'s study<sup>16</sup>, most patients who were diagnosed with SpCC had triple-negative histo-type (i.e., negative ER, PR, and HER2/neu receptor), while only 15% had ER+ receptor status. The same results were reported in the study conducted by Vranic et al. 18, where 21 out of 23 patients had triple-negative breast SpCC. Similarly, our patient had triple-negative ER-, PR-, and HER2/neu histotype. Therefore, the major drawback of this histo-type is the lack of hormone receptors (i.e., target cells) and, consequently, hormonal therapy would not be effective.<sup>19</sup> Neoadjuvant chemotherapy is still an option, mostly for big-sized SpCC tumors, which can help the surgeons to achieve negative resection margins.<sup>20</sup> However, Chen et al. applied a neoadjuvant chemotherapy setting and their study showed no benefits in terms of tumor shrinkage and downstaging.<sup>21</sup> Furthermore, chemotherapy for metastatic disease showed a very low response rate.<sup>22</sup> However, chemotherapy was offered to our patient, but the side effects of the chemotherapy and the low response rate were the reasons behind the patient's refusal to proceed with further treatment after surgical resection.

Nevertheless, in the pioneering study conducted by Zhou et al., apatinib -tyrosine kinase inhibitor (TKI)- was used after unsuccessful treatment of SpCC by using chemotherapy plus cyclophosphamide and epirubicin.<sup>23</sup> The study showed promising results in terms of nearly complete response to treatment accompanied by tolerable toxicity. These results raise hope as they suggest an effective treatment option for resistant cases. Vranic et al.'s study is considered influential as they elucidated prospective biomarkers for SpCC. Out of 23 patients, 21 had specific mutations, such as PIK3CA, TP53, HRAS, NF1, and PTEN.<sup>18</sup> This is an evident indicator that personalized medicine is the future of onco-patient treatment, especially for rare types of cancers. We did not perform genetic profiling for specific gene mutations; hence, our patient was planned to be treated as triplenegative breast cancer. This treatment option consists of chemotherapy in combination with taxane and anthracycline in the adjuvant setting.<sup>24</sup> In case of recurrence, dual therapy (i.e., chemotherapy plus TKIs, such as lapatinib or apatinib) can be incorporated.

The benefits of adding radiation therapy (RT) to surgery is still unclear as the results reported by



several studies are still controversial. Tseng *et al.*'s study <sup>25</sup> demonstrated improved survival rates in patients who received RT in combination with surgery, while Moten *et al.* showed worse survival rates with the same combination of treatment.<sup>16</sup> Consequently, in order to decide on which treatment is the best option, this can potentially be done on a case-by-case basis. This means that personalized medicine in combination with further investigations for each patient is the best approach in the current situation. For our patient, the multidisciplinary team is aiming to decide on a future treatment plan.

#### CONCLUSION

Owing to the heterogeneity of SpCC, there is no exact treatment protocol for this type of cancer. Nevertheless, mastectomy or conservative surgery can be performed in certain groups of patients depending on tumor size, stage, and lymph node involvement. Before surgery, AUS and FNA must be

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performed to find out whether ALND is required or not. Chemotherapy is a treatment option in adjuvant and neoadjuvant settings. However, the neoadjuvant setting did not show satisfactory effectiveness in terms of downstaging. A novel drug, such as apatinib (TKI), is a promising option for patients with previously ineffective chemotherapy treatment. Biomarkers are also essential in choosing the appropriate drugs for each patient. However, further research is required to declare which treatment option is most appropriate.

# ETHICAL CONSIDERATIONS

Written informed consent was obtained from the patient herself for publication of this case report and any accompanying images.

**CONFLICT OF INTEREST** None.

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