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

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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 ≥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≥75 years and BMI≥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 ≥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.

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.[1, 2] While the radioisotope (RI) and dye-combined method has been considered the standard technique, an SNB technique using superparamagnetic iron oxide nanoparticles (SPIO) and a handheld magnetometer has been reported.[3] 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.[4] Several factors, including obesity and age[5-8], 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

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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[9, 10], the optimal number of lymph nodes to be removed is controversial based on the false negative rate and prognosis.[8, 11-17] 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 ≥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.[4] 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 ≥0.5 μT 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 ≥75 years and BMI ≥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.

Table 1. Patient characteristics

Group		A	B	C	D	Total	P
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)							
	(mean)	56.4	53.4	59.8	57.2	56.6	0.6299*
	≤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 axillary lymph node	US/MRI/CT	91	15	11	45	162	0.4241
	Confirmed by FNA	4	1	0	0	5	
Surgery	Partial mastectomy	50	9	7	27	93	0.8055
	Mastectomy	45	7	4	18	74	
Histology	DCIS	14	4	2	11	31	0.7580
	IDC	63	10	6	25	104	
	Others	18	2	3	9	32	

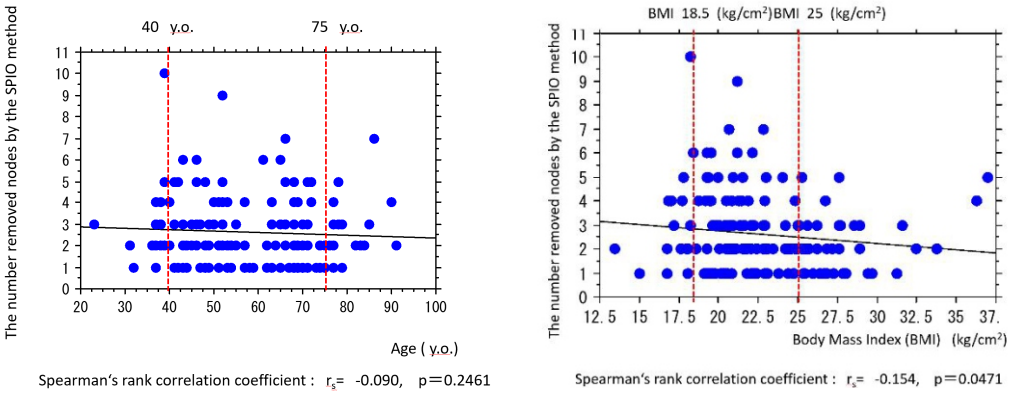

Figure 1 The scatter plots show the relationship between the number of SPIO nodes and age (Figure 1-1) or body mass index (BMI) (Figure 1-2). The two scatterplots demonstrate that the age ≥75 years and BMI ≥25 subgroups had a small number of SPIO nodes. This subgroup might have slow lymphatic drainage.

Table 2. The results of SNB

Group		A	B	C	D	Kruskal-Wallis test P
Cases		95	16	11	45	Total 167
Count on the skin surface	(mean)	1.5	16	11	45	P<0.0001
	(range)	1 ~ 7	2.2	2.7	3.4	
Nodes removed by SPIO method	(mean)	2.3	1.5 ~ 3.5	2.5 ~ 3	2 ~ 6	P=0.0329
	(range)	1 ~ 7	3.3	3.2	2.9	
Total count of nodes removed by SPIO method (μT)	(mean)	3.3	1 ~ 6	2 ~ 5	1 ~ 10	P<0.0001
	(range)	1.2 ~ 11	5.6	6.6	6.9	
Nodes removed by RI method	(mean)	1.8	1.8 ~ 12.2	3.5 ~ 11	1.5 ~ 22.4	P=0.2818
	(range)	0 ~ 7	2.4	1.6	2.0	
Metastasis of SLN	None (136)	78	1 ~ 5	0 ~ 3	0 ~ 9	P=0.7001*
	Micrometastasis (6)	3	15	9	34	
	Macrometastasis (25)	14	0	0	3	
			1	2	8	

*: χ2 test

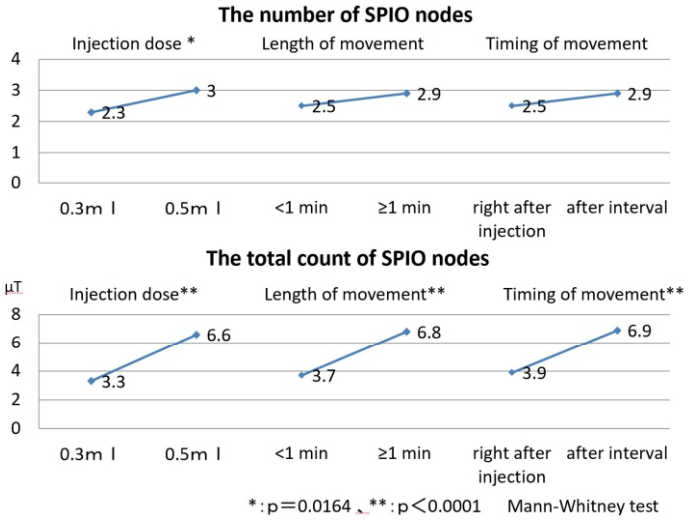

Figure 2. The relationship between the results of SNB and the magnet movement procedure in the SPIO method (injection dose, length of movement and timing of movement)

Table 3. Removed nodes and biopsy method

	Cases		Removed nodes		P, Wilcoxon's signed-rank test
			SPIO method	RI method	
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	2.1 # 0 ~ 9	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 ≥75 years or BMI ≥25 vs. age <75 years and BMI <25). The average number of nodes removed among the age ≥75 years or BMI ≥25 subgroups was significantly lower in comparison to the age <75 years and BMI <25 subgroup with both methods.

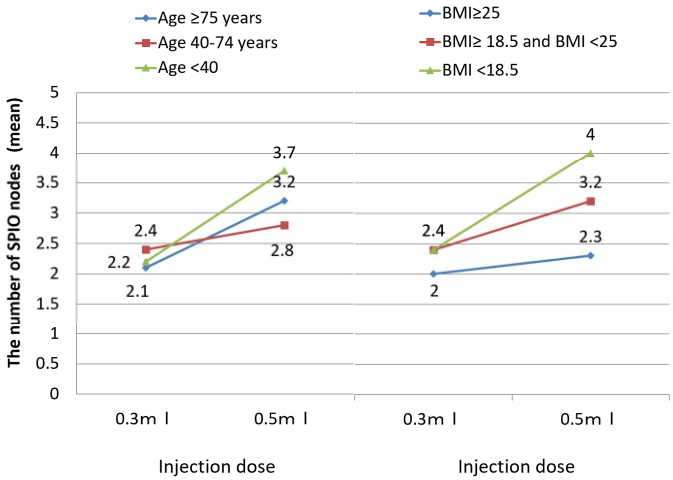

Figure 3. Injection dose and the number of SPIO nodes. The number of SPIO nodes was increased by dose escalation in each subgroup.

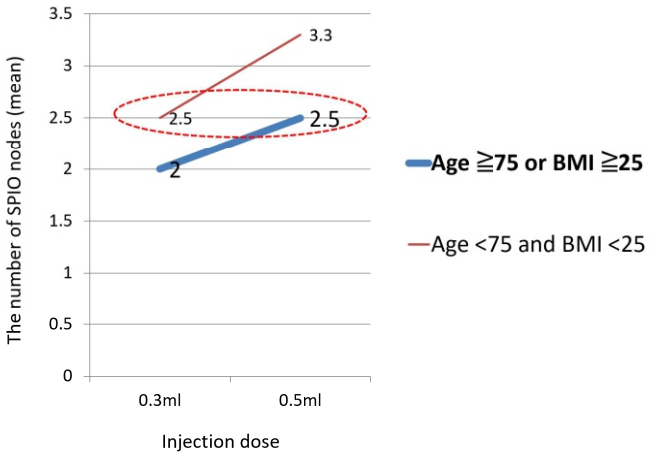

Figure 4. The relationship between the number of SPIO nodes and the injection dose. The average number of SPIO nodes in the age ≥75 years and BMI≥25 subgroups after injection of 0.5 ml was almost the same as that of the age <75 years and BMI<25 subgroups after injection of 0.3 ml.

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 ≥75 years and BMI ≥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[6, 18] and massage after injection have been previously applied as methods to improve tracer infiltration into the lymphatic vessels.[19] 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.[4] 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 ≥75 years and BMI ≥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 non-obese and non-elderly patients, in line with previous reports.[5-8]

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[14,16], 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[17]. 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.[15] 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.[4] 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 ≥75 years and BMI ≥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.[4] 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 ≥75 years or BMI ≥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 ≥75 years and BMI ≥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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