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Neoadjuvant Chemotherapy Effect on Predictive Value of Sentinel Lymph Node Biopsy using Single Method Methylene Blue in Breast Cancer Patients at Low-resource Country

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Abstract

AIM: This study aimed to examine the application of neoadjuvant chemotherapy (NAC) effect on sentinel lymph node biopsy (SLNB) using single method methylene blue 1% in breast cancer patients at low-resource country.

METHODS: This analytical observational study employed a retrospective case–control approach. The total sampling method was used by involving the entire population of Stage-I and -II breast cancer patients after performed core biopsy or open biopsy with clinically negative axillary lymph nodes that had performed SLNB and axillary lymph nodes dissection at several hospitals in Surakarta from January to May 2020. The descriptive data were presented in the frequency table. Sensitivity, specificity, positive predictive value, negative predictive value (NPV), and diagnostic values were reported with 95% confidence of interval (CI).

RESULTS: From a total of 161 patients, 100 patients were given NAC. The identification rate of the non-NAC was 91.3% and the NAC group 80.6%. Non-NAC group obtained a false-negative rate of 24.4% with NPV of 94.4% (95% CI 85–100), while the false-negative rate of the NAC group was 10.8% with NPV of 74% (95% CI 65–80).

CONCLUSIONS: NAC with single method methylene blue 1% injection in SLNB can reduce the identification and false-negative rates in breast cancer patients.

Introduction

Sentinel lymph nodes (SLN) are nodes that receive the first spread of breast cancer metastases [1]. Surgical techniques can be performed with axillary lymph node dissection (ALND) or SLN biopsy (SLNB) [1]. ALND remains a standard procedure but is less useful for 70–80% of early-stage breast cancer patients with negative axillary nodules so that a better treatment is needed, that is, SLNB [2]. In breast cancer patients with clinically negative nodes, SLNB has better diagnostic accuracy than ALND by reducing morbidity and becoming a standard procedure for axillary with an accuracy of >90% [3]. Based on these results, SLNB becomes negatives because mostly, ALND procedures are not performed. However, a study conducted by Lee *et al.* [4] showed a false-negative rate, ranging from 4.6% to 16.7% after SLNB. Several studies explain that the high false-negative rate of SLNB allows experts to perform ALND further [5], [6].

Neoadjuvant chemotherapy (NAC) is not only used in patients with advanced breast cancer and breast cancer with positive axillary nodules but it can also be used in early-stage breast cancer patients with negative axillary nodules [7], [8]. According to Mamounas *et al.* [9], the accuracy of SLNB in post-NAC patients can be reduced with an average identification rate of 83% and an average false-negative rate of 11%. NAC causes damage to lymphatic drainage due to shrinkage and fibrosis by inducing fat degeneration as a result of the tumor cell apoptotic process. This decreases SLNB accuracy in post-NAC patients [10].

In developed countries, the optimal technology for SLNB uses patent blue dye, preoperative lymphoscintigraphy, and radioisotope tracer, which are used as a single or combination technique [11]. Limited access to the optimal technology for SLNB is the main problem in Indonesia. The cost to provide nuclear medicines in every hospital has contributed to the difficulty for administering SLNB optimally. As an alternative to these devices, we sought to evaluate

NAC effect on the identification rate and false-negative rate of SLNB using single method methylene blue 1% in breast cancer patients at low-resource country.

Materials and Methods

This analytical observational study employed a retrospective case–control approach. The total sampling method was used by involving the entire population of Stage-I and -II breast cancer patients after performed core biopsy or open biopsy with clinically negative axillary lymph nodes that met the study requirements at several hospitals in Surakarta from January to May 2020. This study has been approved by the Ethics Committee of the Sebelas Maret University Indonesia number 620/III/HREC/2020.

The data were obtained from the secondary data of patients with clinically node negative without perform an axillary biopsy if the axillary ultrasonography found a suspicious lymph node, we performed SLNB using single methylene blue 1% and ALND. The samples were then assigned into two groups, namely, the NAC group, who underwent NAC, and the control group or non-NAC group, who did not undergo NAC.

The samples from both groups were given a periareolar or peritumoral injection of single methylene blue 1% 20 min before operation and then massaged for 5 min. And then observed to find out the results intraoperatively, positive or negative, by observing whether the color changed to blue or not. The blue nodes and the palpable suspicious nodes were taken. Then, the samples underwent a histopathological examination.

The data were then analyzed statistically to determine the identification rate and false-negative rate. The identification rate was known by counting the number of patients with positive SLNB results histopathologically. The false-negative rate was known by counting the number of patients with negative SLNB results but positive tumor histopathologically after ALND. The descriptive data were presented in the frequency table. Sensitivity, specificity, positive predictive value, negative predictive value (NPV), and diagnostic values were reported with 95% confidence of interval (CI). Data analysis was performed using SPSS version 25.0.

Results

Patient characteristics

Retrospectively, 161 patients were obtained from January to May 2020. Of the 148 patients included in the final analysis, they were assigned to the NAC group and non-NAC group (Figure 1). The average age was 47 years (27–81 years) in the NAC group and 50 years (26–82 years) in the non-NAC group. Forty-seven patients in the non-NAC group (77%) underwent a core biopsy, while 92 patients (92%) in the NAC group underwent open biopsy. Periareolar injection of methylene blue 1% was administered to most patients, 57 patients (93%) in the non-NAC group, and 90 patients (90%) in the NAC group. The median size of the tumor was 3 (1–6 cm). Invasive ductal carcinoma was the most common outcome found in 49 non-NAC patients (80%) and 70 NAC patients (70%). Mastectomy was the most common surgical procedure performed to 32 non-NAC patients (52%), while the breast conservative surgery (BCS) procedure was performed to 71 NAC patients (71%) (Table 1).

Table 1: Patient characteristics (n=161)

Patient characteristics	Neoadjuvant chemotherapy Group (n: 100)	Percentage	Non-neoadjuvant chemotherapy Group (n: 61)	Percentage	p-value
Age (years)					
<40	32	32	20	33	0485
>40	68	68	41	67	
Biopsy					
Core	8	8	47	77	0502
Open	92	92	14	23	
Injection location					
Periareolar	90	90	57	93	0285
Peritumoral	10	10	4	7	
Histopathology results					
IDC	70	70	49	80	
ILC	28	28	11	19	0125
Others	2	2	1	1	
T status					
T1	18	18	13	22	0973
T2	82	82	48	78	
Tumor grade					
1	-	-	-	-	
2	61	61	46	90	0275
3	39	39	15	10	
Operation					
BCS	71	71	29	48	0203
Mastectomy	29	29	32	52	

Remark: BCS: Breast-conserving surgery, IDC: Invasive ductal carcinoma, ILC: Invasive lobular carcinoma.

SLNB and histopathological examination

In this study, SLN was obtained from 46 patients in the non-NAC group and 56 patients from the NAC group. Cases of SLN with positive metastases were found in 27 of 46 patients in the non-NAC group, which resulted in an identification rate of 91.3%. Meanwhile, in the NAC group, SLN cases were found with positive metastases in 24 of 56 patients, which resulted in an identification rate of 80.6%. The average number of SNs that could be identified was two (1-8) in both groups.

In the non-NAC group, 19 patients could be identified without metastases. Eleven patients had metastases in their axillary lymph nodes, which resulted in a false-negative rate of 24.4% with NPV of 94.4% (95% CI 85–100) (Tables 2 and 4). In the NAC group, SLN was obtained without metastases in 32 patients with nine patients experiencing metastases in axillary lymph nodes, which showed a false-negative rate of 10.8% with NPV of 74% (95% CI 65–80) Tables 2 and 4). In the non-NAC group, there were 27 patients with metastases in SLN and axillary lymph nodes. Meanwhile, in the NAC

group, 24 patients with SLN metastases were found, 13 of who showed axillary metastases.

Table 2: Characteristics of patients with false-negative rate

Patient Characteristics	Neoadjuvant chemotherapy Group	Percentage (%)	Non-neoadjuvant chemotherapy group	Percentage
Age (years)				
<40	3	33	3	28
>40	6	67	8	72
T Status				
T1	-	-	1	10
T2	9	100	10	90
Histopathology results				
IDC	6	67	8	72
ILC	3	33	2	19
Others	-	-	1	9
Tumor grade				
1	-	-	-	-
2	2	12	4	37
3	8	88	7	63

Remark: IDC: Invasive ductal carcinoma, ILC: Invasive lobular carcinoma.

Unidentified SLN

In the non-NAC group, there were 15 patients with unidentified SLN with an average age of 50 years (36–63 years). Four patients (27%) had a Grade-II tumor, and 11 patients (73%) had Grade-III tumor. Based on the histopathology, 12 patients (80%) had invasive ductal carcinoma. Five patients showed axillary lymph node metastases, and others were negative.

In the NAC group, 44 patients presented with unidentified SLN were with an average age of 42 years (28–60 years). There were seventeen patients (39%) with Grade-II tumors and 27 patients (61%) with Grade-III patients. Invasive ductal carcinoma was the most histopathological found in 30 patients (68%). Ten patients had metastases in their axillary lymph nodes (Table 3).

Table 3: Characteristics of patients with unidentified sentinel lymph node

Patient Characteristics	Neoadjuvant chemotherapy group	Percentage	Non-neoadjuvant chemotherapy Group	Percentage
Age (years)				
<40	11	25	5	34
>40	33	75	10	66
Histopathology results				
IDC	30	68	12	80
ILC	12	27	2	13
MC	2	5	1	7
Tumor grade				
1	-	-		
2	17	39	4	27
3	27	61	11	73
Axillary lymph node metastasis				
Positive	10	77	5	34
Negative	34	23	10	66

Remark: IDC: Invasive ductal carcinoma, ILC: Invasive lobular carcinoma, MC: Medullary carcinoma.

Statistical analysis

The results of the analysis showed that the parameters of age (p = 0.485), tumor size (p = 0.973), tumor grade (p = 0.275), biopsy type (I=0.502), and injection location (p = 0.285) were not related to SLN identification.

Discussion

Most breast cancer cases in Indonesia are of the advanced stage when compared to some developed countries [12], [13]. It is what makes ALND is generally performed by surgeons in Indonesia. This study had a retrospective case-control research design, which aimed to determine whether NAC could affect the SLN identification rate and false-negative rate using methylene blue 1% as a lymphatic mapping standard before SLNB. Although the standards for lymphatic mapping in developed countries have supported combination techniques, limited access to radioisotope tracking and nuclear medicine in Indonesia is currently a constraint. The study conducted by Brahma *et al.* [14] showed that the use of methylene blue 1% was effective in identifying SLN with a sensitivity of 92% so that it could still be applied in Indonesia if no sophisticated lymph node mapping facilities were found. Fifty-seven patients (93%) in the non-NAC group and 90 patients (90%) in the NAC group had a periareolar injection of single methylene blue 1%. It is following the study of Borgstein *et al.* [15] and Shimazu *et al.* [16], which compared peritumoral injection with periareolar injection, which is an ideal technique in the identification of SLN in early-stage breast cancer patients.

In this study, the identification rate was 91.3% in the non-NAC group, but it decreased in the NAC group by 80.6%. In the NAC group, SLNs were identified as having decreased, that is, 23 patients (71%) had negative lymph nodes for axillary metastases with a false-negative rate of 10.8%, lower than that of the non-NAC group, which was 24.4%. According to the previous study, chemotherapy causes lymphatic drainage damage due to shrinkage and fibrosis by inducing fat degeneration due to the process of tumor cell apoptosis [11]. It causes SLNB accuracy in post-NAC patients to decrease. With this in mind, it is recommended that future studies can reduce ALND to save patients who have received NAC from having the risk of lymphedema and other morbidities.

The AMAROS and IBCSG 23-01 studies explain a new perspective for eliminating ALND after positive SLN [17]. Based on these studies, with specific criteria for small-sized tumors, BCS, and breast radiation can be performed. This selection criterion does not fit most of the characteristics of the patients in the non-NAC group, as shown in this study, where there were 48 patients (78%) with larger tumor size, T2, and 46 patients (90%) with a Grade-II tumor. In the NAC group, 82 patients (82%) had T2 tumors, and 61 patients (61%) had a Grade-II tumor, and mastectomy was preferred by a non-NAC group than BCS. In this study, the histopathological results showed that 119 patients (73%) had invasive ductal carcinoma. In another study conducted by Soebhi *et al.* [18], the same results were obtained, in which there were 687 breast cancer patients

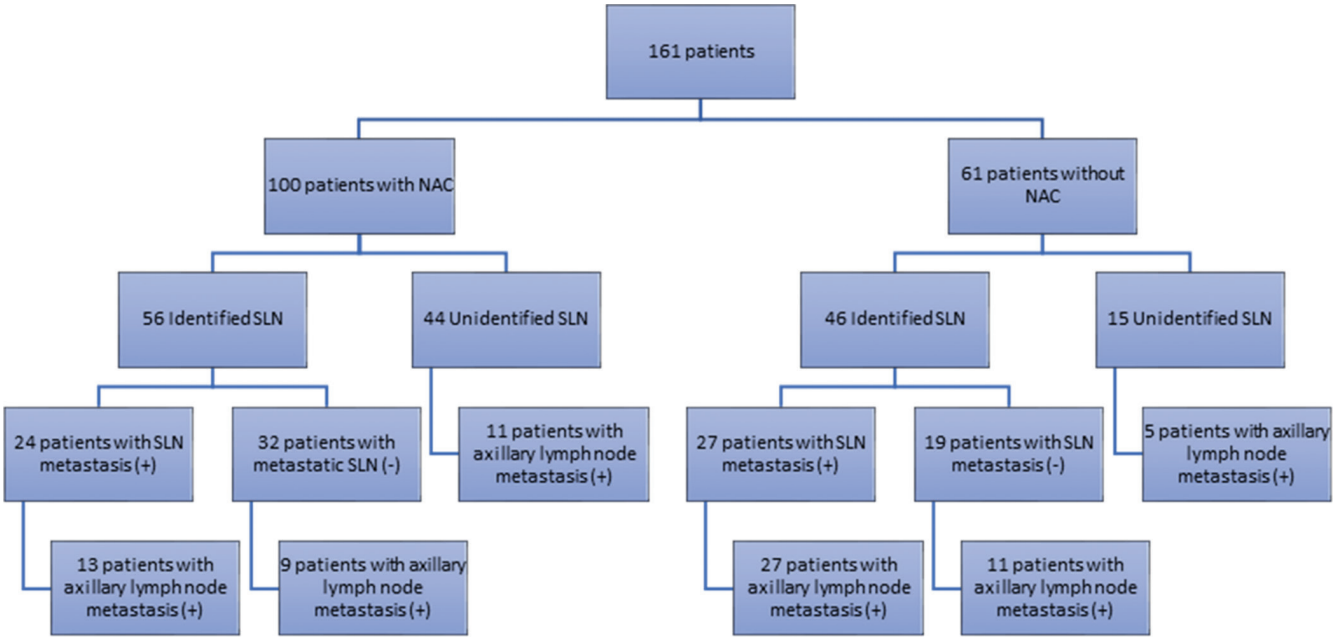

Figure 1: *Chart of patient. Remark: NAC: Neoadjuvant chemotherapy, SLN: Sentinel lymph node*

Table 4: Diagnostic score sentinel lymph node

Groups	Se	Sp	Positive predictive value	Negative predictive value	FNR
Neoadjuvant chemotherapy group	80.6	74.0	79.8	74.0	10.8
	95% CI (71-89)	95% CI (65-80)	95% CI (71-89)	95% CI (65-80)	
Non-neoadjuvant chemotherapy group	91.3	94.4	91.3	94.4	24.4
	95% CI (81-99)	95% CI (85-100)	95% CI (81-99)	95% CI (85-100)	

with an average age of 48.5 years in Indonesia, 89% of whom had invasive ductal carcinoma outcomes.

Brahma *et al.* [14], in their study, found an NPV value of 91.1%. The NPV in the non-NAC group of the current study was realized higher than that in another study of 94.4%, which might have been caused by 11 false-negative cases that had a higher median tumor size (4 cm) in about ten patients (90%), and higher tumor grade (63% with the Grade-3 tumor). Thus, patients with tumor size >3 cm and high-grade tumors may have a higher risk of developing metastases and blockage of the lymphatic system in SLN, switches to pseudo SLN [15]. However, it is different from the NAC group, in which their NPV tended to be low by 74% with nine false-negative cases with the same factors, nine patients were with tumor size more than 4 cm (100%), and eight patients (88%) were with Grade-III tumor. Therefore, the surgeon must be careful in performing SLNB with MBD to patients with Grade-3 tumors and tumor size more than 3 cm.

In this study, SLN could not be found in 15 patients in the non-NAC group. The average age of the unidentified SLN group was 50 years, and this older condition could be one of the factors that caused identification failure in the final results. Increased fat tissue in the breast among older patients can decrease lymphatic flow and failure to identify SLN [14]. Higher tumor grade is known to be a negative factor for SLN identification in univariate analysis [14]. In this study, there were approximately 81% of Grade-3 tumor cases found in the non-NAC group. This characteristic is following the study by Widodo *et al.* [13], showing that 54.8% of breast cancer patients in Indonesia had a Grade-III, 32.1% Grade-II, and 13.1% Grade-I tumors. However, in this study, there was no statistically significant relationship between age (p = 0.973), tumor grade (p = 0.275), and SLN identification. Meanwhile, in the NAC group, the identification of SLN on 44 patients was failed. The use of NAC can cause lymph node atrophy, which is microscopically proven by the study of Fan [10], in which there were lymphocyte loss, fibrosis, and histiocyte collection in lymphocytes.

This study has some limitations. The researchers only included clinically negative patients, but they did not perform an axillary biopsy if the axillary ultrasonography found a suspicious lymph node. Ultrasound-guided axillary lymph node biopsy will select patients with true negative axillary lymph nodes before surgery. We did not perform the clinical and pathological response evaluation in the NAC group with positive and negative metastases

Conclusion

This study proves that the NAC effect on SLNB technique with single methylene blue 1% can reduce the identification and false-negative rates of SLNB in breast cancer patients at low-resource country. Thus, the use of single methylene blue 1% injection in combination

with NAC cannot be completely reliable as a diagnostic tool for lymph node mapping.

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