

Sentinel lymph node biopsy for breast cancer using methylene blue: a new anatomical landmark involving intercostobrachial and medial pectoral nodes

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ABSTRACT

BACKGROUND Sentinel lymph node biopsy (SLNB) using blue dye is becoming popular in Indonesia given that knowledge on new anatomical landmarks involving intercostobrachial and medial pectoral nodes have replaced the need for radioisotope tracers. This study aimed to evaluate the utility of the proposed landmark involving intercostobrachial and medial pectoral nodes to determine axillary lymph node status during SLNB.

METHODS A prospective study was conducted involving 55 patients with early-stage breast cancer who had clinically negative lymph nodes (T1–T2, cNo) between 2018 and 2019 at Cipto Mangunkusumo Hospital. During SLNB, methylene blue 1% was injected at the subareolar area to identify intercostobrachial and medial pectoral nodes followed by axillary lymph node dissection (ALND). Histopathological results of sentinel nodes (SNs) were then compared to those of other axillary nodes.

RESULTS SNs were identified in 54 patients (98%), 33 (61%) of whom had both intercostobrachial and medial pectoral SNs. Among patients with SNs, there were 1 patient without intercostobrachial SNs, 10 patients without medial pectoral SNs, and 1 patient with medial pectoral SNs but no intercostobrachial SNs. Accordingly, SNs had a negative predictive value (NPV) of 96.77% for axillary metastasis (95% confidence interval = 81.54–99.51), with a false negative rate of 4.7%. No serious adverse events was observed.

CONCLUSIONS The high identification rate and NPV, as well as the low false negative rate of the new anatomical landmark involving intercostobrachial and medial pectoral nodes during SLNB, suggest its reliability in determining axillary lymph node status.

KEYWORDS breast neoplasm, intercostobrachial, methylene blue, sentinel lymph node biopsy

Sentinel lymph node biopsy (SLNB), a minimally invasive procedure, has been shown to accurately stage the axilla and promote lesser morbidity compared to axillary lymph node dissection (ALND).¹ The mapping method has been among the most important factors affecting the identification rate and false negative rate of SLNB in breast cancer. A combination of lymphoscintigraphy, blue dye, and intraoperative gamma probe for SLNB has currently been considered the gold standard for detecting

sentinel nodes (SNs) in early-stage breast cancer.^{2,3} In Indonesia, however, SLNB for breast cancer has yet to become popular considering several factors, including patient characteristics (most already had the advanced-stage disease), unclear recommendations, and limited facilities for radioisotope use due to high costs and unavailability in most cancer centers. The aforementioned factors are compounded by the fact that Indonesia consists of many islands, making access to cancer centers even more difficult.⁴ All such factors

have contributed to the difficulty in the application of SLNB. Hence, studies that attempt to address the aforementioned limitations in performing SLNB are imperative.^{4,5}

New knowledge on the proposed landmark involving intercostobrachial and medial pectoral nodes as the first group of axillary lymph nodes that receive lymphatic flow from the primary tumor has allowed the utilization of a blue dye as a single tracer for detecting SNs. Lymphatic drainage from the primary tumor has been suggested to consecutively spread in a stepwise fashion to the intercostobrachial and medial pectoral nodes. Several blue dyes, such as patent blue, isosulfan blue, and methylene blue, have been commonly used in SLNB to map the axillary lymph nodes. Considering its better accessibility and considerably lower allergic risk compared to other blue dyes, some cancer centers have preferred to use methylene blue. The present study primarily aimed to evaluate the utility of SLNB for breast cancer using methylene blue to predict axillary lymph nodes metastasis based on the proposed landmark involving intercostobrachial and medial pectoral nodes.

METHODS

This prospective study included a total of 55 patients with clinically No breast cancer, among whom 20 and 35 had T1 and T2 disease, respectively, who received treatment at the Surgical Oncology Subdivision, Department of Surgery, Cipto Mangunkusumo Hospital from August 2018 to March 2019. Malignancy was diagnosed through an ultrasound-guided core biopsy of the breast tumor. All patients had no history of previous surgery on their ipsilateral breast. Preoperative No axillary lymph node status was confirmed through physical examination and ultrasound. Patients with distant metastases found during the initial staging workup and a history of allergic response to blue dye were excluded. This study was approved by the Ethics Committee of the Faculty of Medicine, Universitas Indonesia, with registration number 0771/UN2.F1/ETIK/2018. All patients were informed regarding the SLNB procedure and provided informed consent.

To avoid bias during SLNB assessment using blue dye, only one surgeon with more than 2.5 years of experience in breast surgery participated in the research. The included surgeon had already obtained

internal validation in performing SLNB, including 20 SLNB procedures with identification rates above 95% and false negative rates below 5%. All patients underwent either breast-conserving surgery (BCS) or mastectomy for local control of the primary tumor. Mastectomy was performed for patients with an estimated defect size after wide excision exceeding 20% of the breast size when multicentricity was observed, and as preferred by the patient.

SLNB was performed using 1.5 ml methylene blue 1% injected at the subareolar site, followed by a 5-min

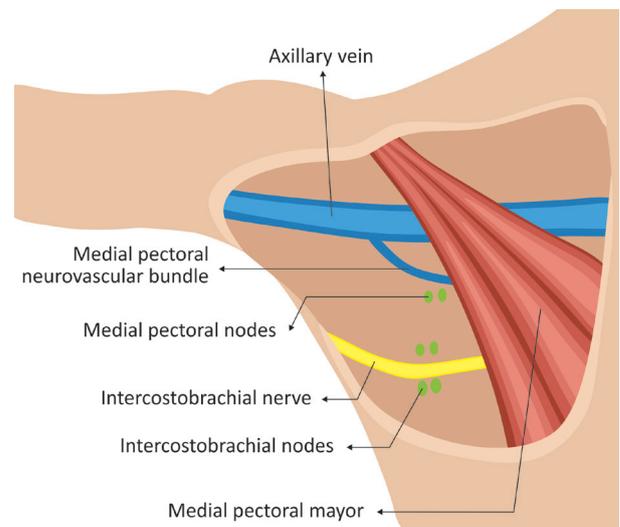


Figure 1. Schematic view of the sentinel node (SN) locations (intercostobrachial and medial pectoral nodes) in the right axillary region

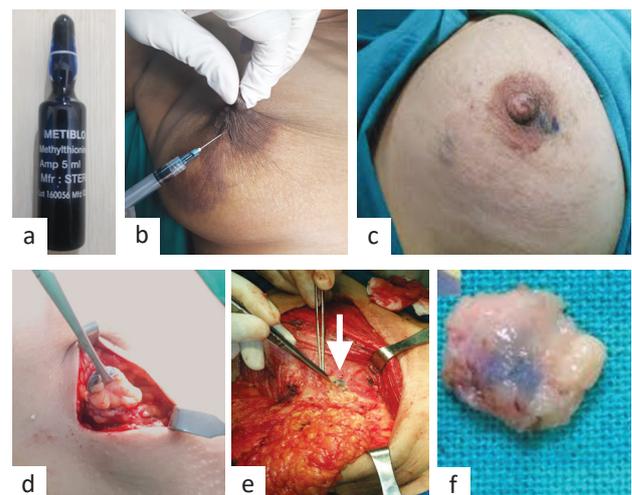


Figure 2. (a) Methylene blue dye; (b) SLNB was performed using a 3-cm³ syringe with 1.5 cm³ of methylene blue 1% injected at the subareolar region; (c) breast appearance after methylene blue dye injection; (d) SLNB using a separate axillary incision in breast-conserving surgery; (e) SLNB during mastectomy; blue-stained SN (arrow); (f) SN specimen. SLNB=sentinel lymph node biopsy; SN=sentinel node

massage around the injection site to allow the blue dye to fill the lymphatic channels into the axillary area. Blue lymphatic channels were meticulously traced down to the SNs guided by the area around the intercostobrachial nerve (intercostobrachial nodes) and medial pectoral bundle (medial pectoral nodes) as an anatomical landmark (Figures 1 and 2).

Level I–II ALND was then performed after completing all procedures. During axillary dissection, SNs were identified and removed. Non-sentinel nodes (NSNs) were defined as lymph nodes harvested during ALND that were not classified as SNs. SNs and dissected axillary specimens underwent histopathologic evaluation, after which metastasis rates were compared.

RESULTS

Patient characteristics

Fifty-five patients with early-stage breast cancer included herein, >60% had T2 tumor (size 2–5 cm). Majority of the patients underwent mastectomy for the primary tumor (74%). Four patients who were formerly scheduled to undergo BCS procedure were intraoperatively converted to mastectomy due to persistent positive margins after reasonable surgical attempts. Primary breast tumors were most commonly located in the upper lateral quadrant (30.9%), with only one patient have a primary tumor located in the axillary tail of the breast. Patient characteristics and the median number of detected intercostobrachial and medial pectoral nodes, as well as the number of NSNs, collected following axillary dissection are summarized in Table 1.

Sentinel and non-sentinel lymph node characteristics

After SLNB, SNs were identified in 54 of 55 patients (SN identification rate 98%), with more than 75% of them having more than one SN. Among patients determined to have SNs, all except one case had intercostobrachial SNs, all of which were consistently located around the intercostobrachial nerve and superficially located within the axillary fat just below the clavipectoral fascia. Medial pectoral nodes were less frequent than intercostobrachial nodes. Among 34 patients with medial pectoral nodes, 20 patients had partially blue-stained SNs, the identification of which was guided by the blue lymphatic channels toward the nodes. The blue lymphatic channel was observed starting

from the direction of the primary tumor through the intercostobrachial region and then toward the medial pectoral region.

The number of patients with intercostobrachial nodes, medial pectoral nodes, and both are presented in Figure 3. After SNs were identified, ALND was performed to search for other remaining NSLNs.

Approximately half of all patients with T1–T2 breast cancer showed no axillary lymph node metastasis for both SNs and NSNs. Among 24 patients with axillary

Table 1. Subject characteristics

Characteristic	n (%) (N = 55)
Age (years), mean (SD)	50.56 (13.20)
≤40	16 (29)
>40	39 (71)
Tumor size (cm), median (min–max)	3 (0–5)
<2	20 (36)
2–5	35 (64)
Location of primary tumor	
Superolateral	17 (31)
Superomedial	10 (18)
Inferolateral	9 (16)
Inferomedial	10 (18)
Central	9 (16)
Histology subtype	
Invasive NST	44 (80)
Lobular invasive	8 (15)
Others	3 (5)
Molecular subtype	
Luminal A	19 (35)
Luminal B	14 (25)
HER2	11 (20)
Triple negative	11 (20)
Operation type	
BCS	14 (26)
MRM	41 (74)
Number of SNs, median (min–max)	3 (0–5)
Intercostobrachial nodes	2 (0–3)
Medial pectoral nodes	1 (0–3)
Number of NSNs, median (min–max)	9 (6–15)
Size of SNs (cm), median (min–max)	0.85 (0.5–1)
Number of SNs with metastases, (n)	23
Number of both SNs and NSNs with metastases, (n)	20

SD=standard deviation; NST=no special type; HER2=human epidermal growth factor receptor 2; BCS=breast-conserving surgery; MRM=modified radical mastectomy; SNs=sentinel nodes; NSNs=non sentinel nodes

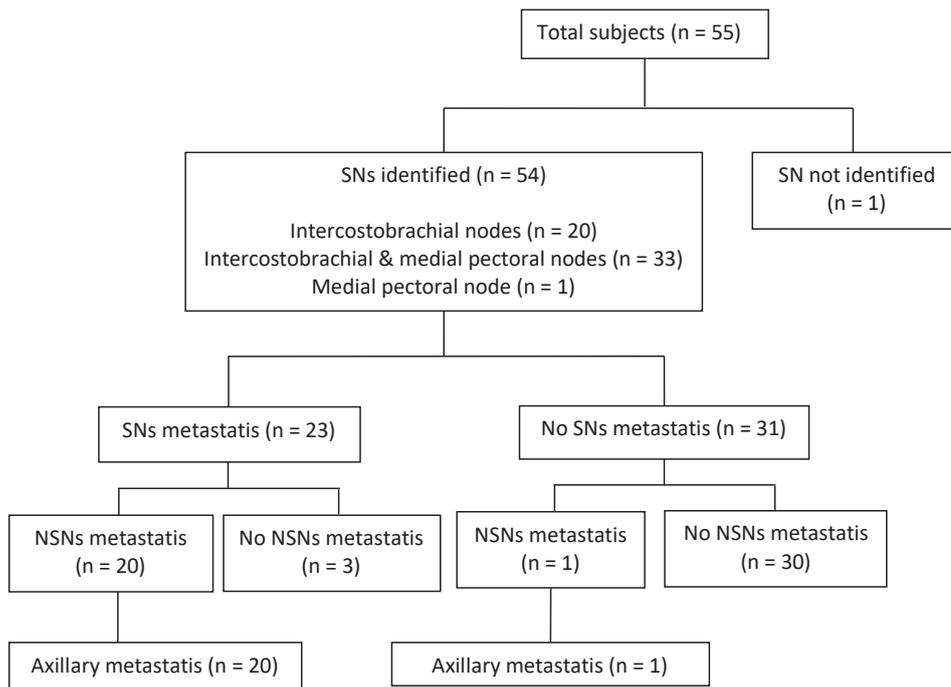


Figure 3. Patients flowchart for recruitment and SN assessment to predict axillary metastasis. SNs=sentinel nodes; NSNs=non-sentinel nodes

Table 2. Relationship between sentinel and non-sentinel lymph nodes

	Result	Non-sentinel lymph node		Total
		Positive	Negative	
Sentinel lymph node	Positive	20	3	23
	Negative	1	30	31
	Total	21	33	54

lymph node metastasis, 21 patients (88%) definitely required axillary lymph node dissection given that only 3 patients had metastases confined to the SNs, while the rest exhibited metastasis to NSNs. Only one NSN metastasis was observed in the SN-negative group, resulting in a negative predictive value (NPV) of 96.77% (95% confidence interval = 81.54–99.51) and false negative rate of 4.7%. Figure 3 and Table 2 show the recruitment of patients and the relationship between SNs and NSNs.

Complications

No systemic anaphylactic reactions and postoperative complications related to the subdermal injection of methylene blue, such as allergic reactions and skin and parenchymal necrosis, occurred throughout the study. Patients who underwent BCS exhibited blue skin staining in the skin around the injection site, which remained for approximately 1–2 months after the procedure.

DISCUSSION

Blue dye SN biopsy using the new anatomical landmark involving intercostobrachial and medial pectoral nodes has transformed surgical staging in the management of the axillary regional basin among patients with breast cancer.⁶ Among 55 patients with early-stage breast cancer included herein, 98% and 61% had intercostobrachial and medial pectoral nodes, respectively. Only one patient had medial pectoral nodes without any intercostobrachial SNs. This supports the notion that lymphatic metastasis from the primary tumor consecutively spreads in a stepwise fashion to the intercostobrachial and medial pectoral nodes. Ong Kong Wee, who proposed a new concept of lymphatic spread from the primary tumor to the axillary lymph nodes, revealed a consecutive pattern of SN drainage starting from the breast to the intercostobrachial region and then finally to the medial pectoral nodal stations.⁷ This further supports the identification of SNs around the intercostobrachial nerve as the first station. This new concept will make SN detection using blue dye as a single tracer much easier.

The proposed method failed to detect SNs from either the intercostobrachial or medial pectoral nodes in only one patient who was a 50-year-old female with a tumor size of 5 cm in the upper outer quadrant close to the axilla. A more lateral tumor location had been

found to promote a higher possibility of lymphatic channel blockage toward the axillary region. Accordingly, the aforementioned patient had been the only one to have a tumor located on the axillary tail of the right breast. The AMAROS trial showed that older age, larger tumor sizes, identification method, and surgeon experience may affect SNs detection rates.⁸

All SLNB procedures utilized herein were performed by injecting 1.5 cm³ of methylene blue 1% around the subareolar region. Current anatomical knowledge of breast lymphatics introduced by Sappey⁹ revealed that the breast lymphatic network collects into the subareolar plexus and then drains toward the axilla via collecting lymphatic vessels. This description has been adopted by most anatomists and has become the theoretical basis for subareolar injection of dyes and/or isotopes for lymphatic mapping as part of SLNB for breast cancer.⁹ Nonetheless, controversies still exist over the role of the subareolar plexus in lymphatic mapping. The FRANSENODE trial suggested that the introduction of mapping agents into the subareolar and peritumoral sites produced a similar SN identification rate.¹⁰ The advantages of subareolar injections include less pain, rapid blue dye uptake, avoiding blue dye overlap with tumors, and the ability to inject impalpable lesions without the need for additional preoperative imaging.⁸ Several studies have also reported that subareolar and periareolar injections promote higher SLN identification rates compared to peritumoral injections.⁹⁻¹²

The optimal methylene blue dosage for SLNB remains controversial, with the most commonly used dosages being 2 and 5 ml.^{13,14} Accordingly, Brahma et al,⁵ who used 5 ml of methylene blue injected at the subareolar or peritumoral areas, obtained an SN detection rate of 91.7%. The present study obtained a comparable identification rate using 1.5 cm³ of methylene blue injected at the subareolar area. To determine the optimal methylene blue dose for SNB, however, a well-designed study is needed.

Apart from the excellent identification rate (98%), the current study also obtained a high NPV (96.77%) with a low false negative rate (4.7%), a result consistent with those obtained from other centers across Indonesia, such as the Dharmas Cancer Hospital (identification rate of 91.7% and NPV of 90%).⁵ Moreover, a study by Nandu and Chaudhari¹⁵ found a similar SN identification rate (100%) and NPV (85.7%).

The identification rate and false negative value obtained herein are considered acceptable according to the 2000 American Society of Breast Surgeons guidelines (identification rate for SLNB $\geq 85\%$ and false negative rate $\leq 5\%$).¹⁶

Among patients included herein, only one developed metastasis in the axilla, although this was not detected in the SNs. Moreover, the only patient with a false negative result in this study was a 70-year-old female with a tumor size of 5 cm, one identified SN, preoperative biopsy results showing no special type invasive carcinoma, and triple negative molecular subtype. Given that only one SN had been found in this patient, a higher false negative rate may be expected. Several studies have stated that the number of detected SNs was one of the significant factors influencing false negative rates during SLNB. A study by Kim et al¹⁷ showed that the optimal number of SNs that needed to be found was two nodes. The current study had a false negative rate of 4.7%, which is considered acceptable, and identified at least three SNs on average, which can be considered an optimal number for reducing false negative rates.

A total of 30 patients whose SNs were negative were also free from regional metastases based on axillary node dissection results. This indicated that in most cases, SLNB using methylene blue dye was reliable in determining axillary lymph node status without dissection, thereby reducing post dissection morbidities, such as lymphedema, arm paraesthesia, and other morbidities. This high identification rate and NPV along with the low false negative rate of SLNB using methylene blue prevented both unnecessary axillary dissection and neglect of patients with lymph node metastases. At present, most of the studies that had assessed the efficacy of SLNB in pathologically free axilla (pNo) also reinforced the favorable impact of SLNB on morbidity and equivalent oncological outcomes.^{5,18,19}

Several blue dyes, such as patent blue, isosulfan blue, and methylene blue, have been frequently used to map the axillary lymph node during SLNB.^{2,18} Among such dyes, methylene blue has been considered to have a lower allergic risk and better accessibility in some cancer centers across Indonesia. Accordingly, none of the patients who underwent SLNB using methylene blue in this study developed postoperative complications, including allergic reactions and skin and parenchymal necrosis. The only methylene

blue-related skin change noted herein was skin discoloration around the injection sites that remained for less than 2 months. However, Brahma et al⁵ reported that among their patients, two experienced skin necrosis around the methylene blue injection site, while none experienced anaphylactic reactions. One study showed that the incidence of allergic reaction following injection of methylene blue was between 0.06% and 2.7%.²⁰ Other studies have shown that methylene blue was safe blue dye with only few cases developing anaphylaxis.^{20,21} Accordingly, none of the patients included herein developed anaphylactic reaction. These results demonstrate that methylene blue is generally a safe blue dye for mapping SNs in breast cancer.

Currently, neoadjuvant treatment has been increasingly utilized as the preferred therapeutic option for advanced and high-risk early-stage breast cancer.²² Given that chemotherapy may adversely affect the lymphatic channel, using methylene blue for SLNB in such settings needs to be explored in future research. Furthermore, the clinical impact of micro- and macrometastases on detected SNs remains to be elucidated.

The current study has several limitations. First, only a limited number of patients had been included considering that most of our patients already had advanced-stage breast cancer upon diagnosis. SLNB is typically indicated for T1 and T2 breast cancer with clinically No disease. Second, the identification technique only relied on blue or non-blue nodes as the criteria for identifying SNs without exploring other suspicious non-blue nodes. SNs may not always appear as a blue node and can occasionally present as a non-blue node with an apparent blue lymphatic channel around it. This usually occurs during peritumoral lymphatic blockage by tumor cells, which prevents the blue dye from staining the SNs.

In conclusion, the current study revealed that applying the proposed anatomical landmark involving intercostobrachial and medial pectoral nodes during SLNB using methylene blue promoted a high identification rate and NPV with low false negative rate, thereby suggesting its reliability in determining axillary lymph node status.

Conflict of Interest

The authors affirm no conflict of interest in this study.

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REFERENCES

1. Verbelen H, Tjalma W, Meirte J, Gebruers N. Long-term morbidity after a negative sentinel node in breast cancer patients. *Eur J Cancer Care.* 2019;28(5):e13077.
2. Li J, Chen X, Qi M, Li Y. Sentinel lymph node biopsy mapped with methylene blue dye alone in patients with breast cancer: a systematic review and meta-analysis. *PLoS One.* 2018;13(9):e0204364.
3. Dogan NU, Dogan S, Favero G, Köhler C, Dursun P. The basics of sentinel lymph node biopsy: anatomical and pathophysiological considerations and clinical aspects. *J Oncol.* 2019;2019:3415630.
4. National Institute of Health Research and Development, Indonesian Ministry of Health. Basic Health Research; RISKESDAS 2013. Jakarta: National Institute of Health Research and Development, Indonesian Ministry of Health; 2013. p. 110–9. Indonesian.
5. Brahma B, Putri RI, Karsono R, Andinata B, Gautama W, Sari L, et al. The predictive value of methylene blue dye as a single technique in breast cancer sentinel node biopsy: a study from Dharmais Cancer Hospital. *World J Surg Oncol.* 2017;15(1):41.
6. Li C, Zhang P, Li R, Wu C, Zhang X, Zhu H. Axillary lymph node dissection versus sentinel lymph node biopsy alone for early breast cancer with sentinel node metastasis: a meta-analysis. *Eur J Surg Oncol.* 2015;41(8):958–66.
7. Ong KW, Sim YR. Sentinel lymph node biopsy in breast cancer. In: Sobri FB, Azhar Y, Wibisana IGN, Rachman A, editors. *Current management of breast cancer.* 1st ed. Jakarta: Media Aesculapius; 2017. p. 144–59. Indonesian.
8. Straver ME, Meijnen P, van Tienhoven G, van de Velde CJ, Mansel RE, Bogaerts J, et al. Sentinel node identification rate and nodal involvement in the EORTC 10981-22023 AMAROS trial. *Ann. Surg. Oncol.* 2010;17(7):1854–61.
9. Sappey M. Injection preparation et Conservation des Vais-Seaux Lymphatics (thes pour le doctorat en medecine, No. 241). Paris: Rignoux Imprimeur de la Faculte de Medecine. 1834. French.
10. Rodier JF, Velten M, Wilt M, Martel P, Ferron G, Vaini-Elies V, et al. Prospective multicentric randomized study comparing periareolar and peritumoral injection of radiotracer and blue dye for the detection of sentinel lymph node in breast sparing procedures: FRANSENODE. *J Clin Oncol.* 2007;25(24):3664–9.
11. Whitman GJ, AlHalawani RH, Karbasian N, Krishnamurthy R. Sentinel lymph node evaluation: what the radiologist needs to know. *Diagnostics.* 2019;9(1):12.
12. Parasuraman M, Girdharan B, Vijaylakshmi. Use of methylene blue for the detection of sentinel lymph node in breast cancer: a systematic review and meta-analysis. *Int Surg J.* 2018;5(1):1–10.
13. Ushadevi JS, Rajaraman R, Subbaih S. Assessment of sentinel nodes with methylene blue dye in carcinoma breast is feasible? A pilot study. *Int J Sci Stud.* 2017;5(1):148–52.
14. Bakhtiar N, Jaleel F, Moosa FA, Qureshi NA, Jawaaid M. Sentinel lymph node identification by blue dye in patients with breast carcinoma. *Pak J Med Sci.* 2016;32(2):448–51.
15. Nandu VV, Chaudhari MS. Efficacy of sentinel lymph node biopsy in detecting axillary metastasis in breast cancer using methylene blue. *Indian J Surg Oncol.* 2017;8:109–12.
16. Lyman GH, Temin S, Edge SB, Newman LA, Turner RR, Weaver DL, et al. Sentinel lymph node biopsy for patients with early-stage breast cancer: American Society of Clinical Oncology clinical practice guideline update. *J Clin Oncol.* 2014;32(13):1365–83.
17. Kim MK, Park HS, Kim JY, Kim S, Nam S, Park S, et al. The clinical implication of the number of lymph nodes harvested during sentinel lymph node biopsy and its effects on survival outcome in patients with node-negative breast cancer. *Am J Surg.*

- 2017;214(4):726–32.
18. Dialani V, James DF, Slanetz PJ. A practical approach to imaging the axilla. *Insights Imaging*. 2015;6:217–29.
 19. Giuliano AE, Ballman KV, McCall L, Beitsch PD, Brennan MB, Kelemen PR, et al. Effect of axillary dissection vs no axillary dissection on 10-year overall survival among women with invasive breast cancer and sentinel node metastasis: the ACOSOG Z0011 (Alliance) randomized clinical trial. *JAMA*. 2017;318(10):918–26.
 20. Muñiz-Díaz E, Puig L. Allergic and anaphylactic reactions to methylene-blue-treated plasma in Catalonia in the period 2008–2013. *Blood Transfus*. 2014;12(4):628–30.
 21. Varghese P, Abdel-Rahman AT, Akberali S, Mostafa A, Gattuso JM, Carpenter R. Methylene blue dye—a safe and effective alternative for sentinel lymph node localization. *Breast J*. 2008;14(1):61-7.
 22. Colomer R, Saura C, Sánchez-Rovira P, Pascual T, Rubio IT, Burgués O, et al. Neoadjuvant management of early breast cancer: a clinical and investigational position statement. *Oncologist*. 2019;24(5):603–11.