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The Application of 1% Methylene Blue Dye as a Single Technique in Breast Cancer Sentinel Node Biopsy

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Cover letter to the editor

Dear editors,

It has been a great opportunity for us to propose a manuscript to your journal. Our work is based on the development of breast cancer sentinel node biopsy (SNB) in our country. For many years, axillary lymph node dissection is the common procedure for axillary staging in Indonesia and SNB has not been a major interest among our surgeons. Perhaps, one of the reason is because of the expensive and unavailability to provide radioisotope tracer and patent or isosulfan blue dye in our country. In order to solve it, we have made a validation study which was based on the utility of 1% methylene blue dye alone technique. It is a quite simple procedure with a favorable results if it is used in selected cases. Although it is not a new and sophisticated technique, but sharing our early experience in a video journal format might be useful for some colleagues who have the same situation like ours.

Bayu Brahma, Rizky I. Putri, and Samuel J, Haryono are contributing for the idea, video making and editing as well as writing and editing the manuscript. The others contribute to the concept of the study design. Mr. Ronald Myers has been very helpful to guide us through the submission process. As for the reviewer here is the list of people that might help:

1. Teguh Aryandono from Gadjah Mada University, teguharyandono@yahoo.com
2. Wirsma Arif Harahap from Andalas University, wirsma_arif@yahoo.com
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6. Tran Viet Phuong Ho Chi Minh City Oncology Hospital tvt.phuong_1999@yahoo.com

Hopefully, the work will contribute to a better breast cancer in every part in the world.

Sincerely yours,

Bayu Brahma, MD

TITLE:

The Application of 1% Methylene Blue Dye as a Single Technique in Breast Cancer Sentinel Node Biopsy

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KEYWORDS:

Breast cancer, sentinel node, methylene blue dye, axillary dissection, SLNB, SNB

SUMMARY:

In Indonesia, sentinel node biopsy (SNB) is not routinely performed for breast cancer surgery because of the limitation to provide radioisotope tracer and isosulfan or patent blue dye (PBD). To overcome this obstacle, we applied 1% methylene blue dye (MBD) as a single agent to map the sentinel nodes (SNs).

ABSTRACT:

In this study, we injected 1% MBD into the subareolar or peritumoral space of the breast. In the case of breast conserving surgery (BCS), a separate incision in the lower axilla hairline was made to find the SNs. In mastectomy, the SNs were identified through the same mastectomy incision. The SNs were described as blue nodes or nodes with lymphatic blue channels. An anatomical landmark in the axilla was used to facilitate SNs identification. The SNs metastases were evaluated by intraoperative frozen section analysis and histopathology examination as it is a gold standard. Here, we described the MBD as the lone technique in breast cancer SNB which could be useful when radioisotope tracer or PBD cannot be provided.

INTRODUCTION:

The status of axillary lymph nodes (ALNs) metastasis is the most important prognostic factor in breast cancer. Axillary lymph node dissection (ALND) was the conventional procedure to assess

metastatic status of ALNs[1,2]. Unfortunately, ALND results in morbidities to the patients which decreases the quality of life, especially by increasing the risk of lymphedema after this procedure[3,4]. Nowadays, sentinel node biopsy (SNB) has replaced ALND for axillary staging because of its minimal morbidities among patients[5]. The most common method for performing SNB is using radioisotope tracer and PBD[6]. In some parts of the world, including in developing countries, these tracer agents could be difficult to procure and searching for an alternative tracer agent is critical to solve the problem.

Initially, MBD was used by Wong et al. as a tracer agent for mapping sentinel nodes (SNs)[7]. In their study using a feline model, intradermal injection of MBD showed poor lymphatic uptake and isosulfan blue was chosen as the preferred dye for sentinel node (SN) mapping[7]. Methylene blue dye has been used in breast cancer SNB since the first successful report by Simmons et al.[8]. Several studies have also reported MBD as the favorable dye for SNs identification, and that the false negative rates of MBD technique were comparable to radioisotope or PBD[9-11]. Fewer allergic reactions and lower price are the other reasons to consider its use in SN mapping[12].

Recently, we studied the use of 1% MBD alone for SNB in clinically node negative breast cancer. In early stages, MBD has a favorable identification rate and negative predictive value[13]. We inject 2 mL of 1% MBD into the subareolar space or peritumoral area if there was an excisional biopsy scar at the upper outer quadrant or nipple areolar complex (NAC) of the breast. The blue nodes and non-blue nodes with lymphatic blue channels are categorized as SNs. The anatomical landmark in the axilla is used as a guidance to find SNs. Intraoperative examination is applied to assess the metastasis and the SNs are sent for histopathology analysis based on American Society of Clinical Oncology (ASCO) Guidelines[14].

If the cases are selected carefully and the skills required for this technique are obtained by the surgeons as well as pathologists, many patients could be saved from the harmful effects of ALND while still having favorable survival.

PROTOCOL:

All procedures including human subjects have been approved by Dharmais Cancer Hospital Ethics Committee with certificate number of 040/KEPK/VII/2017. All patients signed the consent forms and expressed agreement to participate in this study.

NOTE: The inclusion criteria are patients with diagnosis of early breast cancer, with tumor stage T1–T2 without palpable and ultrasonography lymph nodes enlargement (cNo). The exclusion criteria are locally advanced breast cancer, no neo adjuvant chemotherapy, and pregnancy.

1. Preparation of 1% methylene blue dye and the injection technique

1.1. Sterilize the surgical field after anesthetizing the patient.

1.2. Aspirate 2 mL of 1% methylene blue dye from its vial with a 3 mL syringe.

1.3. Draw a line to mark the lower axillary hairline below the lateral border of pectoralis major muscle.

1.4. Inject 2 mL of 1% methylene blue dye into the peritumoral site of the breast with a 23 G needle under ultrasound guidance with a linear probe (12 MHz).

1.5. Massage the breast circularly at the injection site for 5 min, and then continue to perform surgery.

2. Sentinel node biopsy technique in breast conserving surgery (BCS)

NOTE: The surgery is performed in a patient who underwent BCS and SNB.

2.1. Prepare the surgical tools: monopolar electrocautery, DeBakey forceps/anatomical forceps, and retractors.

2.2. Incise the skin, subcutaneous tissue, and fascia.

2.3. Find the blue nodes or blue lymphatic tracts. Follow the blue tracts until the blue nodes or non-blue nodes with lymphatic blue tracts are identifiable.

2.4. Search for the sentinel nodes along the intercostobrachial nerve and lateral thoracic vein if the blue nodes or blue lymphatic tracts cannot be found.

2.5. Resect the sentinel nodes carefully and avoid damaging the nodes.

2.6. Palpate the axillary space to find additional suspicious malignant lymph node enlargement.

3. Sentinel node biopsy technique in mastectomy

NOTE: The surgery is done in a patient who underwent mastectomy and SNB.

3.1. Incise the skin and subcutaneous tissue.

3.2. Create skin flaps.

3.3. Remove the breast from pectoralis major until axillary fossa can be fully exposed.

3.4. Incise the clavicopectoral fascia to find the sentinel node.

3.5. If the blue lymphatic tracts cannot be found, find the sentinel node along the intercostobrachial nerve and lateral thoracic vein area.

3.6. Remove the sentinel node.

3.7. Look for additional suspicious lymph nodes by palpation.

4. Intraoperative examination

4.1. Slice the lymph nodes no thicker than 2 mm, parallel to the long axis.

4.2. Make touch imprint cytology from each node.

4.3. Place the surgical specimen on a metal tissue disc and embed in a gel-like medium with the same density as frozen tissue.

4.4. Submit all of the nodes for frozen section (FS) examination.

4.5. Categorize the metastatic status of sentinel nodes into positive or negative, and report it to the surgeon during the surgery.

5. Pathological examination

5.1. Perform the final pathologic evaluation of the sentinel nodes on formalin-fixed and paraffin-embedded tissue sections.

5.2. Classify the sentinel nodes metastasis according to the 6^{th} edition of American Joint Committee on Cancer (AJCC) manual. Macrometastasis (MAC) is defined as tumor deposits larger than 2 mm, micrometastasis (MiC) is defined as tumor deposits between 0.2 and 2 mm, isolated tumor cells (ITC) are defined as cell clusters no larger than 0.2 mm.

5.3. Perform the serial sectioning and immunohistochemistry analysis for cytokeratin when there are doubts over defining ITC.

5.4. Examine the rest of axillary lymph nodes in a similar manner.

REPRESENTATIVE RESULTS:

Here, we describe the results from the presented technique. Two milliliters of 1% MBD were injected at the deep subareolar space as the standard technique of injection (**Figure 1A**). If the peritumoral injection is indicated, it should be performed under ultrasound guidance (**Figure 1B**). The blue nodes or lymphatic blue tracts were seen after entering the axillary space. Following the lymphatic blue tracts lead to finding the SNs (**Figure 2A**). If the blue nodes or lymphatic blue tracts could not be seen, we used the intercostobrachial nerve and lateral thoracic vein as the anatomical landmarks. The SNs were usually located around those areas (**Figure 2B**,**C**).

Once the SNs were localized, they were sent immediately to the laboratory for intraoperative frozen section examination. The sentinel nodes were sectioned into 2-mm-thick slices, parallel to the long axis (**Figure 3A**). The specimen was then immediately frozen and thin sections were cut

on a cryostat machine (**Figure 3B**). An intraoperative analysis was used to categorize SNs as positive or negative for metastases (**Figure 3C**). The rest of SNs were formalin-fixed and paraffin-embedded for hematoxylin-eosin staining.

The results of intraoperative frozen section assessment were then compared to the permanent section of pathological examination with regard to nodal oncological status. The tumors were histologically classified according to the World Health Organization (WHO) Histological Classification of Breast Tumors, and grading was defined according to Elston and Ellis modification. The nodal metastasis status was classified according to the 6th edition of American Joint Committee on Cancer (AJCC) manual.

FIGURE LEGENDS:

Figure 1: The 1% methylene blue dye injection technique. (A) The deep subareolar space is the standard site of injection in this study. **(B)** Peritumoral injection is used in cases with previous excisional biopsy scar at the upper outer quadrant of the breast. The injection is performed under an ultrasound guidance to ensure the methylene blue dye is injected at the breast parenchyma.

Figure 2: The anatomical landmarks and technique to find sentinel nodes. (A) Finding and following the blue lymphatic tracts led to the SNs. **(B, C)** The intercostobrachial nerve and lateral thoracic vein were identified, because blue nodes or lymphatic blue tracts could not be found after entering the axillary space. The SNs were located around these landmarks.

Figure 3: Frozen section analysis. (A) The sentinel nodes were sliced no thicker than 2 mm. **(B)** Each part was included for frozen section examination. **(C)** The frozen section analysis showed positive result for metastasis; original magnification was 40x.

Figure 4: Sentinel lymph node metastases. (A) Macrometastasis (MAC) is defined as tumor deposits that are larger than 2 mm; original magnification 4x. **(B)** Tumor deposits found between 0.2 and 2.0 mm are defined as micrometastasis (MIC); original magnification 4x. **(C)** Immunohistochemistry (IHC) for cytokeratin was performed when there was some doubt when defining the metastasis; original magnification 40x.

DISCUSSION:

In the modern era of breast cancer surgery, SNB has replaced ALND as the standard of care for axillary staging in early breast cancer and ALND should be abandoned if the SNs are free from metastasis[14-5]. The lymphatic mapping technique which is commonly used in developed countries is the application of radioisotope tracer and PBD as a combined or single technique[16-7]. The question on how to perform SNB is raised when there is no access to radioisotope or even PBD. Our described technique with MBD alone is addressed to solve the limitation of unavailability of these tracer agents.

Methylene blue dye is methylthionine hydrochloride and a dark green crystalline compound which turns dark blue in solution. In medicine, it is commonly utilized as a diagnostic tool such as

in fistula and as a treatment, for example in methemoglobinemia[18]. Although PBD is the preferred blue dye after the study in the feline model[7], MBD has been used more frequently in breast cancer SNB after the study by Simmons et al.[8]. We chose MBD because until now, it is the only available blue dye for lymphatic mapping in our country and our initial study revealed a favorable result with the identification rate of 95.8%[19].

There are several important points that can be highlighted regarding to this technique. The subareloar injection site is based on the data that support the use of superficial injection[20,21]. This anatomical background is supported by Sappey's theory of the breast lymphatic system. The study concluded that the lymphatic system of the breast will drain to the axilla through the subareolar plexus[22]. It was proven in the recent meta-analysis demonstrating that the concordance rate of SNs mapping between superficial and peritumoral injection was fairly high[23]. So, based on these findings and the simplicity of the procedure, we applied subareolar injection as the standard technique in our study. The intra-parenchymal injection will be used if there is an excisional biopsy scar at the upper outer quadrant or NAC. It is because of the possibility that the lymphatic tracts from subareolar plexus to axilla were disrupted by the previous biopsy. By performing this technique, we could identify the SNs in 91.7% of the cases with the negative predictive value of 90% in predicting axillary metastasis[13].

The next important issue to be discussed is the SNs anatomical landmark. Every surgeon would expect to see the blue nodes or lymphatic blue tracts immediately after opening the axillary space. However, if the blue signs could not be found, an anatomical landmark is needed to find the SNs without creating unnecessary dissections that may increase the morbidity of SNB. Our method in identifying SNs is based on Clough's study which revealed that the second intercostobrachial nerve and lateral thoracic vein are constant anatomic structures in axilla which can be used as guidance to find the SNs[24]. In our experience, the SNs are usually located around these structures. The anatomical location of the SNs has become an interesting field of study. Some anatomical models for SNs localization were created based on the methods of injection using radioisotopes[25,26]. However, when blue dye alone is the method of choice for SN mapping, we consider the intercostobrachial nerve as the most reliable landmark for SN identification. In our surgical method, removing non-blue suspicious nodes is recommended to avoid false negative results. Special attention must be paid when palpable nodes are identified in the lateral axillary region, because these nodes could be the draining arm nodes. It is suggested to dissect the nodes only when there is a high suspicion of metastasis, in order to reduce the risk of lymphedema[24].

There are some critical steps in this technique. (i) When performing SNB using MBD alone, it is important to avoid injecting excessive volumes. The high concentration of MBD would color additional SNs which may not be the true SNs and resecting all these nodes could increase the risk of lymphedema. (ii) Attention should be paid when performing the peritumoral injection technique. It is difficult to determine the resection margin if we inject a large volume of MBD, because the margin will be stained blue. (iii) If there are no blue nodes found, look at the anatomical landmark and find the nodes in that area. Look carefully at the lymphatic channel surrounding the nodes. If it is blue-stained, the lymph nodes can be considered as SNs. However,

if the nodes still cannot be found, then ALND must be performed.

We use frozen section analysis to assess SNs metastasis to avoid a second surgery if the SNs are positive. This procedure has high sensitivity and specificity to detect macrometastasis. On the other hand, the false negative rate varies (up to 24%) because of the presence of micrometastasis[27]. In the histopathology assessment, the SNs were sectioned no thicker than 2 mm, in order to ensure that all macrometastasis were identified[28]. Micrometastasis, isolated tumor cells, and low-grade lobular carcinoma are doubtful conditions that can be the cause of false negative results[29]. We perform immunohistochemistry for cytokeratin when we find such cases.

Overall, this technique has demonstrated some advantages. Firstly, MBD is cheaper than PBD and is easy to acquire than radioisotope traces. Secondly, the patients that have undergone SNB with this technique have very low complication rates, especially anaphylactic shock. If the procedure is performed carefully, the false negative result can be low, as we have mentioned previously[13].

Based on our experience, there are some limitations considering the MBD-alone method. The anatomical landmark identification may not be easy, especially in obese patients. Thus, it demands a large incision to explore the axillary space to find the anatomical landmark. A steep learning curve is needed to achieve favorable results. Another difficulty is in treating cases with previous excision biopsy. Sometimes, MBD cannot flow to SNs because the surrounding excisional biopsy scar has resulted in fibrosis. So, this procedure is recommended for patients without previous excisional surgery. Lastly, skin irritation has been reported as a complication caused by MBD injection[30,31]. Injecting MBD closer to the skin must be avoided to prevent skin necrosis. We inject MBD into deep subareolar space for reducing risk of such complications.

In conclusion, the MBD technique alone could be considered as an alternative technique for SN mapping in early breast cancer, especially in a situation when radiotracer agents or PBD are not available. A future study to evaluate its oncological safety would benefit the field of breast cancer SNB.

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DISCLOSURES:

The authors have nothing to disclose.

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Figure 1

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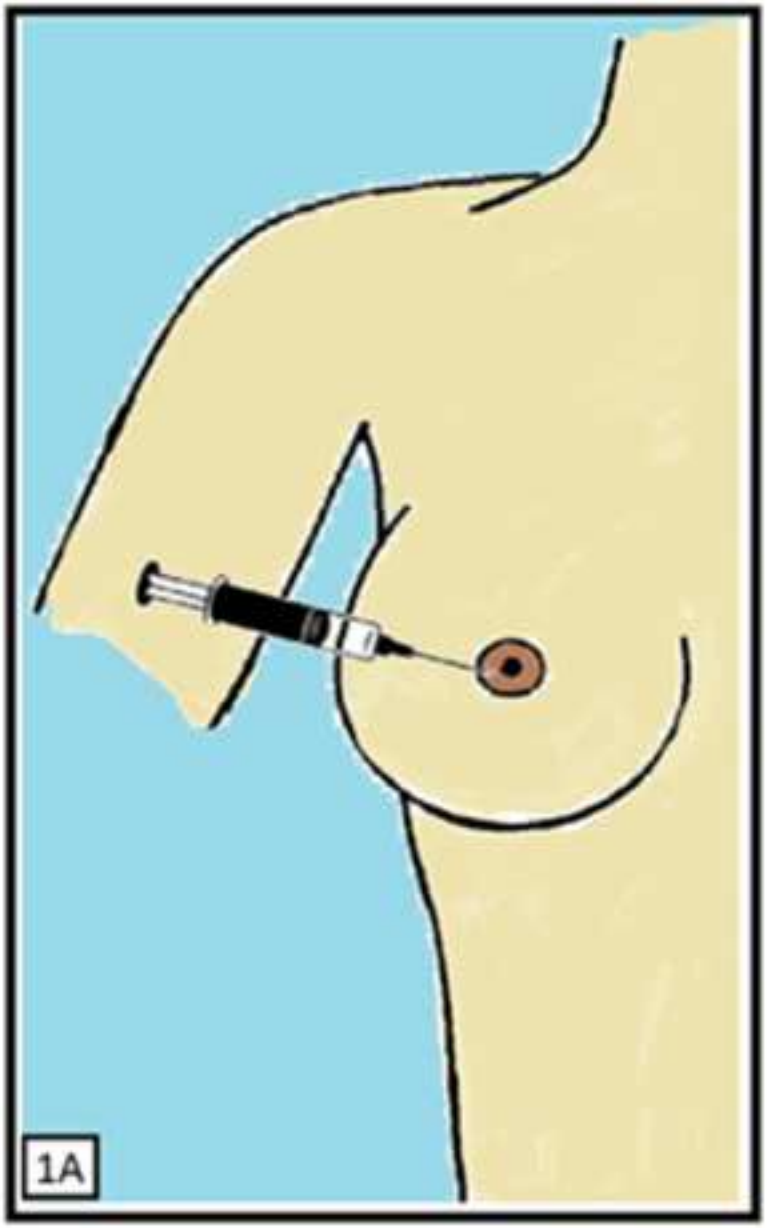

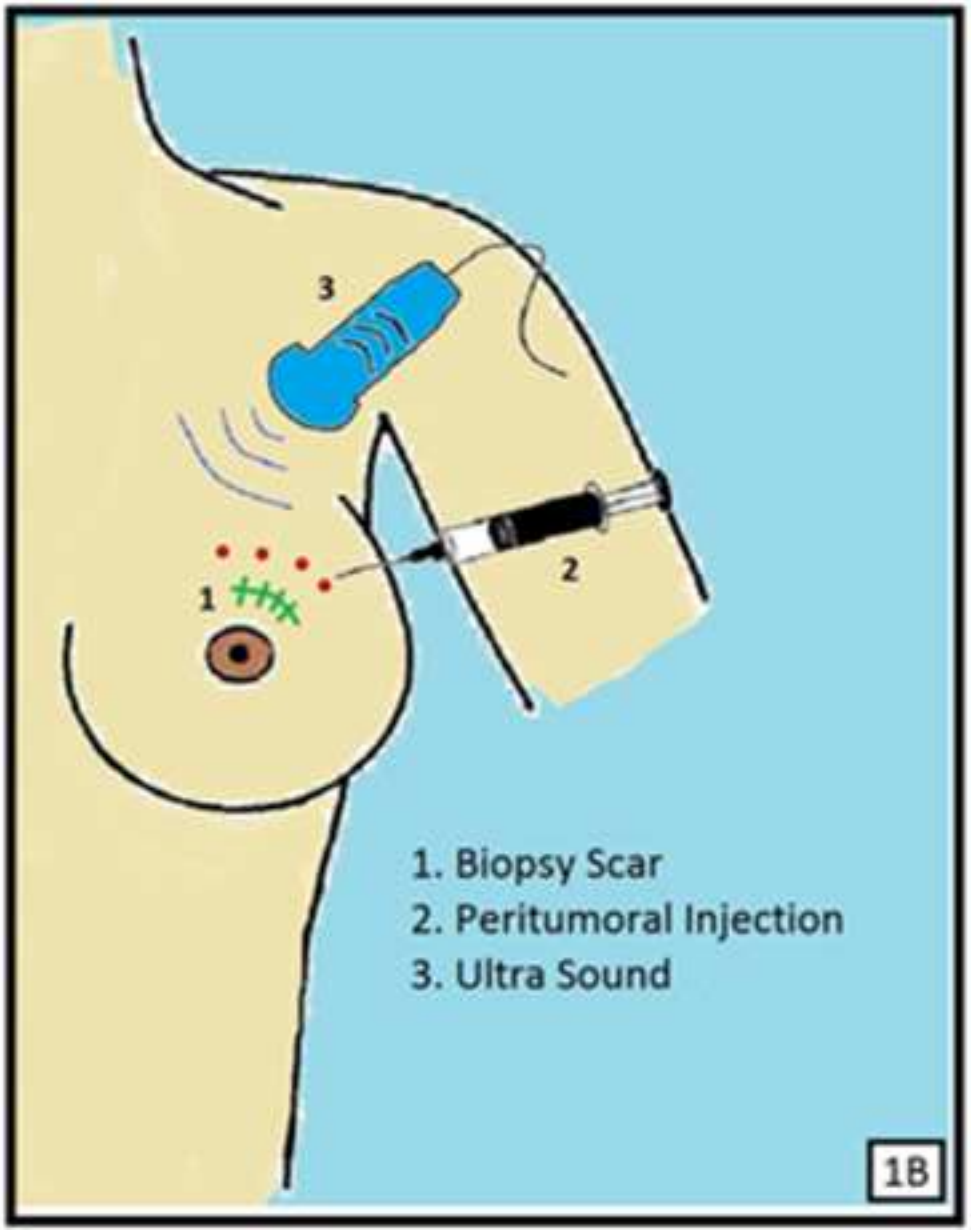

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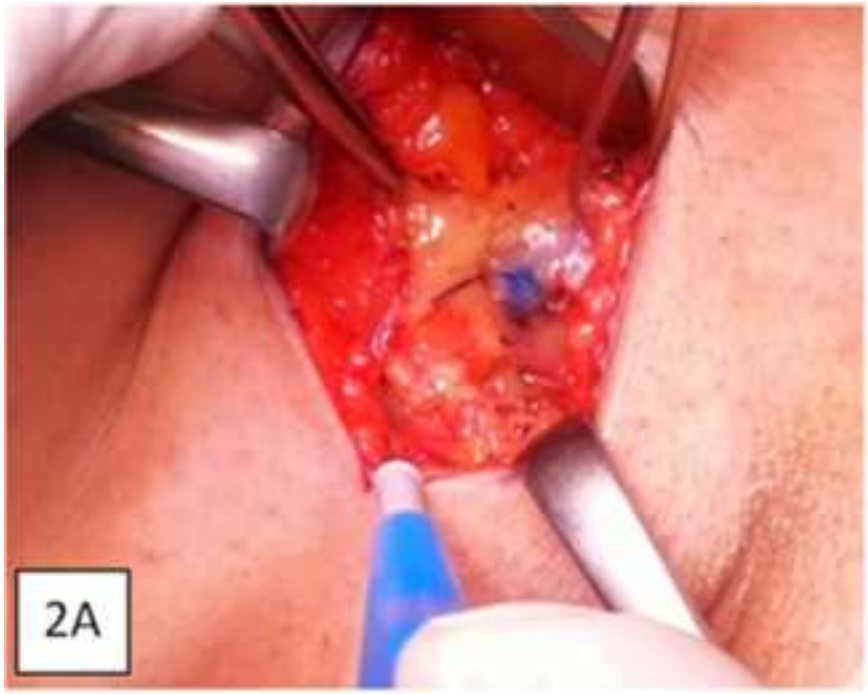

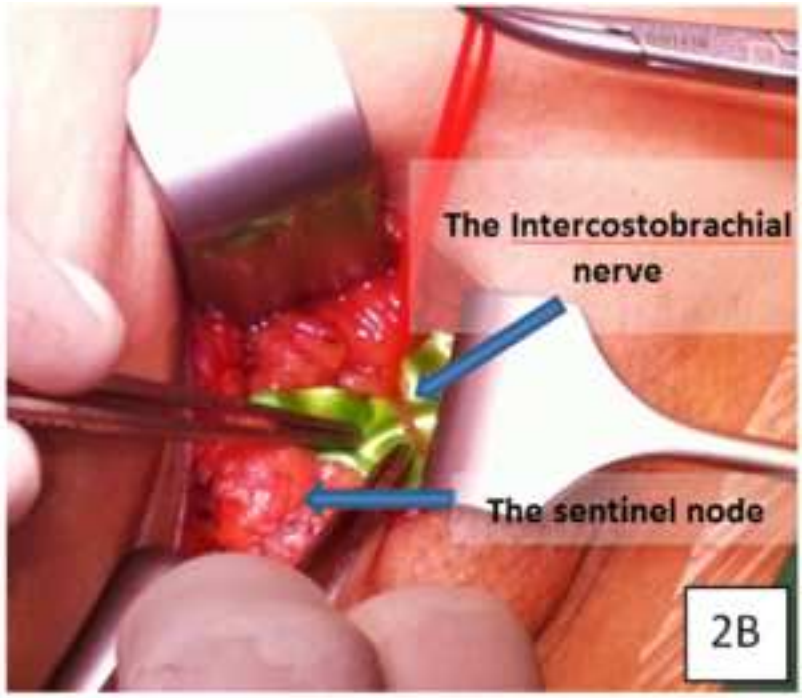

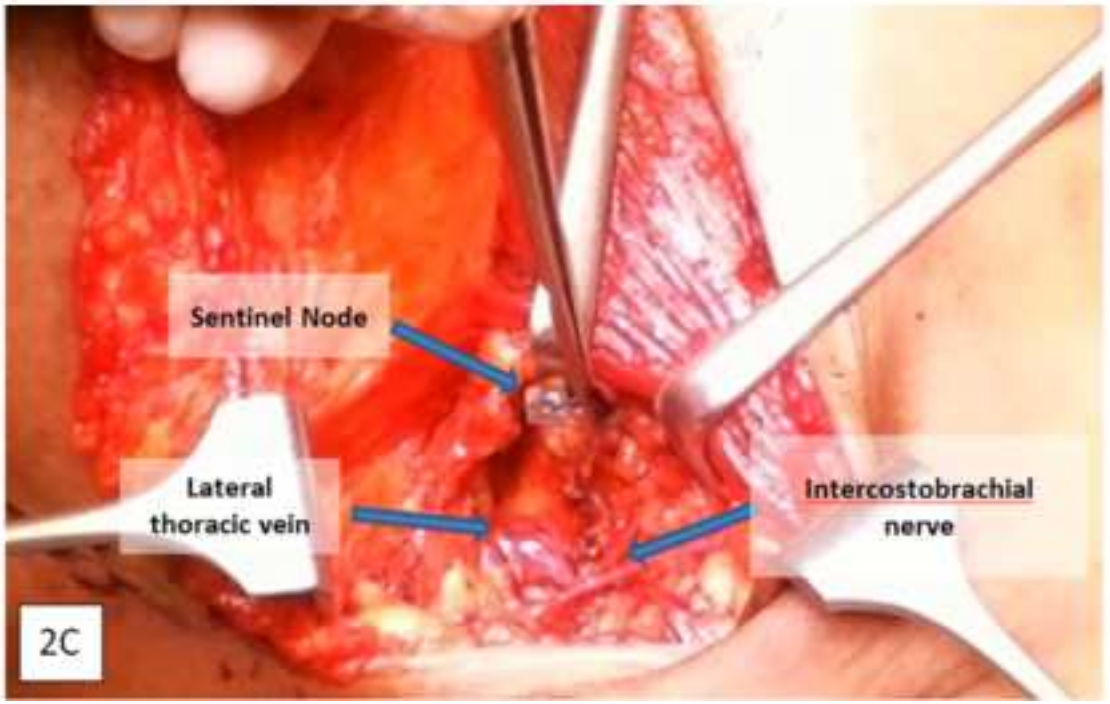

Figure 3

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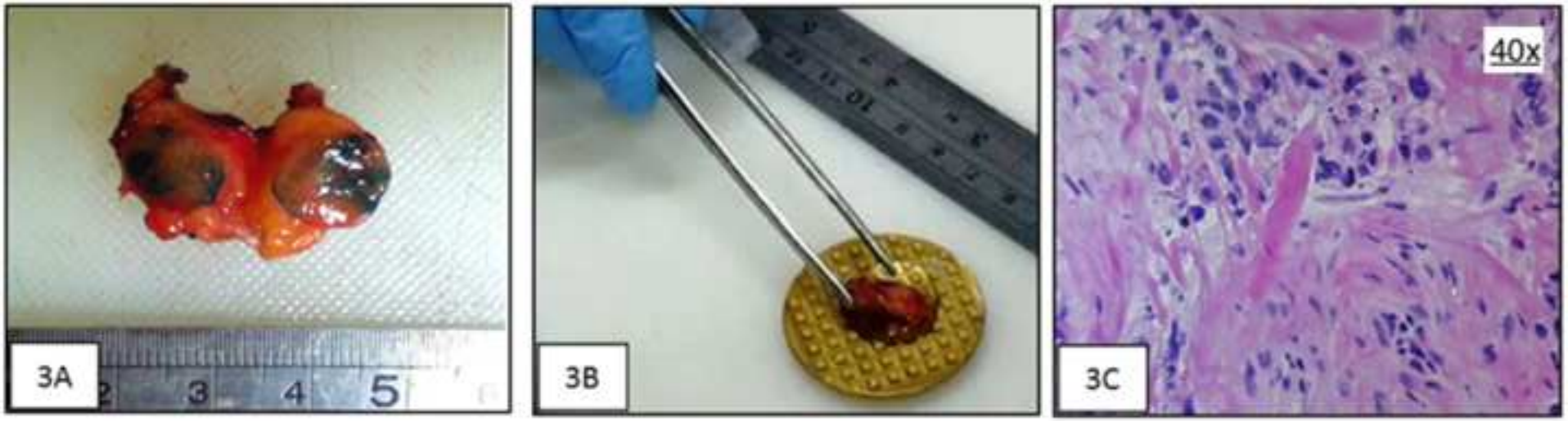

Figure 4

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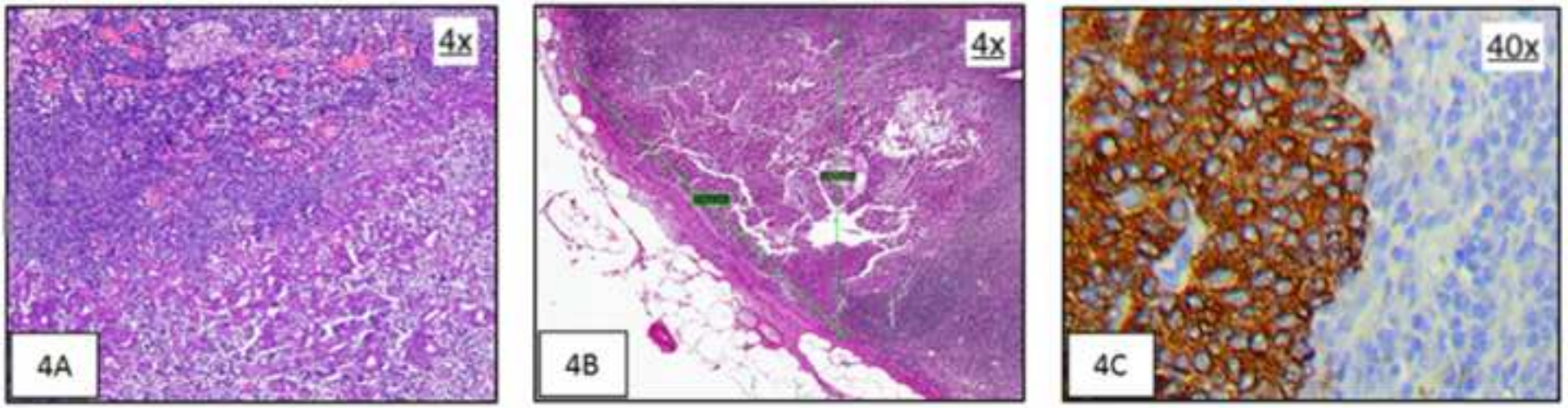

Table of Materials/Equipment

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Name of Material/ Equipment	Company
Metiblo 50mg/5ml	Laboratories Sterop NV
Disposable syringe with needle - 3mL (Luer Lock Tip)	Terumo Europe NV
ForceTriad energy platform	Medtronic
Shandon Cryomatrix embedding resin	Thermo (scientific)
Cryotome FSE	Thermo (scientific)
HistoStar Embedding Workstation	Thermo (scientific)
Finesse Me+	Thermo (scientific)
Gemini AS	Thermo (scientific)
Benchmark GX	Ventana Medical Systems
Benchmark XT	Ventana Medical Systems
Microscope	Olympus
Ultrasound	Phillips

Catalog Number	Comments/Description
BE475217	Methylene Blue Dye
dvr-3414	Syringe for Injection
ForceTriad	Surgical cautery
6769006	Frozen Section
77210153	
A81000001	Histopathological Examination
A77510272	
A81500002	
750-850	Immunohistochemistry
750-700	
Model BX53	
HD 7XE	

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Title of Article: The Application of 1 % Methylene Blue Dye as a Single Technique in Breast Cancer Sentinel Node Biopsy

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Signature: [signature] Date: August 25th 2017

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Title: The Aplication of 1% methylene blue dye as a single technique in breast cancer sentinel node biopsy
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We are grateful for your and the reviewer's comments, and the positive evaluation of our work. We have revised and modified the video according to the editor's critique. As a consequnce we provide new video about "The Aplication of 1% methylene blue dye as a single technique in breast cancer sentinel node biopsy" with title cards to indicate each section; title card with aproval from reseach ethics comitee; representative results section; subtitles for audio; retake the failed surgical clip; add concluding statement after result; and adding article ID number in the video file name.
These changes have improved the video manuscript considerably and we hope that it can be published without delay.

Sincerely,

Bayu Brahma, MD