THE EFFICACY OF RADIOCOLLOID IN SENTINEL LYMPH NODE BIOPSY IN EARLY-STAGE BREAST CANCER

By

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DECLARATION

I hereby declare that this research was sent to Universiti Sains Malaysia for the Degree of Master of Medicine (Nuclear Medicine). It has not been sent to other universities. With that, this research can be used for consultation and will be photocopied for reference.

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DISCLAIMER

I hereby certify that the work in this dissertation is my own and of my own composition.

I declare that I have no financial interest in the instruments or materials used in this study.

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First and foremost, اَلْحَمْدُ لِلّٰهِ praises and thanks to Allah سُبْحَانَهُ وَ تَعَالَى the most glorified and exalted be He, for His showers of blessings throughout my work to complete the research successfully. The wisdom He bestowed upon me, the strength, peace of mind and good health to finish this research during this trying time.

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Abbreviations

BD	Blue dye
BMI	Body mass index
BP	Blood pressure
CKD	Chronic kidney disease
ER	Estrogen receptor
HER2	Human epidermal growth factor receptor 2
HPJ	Hospital Putrajaya
IHC	Immunohistochemistry
IKN	Institut Kanser Negara
IV	Intravenous
MO	Medical officer
PR	Progesterone receptor
RC	Radiocolloid
SLN	Sentinel lymph node
SLNB	Sentinel lymph node biopsy

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ABSTRAK

Latar belakang: Kajian ini bertujuan untuk menilai prestasi agen pengesan radiokoloid, pewarna biru dan kaedah gabungan untuk mengesan nodus limfa sentinel melalui prosedur biopsi nodus limfa di kalangan pesakit kanser payudara peringkat awal.

Metodologi: Senarai maklumat 172 orang pesakit kanser payudara peringkat awal yang menjalani prosedur biopsi nodus limfa sentinel yang menggunakan radiokoloid dan pewarna biru berdasarkan protokol standard hospital telah dikaji. Bilangan "nodus panas" dan "nodus biru" dianalisis berdasarkan kekerapan edaran dalam setiap pesakit, nodus dan pesakit nodus positif yang dijumpai. Kadar pengesanan nodus limfa sentinel, kadar metastasis, dan kadar kegagalan daripada teknik gabungan, radiokoloid dan pewarna biru juga telah dikira. Hubungan perkaitan antara factor kliniko-patologi dan pengesanan nodus limfa sentinel juga dikaji.

Keputusan: Daripada 172 pesakit, bilangan "nodus panas" dan "nodus biru" yang dijumpai adalah masing-masing sebanyak 98.2% (169/172) dan 95.9% (165/172). Kadar keseluruhan pengesanan nodus sentinel limfa melalui teknik gabungan adalah 99.4% (171/172) manakala kadar metastasis ialah sebanyak 19.2% (33/172). Daripada 33 orang pesakit yang mengandungi nodus limfa positif tumor, kaedah gabungan dan juga radiokoloid telah gagal mengesan satu kes nodus positif tumor manakala pewarna biru pula telah gagal mengesan sebanyak 4 kes, memberikan kadar kegagalan masing-masing sebanyak 3.0% (1/3), 3.0% (1/33) dan 12.1% (4/33). Hubungan perkaitan antara

faktor-faktor kliniko-patologikal dengan kadar pengesanan nodus limfa sentinel adalah tidak signifikan.

Kesimpulan: Kajian ini telah menunjukkan kadar pengesanan nodus limfa sentinel yang paling tinggi melalui teknik gabungan jika dibandingkan dengan teknik radiokoloid atau pewarna biru sahaja. Lebih banyak nodus limfa sentinel dikesan oleh radiokoloid berbanding pewarna biru. Oleh itu, menggunakan teknik radiokoloid bersama-sama pewarna biru akan meningkatkan kadar pengesana nodus sentinel limfa serta mengurangkan kadar kegagalan dalam mengenal pasti pesakit nodus limfa yang positif tumor.

ABSTRACT

Purpose: This study aims to evaluate the performance of radiocolloid, blue dye and combined method in sentinel lymph node biopsy in early breast carcinoma.

Methods: One hundred seventy-two clinically node-negative, early-staged breast cancer patients who underwent SLNB with combined radiocolloid and blue dye were recruited. The numbers of hot node and blue node were analysed in all patients, all excised nodes and metastatic nodes patients, giving rise to the detection rate, metastatic rate and failure rate of combined method, radiocolloid and blue dye method. The association between the clinicopathological factors and the SLN detection was also analysed.

Results: Out of 172 patients, the presence of a hot node and a blue node were 98.2% (169/172) and 95.9% (165/172) respectively. The overall SLN detection rate through combined method was 99.4% (171/172) and metastatic rate was 19.2% (33/172). Among the 33 metastatic-node patients, the combined method, radiocolloid and blue dye method failed to identify 1, 1 and 4 patients respectively. Therefore, the failure rate of the combined method, radiocolloid and blue dye method were 3.0% (1/33), 3.0% (1/33) and 12.1% (4/33), respectively. No significant finding was observed to associate clinicopathological factors and the SLN detection.

Conclusion: This study showed the combined method of SLNB yielded the highest detection rate when compared to radiocolloid-alone and blue dye-alone method. More SLN was detected by radiocolloid method than blue dye method. Therefore, incorporating radiocolloid method in the blue dye method will improve SLN

identification rate and reduce the failure rate of metastatic detection.

CHAPTER 1

INTRODUCTION

1.1 Introduction

In breast cancer, the presence and extension of the axillary lymph nodes involvement signify a vital prognostic factor and carry a significant impact on therapeutic decisions (Gherghe *et al.*, 2015). Early-stage breast cancer patients often present with non-palpable axillary nodes. Early stage breast cancer is defined as disease confined to the breast with or without regional lymph node involvement, and the absence of distant metastatic disease (WHO, 2014). Hence, the presence of nodal involvement was difficult to detect the presence of lymph nodes metastases clinically. In the past, it was initially determined by axillary lymph node dissection (ALND), but the procedure has been replaced by sentinel lymph node biopsy (SLNB) since randomised trial showed that the minimally invasive procedure is able to reflect the overall lymph node status (Gherghe *et al.*, 2015). This procedure has become the standard method for axillary lymph node staging in patients with early-stage breast cancer with clinically negative axillary lymph nodes (Lyman GH *et al.*, 2015).

According to Malaysian National Cancer Registry Report (2007 – 2011), the incidence of breast cancer accounts for 32.1% of all cancer among females in Malaysia. The incidence differs among different races. The cumulative risk was highest among Chinese and lowest among Malay. The lifetime risk was 1 in 30, while for Chinese was 1 in 22, Indian 1 in 24 and the lowest is among Malay which is 1 in 35.

In identifying the SLN, intradermal or subdermal injection of radiocolloid tracer and blue dye are commonly used. The SLN was first described by Morton *et al.* in 1992 as any lymph nodes receiving direct lymphatic drainage from the primary tumour, and therefore, it was the first node to be a tumour metastasis (Morton

et al., 1992). The idea behind SLNB was that the sentinel node status predicted the histological status of the regional lymph nodes. If the sentinel node did not contain tumour metastasis, the draining nodal basin was highly unlikely to harbour metastases, and complete nodal dissection is not required (Somasundaram *et al.*, 2007).

The practice of SLNB was first reported in 1993 when they successfully detected sentinel lymph nodes by radioisotope (Krag *et al.*, 1993). It was a year after that, in 1994 when Guiliano *et al.* had successfully developed a blue dye method to localize SLN (Guiliano *et al.*, 1994). In 1996 (Albertini *et al.*, 1996), there was a practice of using both radiocolloid and blue dye to identify SLN and it was proven in many studies that the detection rate and the false-negative rate were lower by using two mapping techniques together when compared to single tracer agent.

In radiolocalisation of the SLN, the radiocolloids are efficiently trapped as the size of the particles ranges from 40 – 80 nm, which is small enough to migrate yet large enough to be trapped (Somasundaram *et al.*, 2007). Technetium^{99m}-nanocolliod (^{99m}Tc-nanocolloid) is the radiopharmaceutical used for pre-operative lymphatic imaging. It emits 114keV gamma rays with half-life of 6 hour, enough for the localisation intra-operatively by gamma probe on the same day or until 24 hours after the injection. However, radiocolloid method requires high set up and maintenance cost and the strict legislation of handling the radioactive materials. This makes radiocolloid method less favourable compared to blue dye method.

As for blue dye method, the injection is given intra-operatively just before the incision of the skin. It enables the surgeon to identify blue-stained lymph node and

lymphatic tracts draining from the tumour. Compared to radiocolloid method, it is relatively cheaper and more widely available, especially in hospitals whereby the access to nuclear medicine facilities is unavailable. However, using blue dye has its own set of risks, ranging from trivial skin rashes (Salhab *et al.*, 2005) to life-threatening anaphylaxis (Thierrin *et al.*, 2008, Kaufman *et al.*, 2008, Wahid *et al.*, 2014). There were also reports on patients who developed peripheral cyanosis that resulted in false oxygen desaturation on the pulse oximetry, which led to false alarm to the anaesthetists and surgeon (Lai *et al.*, 2011). Nonetheless, the case of anaphylactic reaction from the blue dye method still very rarely reported (Bezu C *et al.*, 2011), and it is still considered safe, simple and cost-effective, especially in centres with resource constraints (Devarokonda *et al.*, 2021).

The efficacy in detecting SLN between radiocolloid method and blue dye method among Malaysian population has not been evaluated. National Cancer Institute, Putrajaya, Malaysia, has utilised the combined technique of radiocolloid and blue dye as lymphatic mapping agents for SLN identification in early-stage breast cancer patients since 2013. Hence, we evaluate the efficacy of radiocolloid, blue dye and combined technique in SLN identification in early-stage breast cancer patients.

1.2 Problem statement

The use of radiocolloid has long been established as a great mapping agent for SLNB, but it is still unpopular in Malaysia. The use of blue dye can cause a spectrum of side effects ranging from trivial skin rash to life-threatening anaphylaxis reaction that may lead to significant morbidity or mortality to the patient. The establishment and steady development of nuclear medicine facilities in Malaysia provides an alternative to detect

SLN using radiocolloid. The efficacy of SLN detection using combined methods compared to radiocolloid and blue dye alone for the Malaysian population needs proper evaluation.

1.3 Justification / benefits

In National Cancer Institute, Putrajaya, Malaysia, the combined method of both radiocolloid and blue dye was employed for SLNB. The availability of the procedure enabled the utilization of data for analysis of the detection rate of both radiocolloid and blue dye in detecting SLN for breast cancer. The study results would provide possibly valuable, if not at least some information in approaching patients of early-stage breast cancer in the Malaysian population, especially in centres/institutes with nuclear medicine facilities. The practice of nuclear medicine in oncological field in Malaysia, particularly in breast cancer will be more widely recognized and this will benefit the patients.

CHAPTER 2

OBJECTIVES

2.1 General Objective

This study aims to evaluate the performance of combined method, radiocolloid and blue dye methods in SLNB in early-staged breast carcinoma.

2.2 Specific Objective

i. To analyse the SLN detection rate of combined method, radiocolloid and blue dye methods.
ii. To calculate the number of hot-blue node, hot only node and blue only node in all patients, all excised nodes and metastatic nodes.
iii. To determine the failure rate of combined method, radiocolloid and blue dye methods.
iv. To assess the association between clinicopathological factors and SLN detection.

2.3 Null Hypothesis

Combined method exhibits no difference in performance compared to radiocolloid and blue dye methods in SLNB.

2.4 Alternative Hypothesis

Combined method is more superior compared to radiocolloid and blue dye methods in SLNB.

CHAPTER 3

METHODOLOGY

3.1 Research location

This study was conducted at the Department of Nuclear Medicine, National Cancer Institute and Department of Surgery, Hospital Putrajaya.

3.2 Study Design

This study was an analysis of a prospectively maintained database from a web-based hospital information system (FiSiCien) in National Cancer Institute, over an 18-month period.

3.3 Sampling

3.3.1 Target Population

The target population included patients with early-stage breast cancer (T1-T2), with clinically no palpable lymph nodes.

3.3.2 Population Sample

Early-stage breast cancer (T1-T2) with clinically no palpable node patients that were referred for radiocolloid injection and lymphoscintigraphy.

3.3.3 Sample Frame

This study was conducted from June 2019 until November 2020.

3.3.4 Sampling Method

This study involved convenience sampling from patients who came to Department of Nuclear Medicine for radiocolloid injection and lymphoscintigraphy.

3.3.4 Sample Size Determination

Sample size calculation was 158 using 2-proportion formulae. Prior data indicated that the proportion rate of detection of blue dye was 0.029, and the estimated proportion of radiocolloid was 0.1. Thus, a minimum sample size of 158 samples is needed to be able to reject the null hypothesis with probability (power) 0.8. The total sample size employed in this study was 173.

3.4 Inclusion and Exclusion Criteria

3.4.1 Inclusion Criteria

i. Breast cancer patient which was proven by pre-operative biopsy

ii. T1 – T2 tumour (<5cm) that was confirmed after definitive histopathology

iii. Clinically non-palpable axillary lymph nodes

iv. Planned for definitive surgery/axillary node clearance

v. Age more than 18 years old

3.4.2 Exclusion Criteria

i. Pregnancy

ii. More than T2 tumour breast cancer

iii. Metastatic breast cancer

iv. Previous breast tissue removal or axillary drainage done

v. Neo-adjuvant chemotherapy or radiotherapy done

vi. Male patient

3.5 Flow chart of study protocol

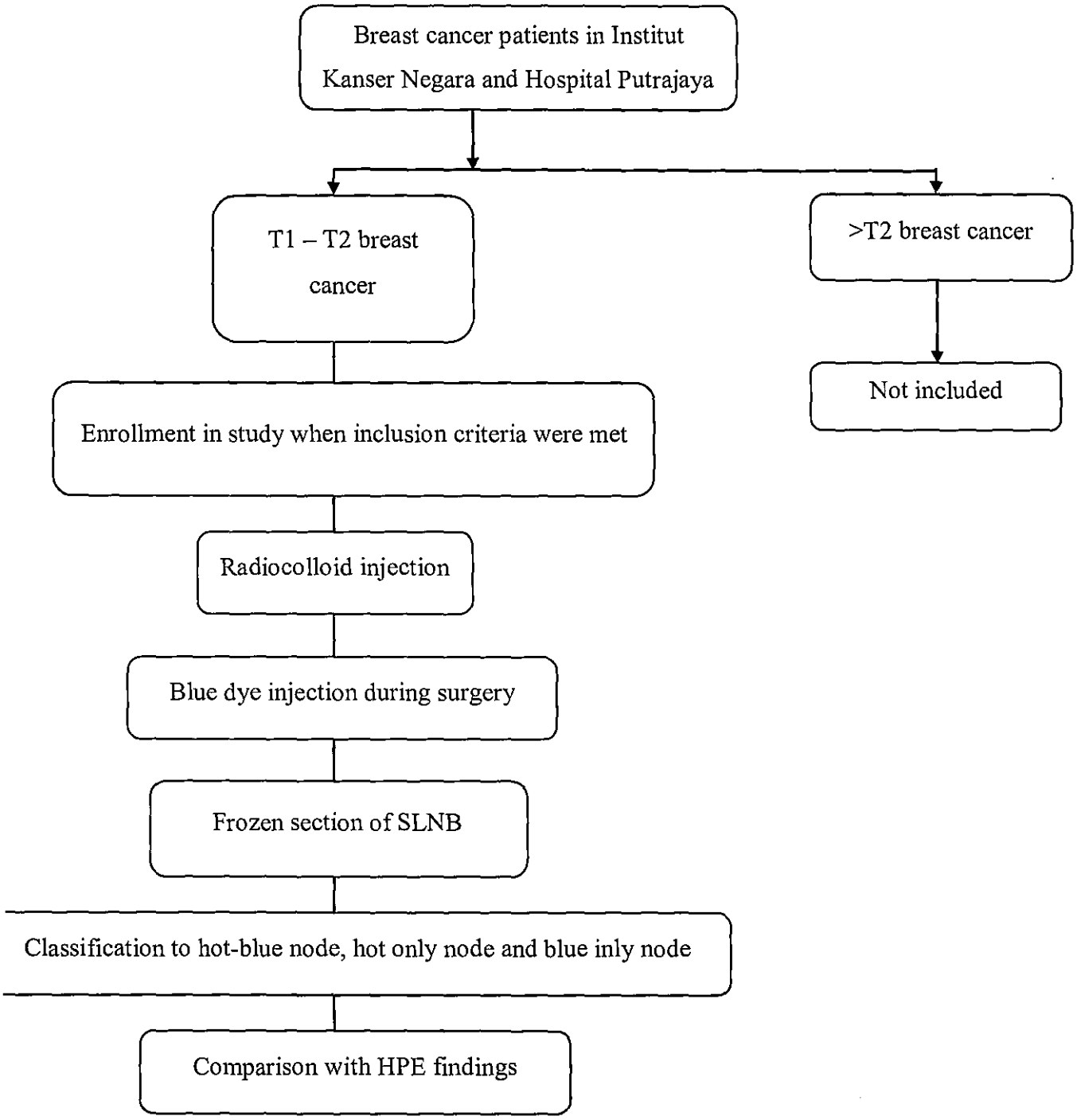

Figure 3.1 Flow chart of study protocol

3.6 SLNB Procedure

3.6.1 Patient referral and recruitment

Patients with early-stage breast cancer who agreed for sentinel lymph node biopsy from the Surgical Department of IKN and Hospital Putrajaya were referred to Nuclear Medicine Department, IKN for radiocolloid sentinel lymph node mapping. Medical officer (MO) at Nuclear Medicine Department IKN clerked the patient and following information were obtained and evaluated:

i. Current symptoms and physical findings.

ii. Previous relevant laboratory results e.g., HPE report, tumour receptor markers.

iii. History of therapy which might affect sentinel node mapping results such as recent procedure, surgery, chemotherapy, or radiotherapy done.

Indication, procedure, complications, side effects, and patient radiation risk to the patient were explained to patient. All patients who fulfilled the inclusion and exclusion criteria received an explanation (from MO in charge of lymphoscintigraphy scan during the day) regarding the study objectives and emphasized to them that only their data will be used for research purposes. The patients were provided with Patient Information Sheet (PIS) as reference (see **Appendix 2**). Patients who agreed that their data can be used in the study signed the informed consent form.

3.6.2 Radiocolloid method

Injection of radiopharmaceutical

Injection of radiopharmaceutical (2 mCi of Tc-99m Nanocolloid (Nanocis) in 0.2mls aliquot) done by MO in charge under the supervision of Nuclear Medicine Physician in charge. One peri-areolar subcutaneous injection (preferably on the tumour contralateral quadrant of the breast) performed under aseptic techniques. After the injection, finger

massage is done to promote lymphatic drainage for minimum of 5 minutes.

Lymphoscintigraphy

The procedure of lymphoscintigraphy was performed according to Standard Operating Procedure (SOP) of Lymphoscintigraphy by Nuclear Medicine Department, National Cancer Institute (see **Appendix 6**). The procedure was carried out in Gamma Camera Room, Nuclear Medicine Department, usually before 4:00 PM, one day prior to the surgery. Scans were reviewed by MO. If necessary, consultation with specialist was done. For marking the sentinel node on patient's axillary region, the physicist assisted MO/Specialist in using gamma probe to count for the injection site and sentinel node area. Once the sentinel node was identified, it was marked as X and the area secured with transparent plaster. If no sentinel lymph node detected from lymphoscintigraphy, second injection with the same dose of radiocolloid followed by lymphoscintigraphy. Approved reports and processed images were given to nurses for dispatch to the primary team, usually on the same day.

Day 1: Radiocolloid injection & Lymphoscintigraphy

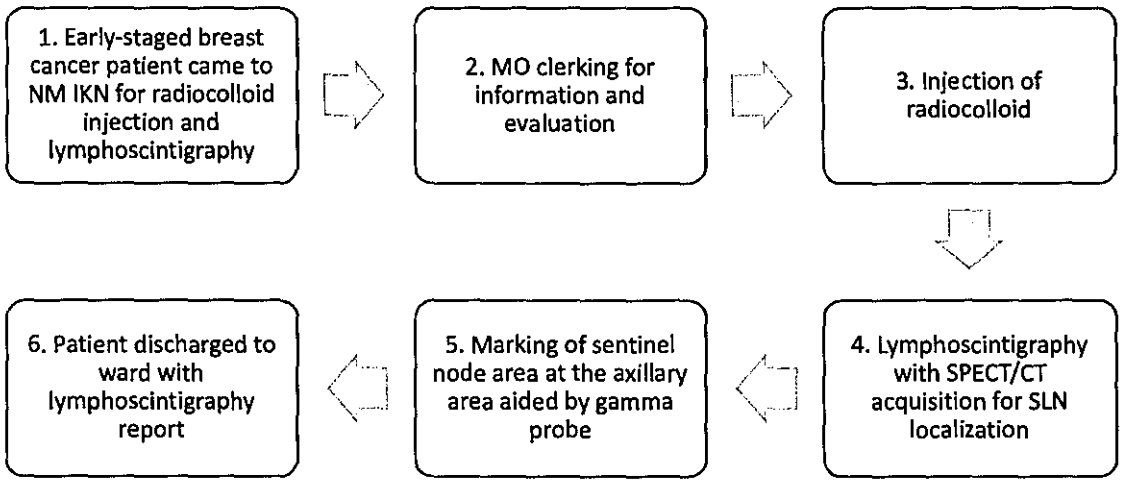

Figure 3.2 *Flow chart of Day 1 of procedure*

3.6.3 Blue dye method

Injection of blue dye

This procedure was done by the Breast & Endocrine surgeon on the operating table during operation day which was usually around 16 – 24 hours after lymphoscintigraphy. About 3 – 5mls of blue dye injected in the peri-areolar area region after the induction of general anaesthesia and the breast was massaged to aid migration of the dye through the lymphatic channels.

3.6.4 Localisation and biopsy of sentinel lymph node

The patient was positioned with the ipsilateral arm abducted. The axillary area was systematically scanned with a gamma probe. The site of highest radioactivity was marked, and a small incision was made. The probe provided audio feedback, which enabled the surgeon to localize the SLN using the principle of 'line of sight'. The surgeon would find node(s) that were:

i. Hot nodes with 5–10 times the background radioactivity or more than 10% of the activity of the hottest node (Hot-only)
ii. Blue nodes with blue afferent lymphatic tracts (Blue-only)
iii. Hot and blue nodes (Hot-Blue)

The sentinel node is identified as any hot, blue or palpable node in the axillary tissue or a node in which a blue lymphatic vessel is seen to enter. After harvesting the sentinel nodes, the probe was used to identify any other hot nodes by checking the residual counts in the axilla, or blue tissue.

3.6.5 Labelling the sentinel nodes

The first node found would be labelled as A, the next node labelled as B and so on together with the characteristic of Hot or Blue. Examples:

- A: Hot-Blue (18090)*
- B: Hot-only (6709)*
- C: Blue-only
- D: Not hot or blue

*The number indicated the radioactivity of the nodes measured by gamma probe. The unit used is cps (count per second).

3.6.6 Histopathology staining

Harvested lymph nodes were sent to Pathology Department of Hospital Putrajaya. The biopsied sentinel lymph nodes were serially sectioned and undergo routine Haematoxylin and Eosin (H&E) staining as per protocol. The status of SLN informed to surgeon by pathologist either positive or negative of tumour deposits. Axillary clearance will be performed if SLN was reported positive of metastasis, and benign axillae were sparred.

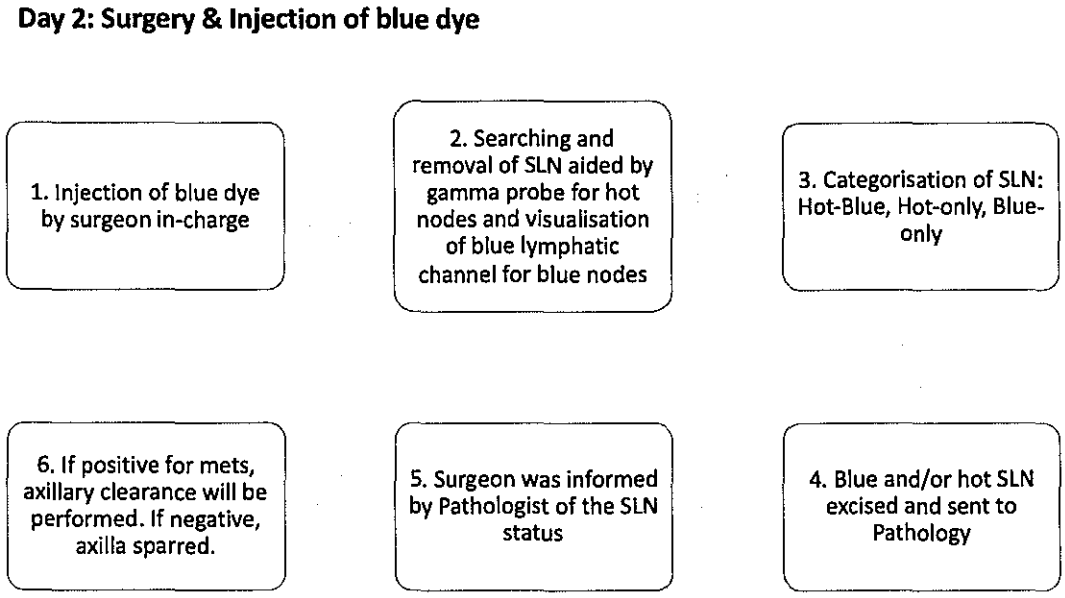

Figure 3.3 *Flow chart of Day 2 of procedure*

3.7 Collection of data postoperatively

A prospectively maintained database (FiSiCien) consisting of data of breast cancer patients who undergo sentinel lymph node biopsy by combined method were analysed. Data collected include age, histology, tumour types, tumour size, tumour grade, presence of oestrogen, progesterone and HER2 receptors.

3.8 Statistical Analysis

The number of a hot or blue node in all patients, all surgically removed nodes and metastatic node were calculated. The detection rate of SLN and failure rate of radiocolloid, blue dye and combined method were also calculated. Detection rate was calculated by the number of patients whose SLN detected divided by total patients. Failure rate of each tracer agent and the combined method was calculated as the proportion of metastatic node patients in which the tracers failed to localize in a sentinel node. As for the metastatic nodes, the presence or absence of hot-blue node, hot-only node, and blue-node was analysed to investigate the association with metastatic nodes frequency.

The association between clinicopathological factors and the presence of a hot-blue node, hot only node and blue only node was analysed using the Kruskal Wallis test or Pearson Chi-square test. The clinicopathological factors analysed in this study were the age, histology, tumour size, tumour grade, presence of estrogen receptor, progesterone receptor and HER2 receptor. Finally, simple linear regression analysis was used to determine any correlation between detection of nodes and clinicopathological factors (pathology, tumour size, tumour grade, the presence of estrogenic receptor, progesterone receptor and HER2 receptors).

Statistical analyses were performed using the IBM SPSS software (Version 26). The average values are presented as mean ± SD. The p value of less than 0.05 is considered as statistically significant.

3.9 Confidentiality and security of source documents and study

No personal identifiers or patient data were obtained in this study. All patients were named by an identity code to maintain confidentiality of the data. The data that has been collected and entered the software (SF-12, SPSS) was stored in an external hard drive with a password. This password was only known to researcher to preserve confidentiality. This external hard drive will be kept in a locked drawer in the researcher's room. The name and contact number of the respondents (patients and their caregivers) were not documented in the SPSS. The respondents will be identified by number coding such as respondent no. 1. The storage of the data is for a duration of five years. The archival of the data would be maintained by researcher without any personal information.

3.10 Ethical issue

This study was approved by the following authorities before the initiation of the study:

1. Medical Research & Ethics Committee, Ministry of Health Malaysia – NMRR-19-594-47141 **(Appendix 3)**
2. Jawatankuasa Etika & Penyelidikan Manusia (JEPeM) of Universiti Sains Malaysia – USM/JEPeM/19030194 **(Appendix 4)**.

CHAPTER 4

MANUSCRIPT

4.0 Manuscript – Title page

The Efficacy of Radiocolloid in Sentinel Lymph Node Biopsy in Early-stage Breast Cancer.

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Abstract

Purpose: This study aims to evaluate the performance of radiocolloid, blue dye and combined method in sentinel lymph node biopsy in early breast carcinoma.

Methods: One hundred seventy-two clinically node-negative, early-staged breast cancer patients who underwent SLNB with combined radiocolloid and blue dye were recruited. The numbers of a hot node and a blue node were analysed in all patients, all excised nodes and metastatic node patients, giving rise to the detection rate, metastatic rate and failure rate of combined method, radiocolloid and blue dye method. The association between the clinicopathological factors and the SLN detection was also analysed.

Results: Out of 172 patients, the presence of a hot node and a blue node were 98.2% (169/172) and 95.9% (165/172) respectively. The overall SLN detection rate through combined method was 99.4% (171/172) and metastatic rate was 19.2% (33/172). Among the 33 metastatic-node patients, the combined method, radiocolloid and blue dye method failed to identify 1, 1 and 4 cases respectively. Therefore, the failure rate of the combined method, radiocolloid and blue dye method were 3.0% (1/33), 3.0% (1/33) and 12.1% (4/33), respectively. No significant finding was observed to associate

clinicopathological factors and the SLN detection. **Conclusion:** This study showed the combined method of SLNB yielded the highest detection rate when compared to radiocolloid-alone and blue dye-alone method. More SLN was detected by radiocolloid method than blue dye method. Therefore, incorporating radiocolloid method in the blue dye method will improve SLN identification rate and reduce the failure rate of metastatic detection.

Keywords: Early-stage Breast Cancer; Sentinel Lymph Node Biopsy; Blue Dye; Radiocolloid

Introduction

The status of the axillary lymph node is crucial in early-stage breast cancer as it determines the staging and treatment strategies. Traditionally, axillary lymph node dissection (ALND) was the standard procedure to evaluate the axillary lymph node status. However, due to a number of significant complications associated with this procedure, it has been replaced by the less-invasive alternative, sentinel lymph node biopsy (SLNB). The concept of sentinel lymph node (SLN) is on the basis of a biological assumption that the primary tumour drains into the afferent lymphatic pathway to a principal "sentinel" node in the regional lymphatic basin that most likely to harbour metastasis if they are present [1]. This concept facilitates the surgeons to stage the axilla from the sentinel node information and avoid the axillary dissection if the sentinel is negative of metastasis.

Despite the widespread practice of the SLNB for the axillary staging of breast cancer, the gold-standard method of performing SLNB is not yet achieved and still

evolving. Currently, the most preferred method is a dual tracer approach using blue dye and radiocolloid (combined method) [2]. According to ACSO guideline, the combined method or dual localization of sentinel lymph nodes (SLNs) with these two reagents is considered the most optimal technique as it has the highest identification rate compared to the single reagent technique [3]. The first study using dual-reagents was in 1996, where they found a high of SLN identification rate of 92% [4]. Multiple studies had been subsequently conducted and reported over the years confirming the claim, making the popularity of this combined method on the rise.

In contrast with the recommendation, a study by Marrow *et. al* in the earlier history of SLNB suggested no advantage for the use combined technique as compared to the usage of blue dye alone [5]. On the other hand, a study by Schmidt *et. al* of 391 SLN biopsy procedures in 2011 suggested the additional application of blue dye is not necessary when the radiocolloid is able to identify the node [6]. These debates regarding which method is superior to the other are still ongoing even to these days [7].

In Malaysia, the radiocolloid method is expensive to be widely practiced on top of the risks of handling radiation and strict legislation regarding radioactive materials. The blue dye method is much cheaper and readily available, but it has associated with serious complications like life-threatening severe anaphylactic reactions [8] and skin necrosis when dye was injected in a subdermal fashion [9]. However, as there are scarcely reported incidents of these severe side effects, blue dye is still widely used due to the convenience of its accessibility compared to radiocolloid [10]. However, the high identification rate of combination radiocolloid and blue dye needs to be validated in the Malaysian population to justify the high cost for technical complexity in terms of radiation handling and facility maintenance. In this study, we evaluated the performance

of radiocolloid, blue dye and combined method of SLNB procedure in early-stage breast cancer patients.

Methods

Patient selection

Clinical and histological data from a prospectively maintained database were analysed on 173 consecutive breast cancer patients who underwent SLNB between June 2018 and November 2020 at the National Cancer Institute/Hospital Putrajaya, Putrajaya, Malaysia. Eligible patients were above 18 years old at registration and diagnosed with T1 to T2 primary breast cancer with clinically non-palpable node. T1 to T2 is defined as tumour not more than 50mm. Histological confirmation of breast carcinoma was done pre-operatively in all cases. This study protocol was approved by MREC (Medical Research and Ethics Committee; NMRR-19-594-47141) and JEPeM (The Human Research Ethics Committee of USM; USM/JEPeM/19030194) as well as the hospital research committee. Patients who already underwent treatment such as axillary drainage procedure or neo-chemotherapy/radiotherapy to axillary area and patients who were pregnant were excluded from this study.

SLN Detection

Radiocolloid mapping was performed on the day prior to the operation. ^{99m}Tc-nanocolloid (Nanocoll, GE Healthcare Ltd. UK) in 0.2mls aliquot injected at a dose of 37 – 74MBq (2 - 3mCi). The approach of subareolar intradermal injection was used. The breast was massaged for 5 minutes following the injection. Localization of SLN was performed on a dedicated SPECT/CT (Philips BrightView XCT, Philips Healthcare, Netherlands) (Figure 1, Figure 2). The hot nodes were marked on the skin

with the aid of a handheld gamma-ray detecting probe (C-Trak® Surgical Guidance System, Care Wise, USA). The same gamma probe was used intraoperatively to locate the hot nodes.

Intra-operatively, about 2 ml of blue dye was injected 5 - 10 min prior to incision, mainly by subareolar injection at four quadrants. The breast was massaged for 5 minutes prior to incision.

Surgical procedure

During axillary exploration, the SLNs were identified by visual inspection of blue colouration and detection of radioactivity using a handheld gamma probe. Apart from that, any suspicious, palpable nodes which were neither hot nor blue, were surgically removed as recommended by international guidelines [11, 12]. Excision of all hot and blue nodes is continued until the background count of the axilla was less than 10% of the hottest excised lymph node. The excised lymph nodes were labelled separately according to the order of removal (A - first node removed, B - second node and so on) and further classified as containing both radiocolloid and blue dye (Hot-Blue node), radiocolloid alone (Hot only node), blue dye alone (Blue only node) and finally neither radiocolloid nor blue dye (No Hot-Blue node). Successful SLNB was defined as true sentinel lymph node was yield to be either blue and/or hot. Any immediate adverse events and reactions after injection of blue dye during surgery were also recorded.

Histopathological examination of sentinel lymph nodes

All SLNs were sent to the frozen section for pathological evaluation intraoperatively which involved haematoxylin and eosin (H&E) staining at serial sectioning 200 –

300μm of cutting interval, in accordance with national protocols.

Patients with positive metastatic SLNs underwent axillary clearance. The definitive pathological reports had been written following the American Joint Committee on Cancer (AJCC) tumor/node/metastasis (TNM) classification. A tumour-positive or metastatic node was defined as micrometastases (>0.2 and ≤ 2.0 mm) or macrometastases (>2.0 mm).

Data analysis

The number of a hot or blue node in all patients, all surgically removed nodes and metastatic-node patients were calculated. The detection rate of SLN and failure rate of combined method, radiocolloid and blue dye methods were also calculated. Detection rate was calculated by the number of patients whose SLN detected divided by total patients. Failure rate of each tracer agent and the combined method was calculated as the proportion of metastatic node patients in which the tracers failed to localize in a sentinel node. As for the metastatic nodes, the presence or absence of hot-blue node, hot-only node, and blue-node was analysed to investigate the association with metastatic nodes frequency.

The association between clinicopathological factors and the presence of a hot-blue node, hot only node and blue only node was analysed using the Kruskal Wallis test or Pearson Chi-square test. The clinicopathological factors analysed in this study were the age, histology, tumour size, tumour grade, presence of estrogen receptor, progesterone receptor and HER2 receptor. Finally, simple linear regression analysis was used to determine any correlation between detection of nodes and clinicopathological factors