Skill validation study on sentinel lymph node biopsy in breast cancer and the challenges of false-negative, in-transit and micrometastatic nodes

Chen Siew Ng, MRCP[1], Sarojah Arulanantham, MS Surgery[2], Joon Joon Khoo, FRCPA[3], Subathra Sabaratnam, MPath[3], Yeong Fong Lee, MD[1], Chin Fang Ngim, MRCP[3]

[1] Department of Nuclear Medicine, Hospital Sultanah Aminah, Johor Bahru, Malaysia, [2] Department of Surgery, Hospital Sultan Ismail, Johor Bahru, Malaysia, [3] School of Medicine & Health Sciences, Johor Bahru Clinical School, Monash University Malaysia

INTRODUCTION

Sentinel lymph node (SLN) is the first lymph node(s) in a regional lymphatic basin to receive the lymphatic drainage from a primary tumor. It has been shown that the lymph flows in an orderly and predicted pattern and any metastatic spread from a primary tumor will involve the sentinel node(s) first, before involving other lymph nodes in that lymphatic basin.[1] The tumor status of the SLN could then help to predict the histopathologic status of other lymph nodes draining the tumor.[2, 3] Although it is possible that at times, breast metastasis may skip pass the SLN to involve the second-tier or a higher-level node, this phenomenon is thought to be an uncommon variant of the lymphatic pattern.[4, 5, 6] Studies have also shown a low axillary recurrence rate in the follow-up of patients with negative SLN.[7, 8, 9]

For many years, removal of primary breast cancer along with axillary lymph node dissection (ALND) was considered a "gold standard" in the treatment of breast cancer with clinically negative node. Although the pathological status of axillary lymph nodes (ALNs) is the most important prognostic factor and plays an important role in planning subsequent adjuvant therapy, prophylactic axillary dissection confers no significant benefit to the overall survival other than increasing the patients' short- and long-term morbidities. A SLN free from tumor metastasis would have excluded tumor spread to the at-risk regional lymphatic basin and conversely, a positive SLN with tumor invasion will require ALND to achieve perhaps marginal benefit of reducing the chance of recurrence.[10, 11, 12]

Over the last two decades, sentinel lymph node biopsy (SLNB) has emerged as a widely accepted treatment option compared to the conventional ALND. It is likely to evolve into a new standard procedure for the management of early breast cancer in the future. SLNB and proper identification of the SLN requires multidisciplinary expertise.[13, 14] Without proper training, there is a steep learning curve in acquiring the skill.[14, 15, 16] Studies have shown a wide range of SLN identification and false-negative rates[17, 18] where success rate is frequently lower in the early stage of introducing this approach. A high false-negative rate in inexperienced hands may compromise the standard of care for patients with early breast cancer. It is thus recommended that sentinel node skills verification should be conducted before introducing SLN based management in any institution.[19, 20] The recommended number of cases to confirm a surgeon's competency in this technique is 20-40 SLNB procedures in combination with completion ALND.[16, 18, 21, 22, 23]

To the best of our knowledge, there are a fair number of surgeons performing SLNB routinely at various institutions in Malaysia but there is no published institutional validation study for Malaysia to date. We hope this study will assist us in evaluating the technical proficiency and collaborative outcome of a multidisciplinary team in this area before offering this option to patients in a tertiary center at the southern region of Malaysia.

MATERIALS AND METHODS

Study design

This prospective study on SLNB skill and performance validation involved a multidisciplinary team of 3 specialties namely surgery, nuclear medicine and pathology. The settings were at the Hospital Sultanah Aminah and the Hospital Sultan Ismail in Johor Bahru which were the two main tertiary referral hospitals in Southern Malaysia. The study was carried out from September 2010 to December 2012. All patients with clinically node-negative breast cancer irrespective of the size of the primary tumor were included in the study. Verbal and written consent were obtained from all patients. Their operations were all performed by the same surgeon who was a qualified breast surgeon.

SLN mapping and SLNB

A standard protocol using a combination of radiotracer and blue dye was used to locate and identify the SLNs. Pre-operative axillary lymphoscintigraphy was carried out a day before the surgery. All patients received a single subareolar injection of radiotracer at the radial position of the tumor. The radiotracer used was 0.2ml of 40MBq ^{99m}Tc-labeled colloidal rhenium sulphide (Nanocis®) consistent with protocols practiced in other centres.[24, 25, 26, 27] In addition, peritumoral injection may be given concomitantly especially to patients whose tumor involved the inner quadrants at the discretion of the nuclear physician where, four peritumoral injections, each with 0.2ml of 20MBq of radiotracer were

This article was accepted: 17 August 2016

Corresponding Author: Chen Siew Ng, Department of Nuclear Medicine, Hospital Sultanah Aminah, Jalan Persiaran Abu Bakar Sultan, 80100 Johor Bahru, Johor, Malaysia Email: chensiewng@hotmail.com

Table I: The clinico-pathological features of patients with clinically node-negative breast cancer (n=24).

Features	No
Age (years)	
Range	34-81
Median	52
< 50 years	11
> 50 years	13
Ethnic background	
Malay	13
Chinese	8
Indian	1
Others	2
Tumor locationa	
Upper outer quadrant	10
Lower outer quadrant	5
Upper inner quadrant	7
Lower inner quadrant	0
Central	2
Tumor sizeb	
Range (mm)	10-55
Median (mm)	27
T1 stage (≤20mm)	9
T2 stage (21-50mm)	14
T3 stage (>50mm)	1
Tumor typeb	
Invasive ductal	23
Invasive lobular	1
Tumor multiplicitya, b	
Unifocal	23
Multifocal	1
Histologic gradeb	
I	7
II	11
III	5
Surgery type	
Total mastectomy	15
Wide local excision	9

[a] Clinical examination findings
[b] Histo-pathological findings

Table II: Histopathological status of SLN and non-sentinel ALN for metastases

Group	SLN status	Non-sentinel ALN status	No. of patients
A	Positive	Positive	3
B	Positive	Negative	5a
C	Negative	Positive	3
D	Negative	Negative	13
		Total:	**24**

a One patient had a positive intramammary node besides her 2 metastatic SLNs.

given around the tumor at 3, 6, 9 and 12 o'clock positions at the depth of the tumor site. All the injected sites were massaged [28] gently for 5 minutes. Static lymphoscintigraphy was performed in anterior and antero-oblique projections at 10 minutes and two hours post-injection. The locations of the axillary SLNs were marked on the skin with indelible ink.

The following day, after induction of anesthesia, a total of 1-2 mls of patent blue V (Guerbet®) was injected. The technique and the methodology of the blue dye injection were left to the discretion of the operating surgeon who may inject at the subareolar, periareolar, intradermal or peritumoral sites singly or in combination. Intra-operative identification of SLN(s) was done with hand-held gamma probe and blue dye visual mapping. The excised SLN were labelled as "hot & blue", if it was identified by both radiotracer and blue-dye, "hot-only" if it contained only radiotracer and "blue-only" if it was detected by blue-dye only. Following SLNB, wide local excision or mastectomy along with completion ALND was performed for all patients.

Histopathological examination

All SLNs harvested were fixed in 10% buffered formalin at a neutral pH of 7 and submitted to the laboratory for processing. The nodes were examined and processed as a whole if the size were less than 5 mm in greatest diameter but

Table III: The clinicopathological features of the 3 cases of false-negative SLN

Features	Case 1	Case 2	Case 3
Age	60 year	53 year	56 year
Tumor size:			
Clinical palpation:	2.5cm	3cm	3.5cm
HPE report:	20x15x20mm	30x40x20mm	25x20x55mm
Tumor location	Upper inner quadrant	Superficially at center quadrant, at the lower outer part of areolar.	Superficially at center quadrant, at retroareolar region
Tumor type	Invasive ductal	Invasive ductal	Invasive ductal
Tumor grade	Grade II	Grade I	Grade III
Injection technique	Subareolar only for radiotracer. Subareolar & peritumoral for blue-dye.	Subareolar only for both radiotracer & blue-dye.	Subareolar only for both radiotracer & blue-dye.
No. of SLN identified during pre-operative lymphosintigraphy	1	1	3
No. of SLN removed & its labeling	SLN1(H&B), SLN2(H-only)	SLN1(H&B)	SLN1(H&B), SLN2(H&B), SLN3(H&B)
No. of lymph node removed during completion ALND	11	12	10
No. of metastatic non-SLN node and HPE report	1 (subcapsular micrometastasis)	1 (metastases reported in all 6 blocks).	1 (5mm macrometastasis)

SLN = sentinel lymph node; H&B = hot & blue; H-only = hot-only.

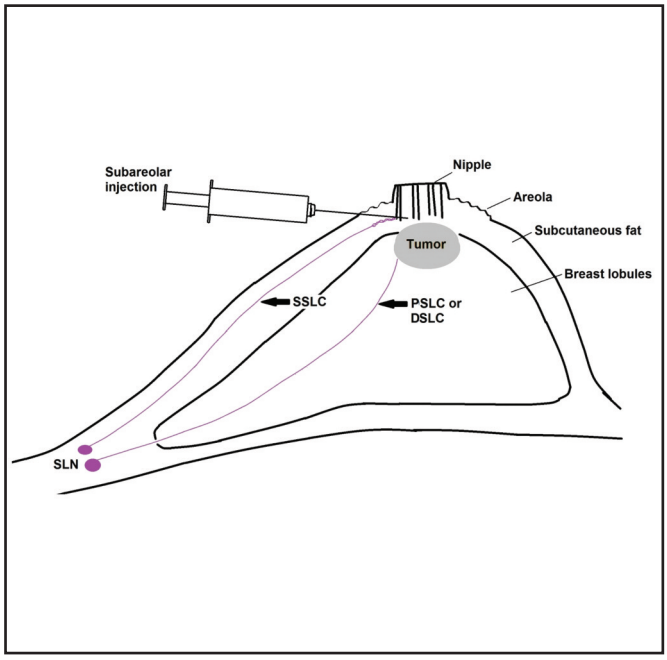

Fig. 1: Illustrates possible types of sentinel lymphatic channels for a superficial and larger subareolar tumor. Lymphatic drainage in the breast tissue tends to flow towards the superficial (subdermal) region and finally join the superficial sentinel lymphatic channel (SSLC) to enter into the axillary nodal basin. Subareolar injection approach helps to map out the SSLC but is unlikely to track the other penetrating sentinel lymphatic channel (PSLC) or deep sentinel lymphatic channel (DSLC).

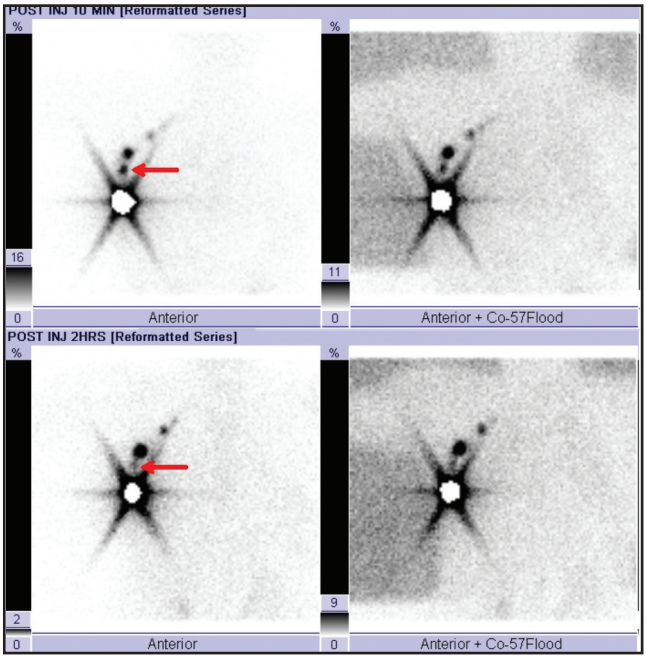

Fig. 2: Illustrates an in-transit node (red arrow) with prominent focal uptake at 10minutes post-injection that quickly faded at images taken 2 hours later. Without careful lymphoscintigraphy, such in-transit node would have passed unnoticed due to the transient radiotracer uptake.

halved if they were 5 to 10 mm in diameter. Nodes larger than 10 mm in diameter were cut into blocks of 5 mm thickness each and processed as individual blocks. For each individual block, one initial hematoxylin and eosin (H&E) stained section was done and screened. If this was negative for metastasis, multiple-level sectioning was done for a further 50 levels. For each level, 4um-thick sections were taken and stained with H&E stain. At every 7th level, immunohistochemical (IHC) stain for AE1/AE3 was done.

All other surgical specimens consisting of breast tissue and lymph nodes from axillary clearance were sent for routine HPE.

Data analysis

The results of this study are presented using descriptive statistics. The two important parameters are the SLN identification rate and the SLN false negative (FN) rate. The SLN identification rate is the proportion of successful SLN localization and removal in all patients undergoing the SLNB procedure. A FN SLN refers to the situation where the patient had no metastasis in the SLN(s) but metastasis was detected in other axillary clearance node(s). The rate for false-negativity in this study is calculated based on the number of FN SLN in comparison to the total number of nodal-positive cases.

This study was conducted in accordance to the ethical standards of the Declaration of Helsinki and received ethical clearance from the Medical Research Ethical Committee of Ministry of Health Malaysia (NMRR-08-575-1730)

RESULTS

A total of 25 patients were enrolled in the study during the period from September, 2010 till December, 2012. One patient was excluded following HPE finding of a benign tumor. The clinico-pathological features of the remainder 24 patients included in this study are depicted in Table I.

All the patients received subareolar radiotracer injection with four patients receiving additional peritumoral injection. Intraoperative blue-dye was also administered to all the patients using different approaches such as subareolar/periareolar and peritumoral (n=8), subareolar/periareolar only (n=10), peritumoral only (n=4) and intradermal only (n=2). SLNs were identified in all the 24 patients giving an identification rate of 100%. No extra-axillary node was identified during the pre-operative lymphoscintigraphy. A total of 48 SLNs were harvested with an average of 2 SLNs removed per patient (range 1-4). The majority of SLNs were identified as "hot & blue" (n=33, 69%), 12(25%) as "hot-only" and the remaining 3(6%) as "blue-only". None of the nodes identified as "hot-only" or "blue-only" was positive for metastasis. All patients had a level-II axillary lymph node clearance. A total of 301 lymph nodes were removed giving a mean of 12.5 non-sentinel ALNs removed from each patient at completion ALND (range 7-22).

The histopathological status of SLNs and non-sentinel ALNs in this study is shown in Table II. Eleven patients (46%) who were those in group A, B and C were positive for nodal metastases on HPE. There were 3 patients who had FN SLN in which metastasis was identified in non-sentinel ALNs but not in their SLNs. The FN rate in this study was calculated to be 3/11 (27%).

The dissected axillary tissues were routinely examined for palpable suspicious nodes. In this study, 5 such non-sentinel ALNs which were "neither hot nor blue" were identified. One node was later confirmed to be positive for metastasis and this case constituted one of the three FN cases.

Metastases were reported in 11 out of the 48(23%) SLNs harvested compared with 9 out of the 301(3%) ALNs removed during completion ALND. If conventional ALND were performed, the detection rate for nodal metastases was calculated to be 6% (20/349) only. Among those patents with axillary nodal metastases, the mean number of positive nodes was 1.7 per patient (range 1–3).

Micrometastases (metastases measuring 0.2 to 2.0mm) were reported in the SLNs of 2 patients. In one of these cases, SLNs were the only positive nodes. The initial sectioning using H&E stain for this patient was reported negative for metastasis but subsequent serial level sectioning of the same node showed positive tumor deposit with IHC staining. Additional H&E staining done for a level adjacent to the above showed similar finding. In the second case, micrometastases were present in both the harvested SLNs alongside with macrometastasis (metastases measuring >2.0mm) in 1 of the 15 nodes removed during completion ALND.

DISCUSSION

The clinico-pathological features of the study cohort mirrored the breast cancer demography in Malaysia where breast cancer tends to be diagnosed with a peak age-standardized rate in the 50-59 years age group.[30, 31] Their ethnicity also reflected those of patients seeking treatment at Ministry of Health hospitals in Malaysia which largely cater to the lower socioeconomic group.[31] A significant number present late with large tumor size (>2cm) at the time of diagnosis as reflected in our study, where 62.5% of our subjects had tumor > 2cm. Consistent with studies reported in other countries, most of the tumors in our cohort were invasive ductal carcinoma located at the upper outer quadrant of the breast.[32, 33, 34]

Technical approach

In most instances, SLNs were identified concordantly by both radiotracer and blue dye. We had observed that in those patients having more than one SLN, the lymphatic channels visualized by blue-dye mapping were frequently destroyed or displaced following surgical removal of the first SLN. The concomitant "hot" radioactivity enabled us to detect subsequent "blue" nodes which may otherwise have been difficult. This approach using a combination of radiotracer and blue dye facilitated the removal of an increased number of labeled SLNs and therefore improve the identification rate and minimize the FN rate. Radiotracer appeared to be more sensitive in identifying multiple SLNs when compared to blue-dye as reflected in our results. The use of blue dye alone

has been shown to be associated with higher FN rate. [35, 36]
Our study showed that SLNB yielded a higher rate for the detection of breast cancer metastases (23%, 11/48) compared to ALND (6%, 20/349). If the SLNB approach is practiced instead of conventional ALND, far less nodal specimens will be submitted for histopathology reporting thus enabling a more comprehensive review.

False-negative node
A high FN rate will adversely affect patients' management and prognosis. We therefore reviewed the 3 cases of FN SLN in our cohort to elicit possible contributory factors as seen in Table III.

Patients' age may affect the ability to identify the SLN where increasing age was associated with greater difficulty in identifying SLNs,[33, 37, 38] but this had not been shown to increase the FN rate39. Though it was reported that there is no significant relationship between FN rate and tumor size when categorized by T-stage, [39, 40, 41] there is also limited data to support the use of SLNB in tumors greater than 3cm. No significant relationship was noted between FN rate and tumor location or tumor histology,[39, 40] but tumor grading39, tumor multifocality[42] and the number of SLN harvested[34, 39] have been found to affect the FN rate.

In case-1 of our study, there was good agreement and concordant finding in the harvested SLN using a combination of radiotracer and blue-dye injected at the subareolar and peritumoral sites. We were unable to identify any distinctive clinico-pathologic feature or modifiable technical approach which could explain the FN SLN seen in this patient. The FN SLN may be attributed to an axillary skip metastasis described previously[4, 5, 6, 43] where as a result of variation in lymphatic drainage or for unknown reasons, the cancer cells appear to "skip" pass the SLN and spread to other non-sentinel ALN.

Both patients described in case-2 and -3 have relatively more superficial and larger primary tumor located at the subareolar region and in both cases, only subareolar injections were given. There may be several explanations for the FN SLNs seen here. Firstly, given the close proximity of the primary tumor next to the subareolar plexus of Sappey, metastasis might have occurred early. In such an advanced tumor, the true SLN might have been heavily infiltrated with metastatic deposits causing blockage of lymphatic channels and diversion of lymph flow to other ALNs. The harvested SLN mapped out by the radiotracer and/or blue-dye may not be the true SLN then. Secondly, as a result of the pressure exerted from the primary tumor itself, the usual pattern of lymphatic flow and communicating vessels from the tumor to the superficial sentinel lymphatic channel (SSLC)[44, 45] might have been compromised. This caused preferential or redirection of flow through the deep sentinel lymphatic channel (DSLC) or the penetrating sentinel lymphatic channel (PSLC)[45] into the axillary basin where the SLNs for these channels may be different from the SLN of the SSLC(Figure 1). The demarcated lymph flow by the radiotracer or blue-dye using the subareolar injection approach alone may not be representative of the actual lymphatic drainage. We therefore proposed that if at all SLNB is being considered for a relative superficial and large tumor at the central quadrant, both the peritumoral and subareolar/periareolar approaches should be used concurrently for SLN mapping. Nevertheless, this hypothesis needs to be substantiated by a larger study.

It was noted that in case-2, our surgeon had identified a palpable suspicious node which was "neither hot nor blue" during surgery. This node was removed along with the dissected axillary tissue and later found to be positive for metastases. On retrospective review, if that suspicious node was identified as a SLN in the first instance, our FN rate would be reduced. This finding support the approach that during SLNB, surgeon should remove any indurated or suspicious node in the surgical bed, even if it is not labeled by radiotracer or blue-dye.[46]

In-transit node
In one of our patients, a metastatic node which was not identified during surgery was found within the upper-outer quadrant of the dissected breast tissue specimen which we identified as an intramammary[47, 48, 49] in-transit[50] node described in previous studies. Intramammary lymph node can be differentiated from a low ALN by the fact that it is surrounded by the breast tissue. However, according to the American Joint Committee on Cancer (AJCC) and Union International Contra la Cancrum (UICC) TNM staging, intramammary lymph nodes are coded as ALN for staging purposes. In-transit lymph nodes are those lymph nodes that are in close contact with a lymphatic channel connecting between the primary tumor and a regional lymphatic basin. They are usually small and may lie in close proximity to the tumor site. Although in-transit node may not be universally recognized as a SLN, it may be the first lymph node to be affected by the tumor cells and if left in situ, in-transit node harboring metastases may pose as potential source for locoregional recurrence.[51]

In this particular patient, besides having a metastatic SLN, a positive intramammary lymph node was also reported and noted to be almost effaced by the tumour cells. Retrospective review of her lymphoscintigram done at 10min post-injection showed a prominent focal uptake close to the injected site which quickly faded at images taken 2 hours later (Figure 2) which was likely due to her in-transit node . Due to its close proximity to the injected site and the transient nature of radiotracer uptake after injection, nuclear medicine physicians may need a high index of suspicion when it comes to identifying its presence with dynamic or sequential lymphoscintigraphy imaging immediately or shortly following injection. A preoperative mammography screening and ultrasound examination may aid in its detection as well.

Micrometastases
Careful and meticulous HPE of the SLN should be carried out with multiple-level sectioning.[51] The importance of doing multiple sectioning cannot be overemphasized. In our study, micrometastases could have been missed in 2 patients if multiple sectioning was not performed. Retrospective reexamination of the involved lymph nodes showed that all nodal tumor deposits could be detected using H&E stain alone but IHC increased the sensitivity of detecting small

metastases. Detailed, definitive HPE of the SLN by serial sectioning and IHC help in the detection of occult metastases and improve the diagnostic accuracy.

In one of these 2 cases with micrometastases, we noted that it is possible for the metastases at the second-echelon or higher-level nodes to appear more extensive than the SLN itself. A micrometastatic SLN may still have up to 15%[52, 53, 54, 55] chance of a non-sentinel, macrometastatic ALN and if this micrometastatic SLN remained undetected, it may contribute to a misleading impression of a skip-lesion or FN SLN. [52, 53, 54, 55]

It has been reported that among the group of patients having SLN metastases of ≤2 mm, solitary axillary macrometastases was found in about 10% of them.[52] A great proportion (90%) of these micrometastases were found in patients having T1c/T2 breast tumors.[52] Another study reported that none of those having a primary tumor size of ≤1 cm had second echelon lymph node involvement.[53] Although many studies have shown that ALND may be avoided if SLNs were found to harbor only micrometastatic tumor, clinicians should consider this recommendation with caution and take into account the existing tumor size[52, 54, 55] and various factors such as tumor multiplicity, possible lymphovascular invasion55 and plan for post-operative radiation therapy in their decision-making.

CONCLUSION

This skill validation study showed an impressive SLN identification rate but an unacceptably high FN rate in the detection of nodal metastases which may be contributed by several factors. A relook into our choice of SLN injection techniques may offer some possible explanation to the FN SLNs where a "one-size-fits-all" injection approach using subareolar technique may not be appropriate for every patient. Peritumoral injection may provide a better option for patients with a relatively superficial and larger tumor at the central quadrant, however further studies on a larger scale will be required to substantiate this hypothesis. The possible existence of intramammary, in-transit nodes should be considered as these nodes may easily remained undetected without a careful pre-operative lymphoscintigraphy, mammography and/or ultrasound examination. Surgeons should regard intraoperative, palpable suspicious nodes as SLNs even if these nodes are not labeled with radiotracer or blue-dye. In addition, multiple sectioning of SLN is important to detect micrometastases. Besides conventional HPE with H&E stain, additional IHC stain may be used to improve diagnosis and reduced false-negativity in selected SLN-negative patients. Decision to proceed or to omit ALND following confirmation of a positive micrometastatic SLN should be evaluated together with other associated compounding factors.

In order to achieve a high standard of accuracy and practice, multidisciplinary collaboration between surgeons, nuclear medicine physicians and pathologists is necessary. Based on the findings of this study, our multi-disciplinary team will review the standard protocols for patient selection, pre-operative lymphoscintigraphy, intra-operative mapping and post-operative HPE in order to improve the outcome of SLNB in the management of early breast cancer. Hopefully, future validation study will show an improved outcome that will support the incorporation of SLNB into our clinical services.

ACKNOWLEDGEMENTS

The authors would like to thank the staff of the Nuclear Medicine Department, Hospital Sultanah Aminah, Malaysia for their dedication and hard work in coordinating and managing the study and the database. We would like to acknowledge the advice, guidance, comments and support given by Dr. Maria Lee Hooi Sean and her team at the Clinical Research Centre, Hospital Sultanah Aminah during the preparation of this manuscript. This study was partly supported by a grant from the Ministry of Health, Malaysia. The authors would like to thank the Director of Health Malaysia for permission to publish this paper.

REFERENCES

1. Reintgen DS, Cruse W, Wells K et al. The orderly progression of melanoma nodal metastases. Ann Surg. 1994; 22: 759-67.
2. Giuliano AE, Kirgan DM, Guenther M *et al.* Lymphatic mapping and sentinel lymphadenectomy for breast cancer. Ann Surg. 1994; 220: 391– 8.
3. Krag D, Weaver D, Alex JC, Fairbank JT. Surgical resection and radiolocalization of the sentinel lymph node in breast cancer using a gamma probe. Surg Oncol. 1993; 2: 335-40.
4. Rosen PP, Lesser ML, Kinne DW, Beattie EJ. Discontinuous or "skip" metastases in breast carcinoma. Analysis of 1228 axillary dissections. Ann Surg 1983; 197: 276-83.
5. Msuya CA, Hartveit F. Skip lesions in the axilla in breast cancer, and their association with micrometastases. Breast Cancer Res Treat. 1991 Nov; 19(3): 277-81.
6. Keskek M, Balas S, Gokoz A *et al.* Re-evaluation of axillary skip metastases in the era of sentinel lymph node biopsy in breast cancer. Surg Today. 2006;36(12): 1047-52.
7. Lyman GH, Temin S, Edge SB *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 Apr 7. [Epub ahead of print]
8. Naik AM, Fey J, Gemignani M *et al.* The risk of axillary relapse after sentinel lymph node biopsy for breast cancer is comparable with that of axillary lymph node dissection: a follow-up study of 4008 procedures. Ann Surg. 2004;240(3):462-8; discussion 468-71
9. Langer I, Marti WR, Guller U *et al.* Axillary recurrence rate in breast cancer patients with negative sentinel lymph node (SLN) or SLN micrometastases: prospective analysis of 150 patients after SLN biopsy. Ann Surg. 2005; 241: 152-8.
10. Bilimoria KY, Bentrem DJ, Hansen NM *et al.* Comparison of sentinel lymph node biopsy alone and completion axillary lymph node dissection for node-positive breast cancer. J Clin Oncol. 2009; 27(18): 2946-53.
11. Glechner A, Wöckel A, Gartlehner G *et al.* Sentinel lymph node dissection only versus complete axillary lymph node dissection in early invasive breast cancer: a systematic review and meta-analysis. Eur J Cancer. 2013; 49(4): 812-25.
12. Galimberti V, Cole BF, Zurrida S et al. Axillary dissection versus no axillary dissection in patients with sentinel-node micrometastases (IBCSG 23-01): a phase 3 randomised controlled trial. Lancet Oncol. 2013 Apr;14(4):297-305. doi: 10.1016/S1470-2045(13)70035-4. Epub 2013 Mar 11.
13. Leong SP, Morita ET, Treseler PA, *et al.* Multidisciplinary approach to selective sentinel lymph node mapping in breast cancer. Breast Cancer. 2000; 7(2):105-13.
14. Classe JM, Curtet C, Campion L et al. Learning curve for the detection of axillary sentinel lymph node in breast cancer. Eur J Surg Oncol. 2003 Jun; 29(5): 426-33.
15. Tafra L. The learning curve and sentinel node biopsy. Am J Surg. 182 (2001) 347-50.
16. Clarke D, Newcombe RG, Mansel RE; ALMANAC Trialists Group. The learning curve in sentinel node biopsy: the ALMANAC experience. Ann Surg Oncol. 2004 Mar;11(3 Suppl): 211S-5S.
17. Sanidas EE, de Bree E, Tsiftsis DD. How many cases are enough for accreditation in sentinel lymph node biopsy in breast cancer? Am J Surg. 2003 Mar; 185(3): 202-10.

18. Sanidas EE, Daskalakis M, Sbyrakis N, Tsiftsis DD. Modifications of the learning curve guidelines for breast cancer sentinel node biopsy. Eur J Surg Oncol. 2005 May; 31(4): 357-63.
19. Zervos EE, Saha S, Hoshaw-Woodard S, Wheatley GH, Burak WE Jr. Localizing the sentinel node outside of the specialty center: success of a lymphatic mapping course in disseminating new technology. Ann Surg Oncol. 2001 Jan-Feb; 8(1): 7-12.
20. Posther KE, McCall LM, Blumencranz PW *et al.* Sentinel node skills verification and surgeon performance: data from a multicenter clinical trial for early-stage breast cancer. Ann Surg. 2005 Oct;242(4):593-9; discussion 599-602.
21. Leong SP. Selective sentinel lymphadenectomy for breast cancer in the United States. Asian J Surg. 2004 Oct; 27(4): 268-74.
22. Ung OA. Australasian experience and trials in sentinel lymph node biopsy: the RACS SNAC trial. Asian J Surg. 2004 Oct; 27(4): 284-90.
23. Mansel RE, MacNeill F, Horgan K *et al.* Results of a national training programme in sentinel lymph node biopsy for breast cancer. Br J Surg. 2013; 100(5): 654-61.
24. Smith LF1, Cross MJ, Klimberg VS. Subareolar injection is a better technique for sentinel lymph node biopsy. Am J Surg. 2000 Dec;180(6):434-7; discussion 437-8.
25. KA Kern. Concordance and validation study of sentinel lymph node biopsy for breast cancer using subareolar injection of blue dye and technetium 99m sulfur colloid. J Am Coll Surg. 2002; 195: 467-75
26. D'Eredita G, Giardina C, Guerrieri AM, Berardi T. A further validation of subareolar injection technique for breast sentinel lymph node biopsy. Ann Surg Oncol. 2006 May; 13(5): 701-7.
27. Kargozaran H, Shah M, Li Y *et al.* Concordance of Peritumoral Technetium 99M Colloid and Subareolar Blue Dye Injection in Breast Cancer Sentinel Lymph Node Biopsy. J Surg Res. 2007; 143: 126-9.
28. Shenov V, Ravichandran D, Ralphs DN. Is massage following dye injection necessary in sentinel node biopsy in breast cancer? Breast. 2002; 11(3): 273-4
29. Kohlberger P, Gantert M, Volk-Orlowska T, Kieback DG, Gitsch G. Immunohistochemical detection of lymph node metastases in node-negative breast cancer patients. Anticancer Res. 2001; 21(1B): 697-9.
30. National Cancer Registry, Ministry of Health (Malaysia). Malaysia National Cancer Registry Report 2007. Kuala Lumpur, Malaysia: National Cancer Registry, Ministry of Health (Malaysia), 2011: 34.
31. Hisham AN, Yip CH. Overview of Breast Cancer in Malaysian Women: A Problem with Late Diagnosis. Asian J Surg. 2004; 27(2): 130-3.
32. Zavagno G, Del Bianco P, Koussis H *et al.* Clinical impact of false-negative sentinel lymph nodes in breast cancer. Eur J Surg Oncol. 2008 Jun; 34(6): 620-5.
33. Chagpar AB, Martin RC, Scoggins CR *et al.* Factors predicting failure to identify a sentinel lymph node in breast cancer. Surgery. 2005 Jul; 138(1): 56-63.
34. Wong SL, Edwards MJ, Chao C *et al.* Sentinel lymph node biopsy for breast cancer: impact of the number of sentinel nodes removed on the false-negative rate. J Am Coll Surg. 2001 Jun; 192(6): 684-9.
35. Pesek S, Ashikaga T, Krag LE, Krag D. The false-negative rate of sentinel node biopsy in patients with breast cancer: a meta-analysis. World J Surg. 2012 Sep; 36(9): 2239-51.
36. Hung WK, Chan CM, Ying M *et al.* Randomized clinical trial comparing blue dye with combined dye and isotope for sentinel lymph node biopsy in breast cancer. Br J Surg. 2005; 92(12): 1494–7.
37. Cox CE, Dupont E, Whitehead GF *et al.* Age and body mass index may increase the chance of failure in sentinel lymph node biopsy for women with breast cancer. Breast J. 2002 Mar-Apr; 8(2): 88-91.
38. Martin RC 2nd, Chagpar A, Scoggins CR *et al.* Clinicopathologic factors associated with false-negative sentinel lymph-node biopsy in breast cancer. Ann Surg. 2005 Jun; 241(6): 1005-12; discussion 1012-5.
39. Goyal A, Newcombe RG, Chhabra A, Mansel RE; ALMANAC Trialists Group. Factors affecting failed localisation and false-negative rates of sentinel node biopsy in breast cancer--results of the ALMANAC validation phase. Breast Cancer Res Treat. 2006 Sep; 99(2):203-8. Epub 2006 Mar 16.
40. Olson JA Jr, Fey J, Winawer J *et al.* Sentinel lymphadenectomy accurately predicts nodal status in T2 breast cancer. J Am Coll Surg. 2000 Dec; 191(6): 593-9.
41. Wong SL, Chao C, Edwards MJ *et al.* Accuracy of sentinel lymph node biopsy for patients with T2 and T3 breast cancers. Am Surg. 2001 Jun; 67(6): 522-6; discussion 527-8.
42. Bergkvist L, Frisell J; Swedish Breast Cancer Group; Swedish Society of Breast Surgeons. Multicentre validation study of sentinel node biopsy for staging in breast cancer. Br J Surg. 2005 Oct; 92(10): 1221-4.
43. Sun J, Yin J, Ning L *et al.* Clinicopathological characteristics of breast cancers with axillary skip metastases. J Invest Surg. 2012; 25(1): 33-6.
44. Wang M, Zhou W, Zhao Y *et al.* A novel finding of sentinel lymphatic channels in early stage breast cancer patients: which may influence detection rate and false-negative rate of sentinel lymph node biopsy. PLoS One. 2012;7(12):e51226. doi: 10.1371/journal. pone.0051226. Epub 2012 Dec 4.
45. Kern KA. Lymphoscintigraphic anatomy of sentinel lymphatic channels after subareolar injection of Technetium 99m sulfur colloid. J Am Coll Surg 2001; 193: 601-8.
46. Choi YJ, Kim JH, Nam SJ, Ko YH, Yang JH. Intraoperative identification of suspicious palpable lymph nodes as an integral part of sentinel node biopsy in patients with breast cancer. Surg Today. 2008; 38(5): 390-4.
47. Fujii T, Yajima R, Matsumoto A *et al.* Implication of an Intramammary Sentinel Lymph Node in Breast Cancer: Is This a True Sentinel Node? A Case Report. Breast Care (Basel). 2010; 5(2): 102-104. Epub 2010 Apr 21.
48. Araújo C, Fougo JL. Intramammary lymph node metastasis of breast cancer after sentinel node biopsy: two cases and a review of the literature. Eur J Surg Oncol. 2009 Dec; 35(12): 1354. doi: 10.1016/j.ejso.2009.06.004. Epub 2009 Jul 9.
49. Intra M, Garcia-Etienne CA, Renne G *et al.* When sentinel lymph node is intramammary. Ann Surg Oncol. 2008 May; 15(5): 1304-8
50. van Deurzen CH, Borgstein PJ, van Diest PJ. In-transit lymph node metastases in breast cancer: a possible source of local recurrence after Sentinel Node procedure. J Clin Pathol. 2008 Dec; 61(12): 1314-6.
51. Jaffer S, Nagi C, Bleiweiss IJ. Occult axillary node metastases in breast cancer are prognostically significant: results in 368 node negative patients with 20-year follow-up. Adv Anat Pathol. 2008; 15(6): 370-3.
52. Dabbs DJ, Fung M, Landsittel D, McManus K, Johnson R. Sentinel lymph node micrometastasis as a predictor of axillary tumor burden. Breast J. 2004 Mar-Apr; 10(2): 101-5.
53. Kamath VJ, Giuliano R, Dauway EL, *et al.* Characteristics of the sentinel lymph node in breast cancer predict further involvement of higher-echelon nodes in the axilla: a study to evaluate the need for complete axillary lymph node dissection. Arch Surg. 2001 Jun; 136(6): 688-92.
54. van Deurzen CH, van Hillegersberg R, Hobbelink MG *et al.* Predictive value of tumor load in breast cancer sentinel lymph nodes for second echelon lymph node metastases. Cell Oncol. 2007; 29(6): 497-505.
55. Bolster MJ, Peer PG, Bult P *et al.* Risk Factors for Non-Sentinel Lymph Node Metastases in Patients with Breast Cancer. The Outcome of a Multi-institutional Study. Ann Surg Oncol.2007;14: 181-9.