WARWICK
THE UNIVERSITY OF WARWICK

Manuscript version: Author's Accepted Manuscript
The version presented in WRAP is the author's accepted manuscript and may differ from the published version or Version of Record.

Persistent WRAP URL:
http://wrap.warwick.ac.uk/142981

How to cite:
Please refer to published version for the most recent bibliographic citation information. If a published version is known of, the repository item page linked to above, will contain details on accessing it.

Copyright and reuse:
The Warwick Research Archive Portal (WRAP) makes this work by researchers of the University of Warwick available open access under the following conditions.

Copyright © and all moral rights to the version of the paper presented here belong to the individual author(s) and/or other copyright owners. To the extent reasonable and practicable the material made available in WRAP has been checked for eligibility before being made available.

Copies of full items can be used for personal research or study, educational, or not-for-profit purposes without prior permission or charge. Provided that the authors, title and full bibliographic details are credited, a hyperlink and/or URL is given for the original metadata page and the content is not changed in any way.

Publisher's statement:
Please refer to the repository item page, publisher's statement section, for further information.

For more information, please contact the WRAP Team at: wrap@warwick.ac.uk.

warwick.ac.uk/lib-publications

A multicentre prospective feasibility study of carbon dye tattooing of biopsied axillary node and surgical localisation in breast cancer patients

Authors: Amit Goyal, FRCS[1], Shama Puri, FRCR[2], Andrea Marshall, PhD[3], Kalliope Valassiadou, FRCS[4], Moin Hoosein, FRCR[5], Amtul R Carmichael, FRCS[6], Gabriella Erdelyi, MD[2], Nisha Sharma, FRCR[7], Janet Dunn, PhD[3], Joanne York, DCR[2]

Institution: Departments of [1]Surgery and [2]Radiology, Royal Derby Hospital, Derby; Departments of [4]Surgery and [5]Radiology, University Hospitals, Leicester; [3]Warwick Clinical Trials Unit, University of Warwick, Warwick; [6]Department of Surgery, Queens Hospital, Burton-On-Trent; [7]Department of Radiology, St James's University Hospital, Leeds

Address for Correspondence:
Amit Goyal, Department of Surgery, Royal Derby Hospital, Uttoxeter Road, Derby, DE22 3NE, United Kingdom
Tel: +44(0)1332 785538
Email: amit.goyal@nhs.net
Twitter: @_amit_goyal

ClinicalTrials.gov:NCT03640819

Shortened version of the title: Tattooing of biopsied axillary node

Category: Original article

Disclosure: There are no conflicts of interest

Key words: carbon dye, breast cancer, sentinel node biopsy, tattooing, neoadjuvant chemotherapy, targeted axillary dissection, axillary staging

Abstract

Background: The primary aim of this prospective, multicentre feasibility study was to determine whether the biopsied axillary node can be marked using black carbon dye and successfully identified at the time of surgery.

Methods: We included patients undergoing needle biopsy of the axillary node. The biopsied node was tattooed at the time of needle biopsy (fine needle aspiration or core biopsy) or at a separate visit with black carbon dye (Spot™ or Black Eye™). Participants underwent primary surgery or neoadjuvant chemotherapy (NACT) and axillary surgery (SNB or ALND) as per routine care.

Results: 110 patients were included. Median age of the women was 59 (range 31 to 88) years. 48 (44%) underwent SNB and 62 (56%) ALND. Median volume of dye injected was 2.0 ml (range 0.2-4.2). Tattooed node was identified intraoperatively in 90 (82%) patients. The identification rate was higher (76 of 88, 86%) in the primary surgery group compared with NACT (14 of 22, 64%) ($p=0.03$). Of those undergoing NACT, the identification rate was better in the patients undergoing SNB (3 of 4, 75%) compared with ALND (11 of 18, 61%) ($p>0.99$). The tattooed node was the sentinel node in 78% (28 of 36) patients in the primary surgery group and 100% (3 of 3) in the NACT group. There was no learning curve for surgeons or radiologists. The identification rate did not vary with timing between dye injection and surgery ($p=0.56$), body mass index ($p=0.62$) or volume of dye injected ($p=0.25$).

Conclusion: It is feasible to mark the axillary node with carbon dye and identify it intraoperatively.

Introduction

We have witnessed de-escalation of axillary surgery in the last two decades. Sentinel node biopsy (SNB) has become the procedure of choice for axillary staging in patients who are clinically node negative at presentation irrespective of whether they undergo primary surgery or neoadjuvant chemotherapy (NACT). For patients with needle biopsy proven positive nodes at presentation, axillary lymph node dissection (ALND) is generally indicated if they undergo primary surgery. However, a subgroup with low volume nodal disease based on number of abnormal nodes on axillary ultrasound and tumour size[1] can be offered sentinel node biopsy rather than ALND. Patients found to have less than 3 involved nodes on SNB may be spared ALND[2;3]. Alternatively, NACT may be given to suitable patients to downstage the axilla followed by SNB to assess the residual tumour burden.

In both scenarios, the concern is that the biopsied node with metastases may be left in the axilla at SNB. For patients undergoing primary surgery, Nathanson et al.[4] reported a 78 per cent concordance between the biopsied node and sentinel node. The false-negative rate of SNB after NACT in cN1 patients was studied in Z1071[5] and SENTINA[6] trials and found to be more than 10%. A recent meta-analysis showed that the combination of SNB with removal of the marked involved node following NACT reduces the FNR to less than 5%[7] (targeted axillary dissection, TAD).

There are a number of techniques being investigated to mark the involved node and then visualise it intra-operatively. A clip can be placed in the needle biopsy positive node and x-ray of the specimen be performed at SNB to ensure that the clipped node is removed. The clipped node can be marked using a wire to guide the surgeon before surgery[8]. However this technique has limitations as nodal shrinkage with chemotherapy may result in clip displacement and post NACT, the clip cannot be visualised on ultrasound scan in around 30% patients[9]. Alternatively, the involved node can be marked using black carbon dye[10-12], magnetic seed (Magseed)[13], reflector (SAVI Scout®)[14], radiofrequency tag (LOCalizer™)[15] or radioactive iodine seed (I^{125})[16]. The black carbon dye is less expensive and single-centre studies have reported more than 90% identification rate of the tattooed node intra-operatively. However, the technique remains to be standardised and tested in prospective multicentre studies.

The primary aim of this prospective, multicentre feasibility study was to determine whether the biopsied node can be marked using black carbon dye and successfully identified at the time of surgery. The secondary aims were to determine the concordance between the tattooed node and sentinel node, migration of black dye into other nodes and standardise the technical aspects of the technique.

Methods

This was an investigator-led multicentre cohort study done at 5 centres in the UK (Derby, Burton-on-Trent, Leicester, Leeds, Plymouth). The study protocol was approved by the South West - Exeter Research Ethics Committee (REC reference: 18/SW/0097) and local research and development offices of participating institutions. All participants provided written informed consent. The study complies with the principles of the Declaration of Helsinki.

Individuals were eligible for inclusion if they were aged ≥ 18 years, had early stage (stage I-IIIA) unifocal or multifocal invasive breast cancer with abnormal looking lymph node/s on preoperative ultrasound scan at presentation and undergoing needle biopsy of the lymph node (fine needle aspiration (FNA) or core biopsy). Individuals were excluded if they had skin tattoo in the drainage area of the axilla, were not undergoing axillary surgery or unfit for axillary surgery. Participants underwent primary surgery or NACT and axillary surgery (SNB or ALND) as per routine care.

Carbon dye tattooing

The biopsied node was tattooed at the time of needle biopsy (FNA or core biopsy) or at a separate routine visit to the hospital. The black carbon dye (Spot™ or Black Eye™ (Omnimed Limited™, Winchester, England)) was injected both in the cortex of the node and the perinodal tissue under ultrasound guidance by a breast radiologist or radiographer. Additionally, in the initial 8 cases the dye was injected in the needle tract but this was abandoned as it resulted in generalised black staining of the axillary contents as observed at the time of surgery. The protocol was not prescriptive about the volume of injection as it was intended that the data generated from this study would inform the recommended volume for future practice. The volume of injection was recorded and varied depending on the node size and the operator. If there were multiple suspicious nodes in the axilla, only the single biopsied node was tattooed.

Axillary surgery

Participants who were node negative on needle biopsy underwent sentinel node biopsy (SNB), while those with involved node/s underwent axillary lymph node dissection (ALND) as per local standard of care. Sentinel node biopsy was performed as per local protocol. The protocol did not dictate the injection technique or tracer/s to be used. Sites were allowed to use a combination of isotope and blue dye or a single tracer (isotope or blue dye). Patients undergoing ALND did not undergo SNB first at the same operation. The surgeon identified and removed the tattooed lymph node/s at the time of planned axillary surgery *(Figure 1)*.

Pathology

All lymph nodes were examined and reported according to predefined local practice that met the UK Royal College of Pathologists guidelines: nodes smaller than 5 mm were bisected, larger nodes were sliced at 2-mm intervals, and single sections assessed using haematoxylin and eosin staining. Immunohistochemical staining was not required, but was used selectively to characterize isolated tumour cells, suspicious or micrometastatic disease, in accordance with local protocol.

Outcomes

The primary outcome measure was identification rate of tattooed node at the time of axillary surgery. Secondary outcomes were concordance between tattooed node and sentinel node (tattooed node removed as part of sentinel node biopsy) and migration of dye into other nodes.

Additionally, the effects of body mass index, the timing of the injection of dye and surgery, volume of dye injected and case volume per surgeon and radiologist performed on identification of tattooed node were assessed. Potential pitfalls of tattooing such as diffusion of the black dye in the axillary contents and skin discoloration were documented.

Data collection and statistical analysis

Study data were collected and managed using REDCap electronic data capture tools hosted at Royal Derby Hospital, Derby. Continuous variables were summarised using medians, interquartile ranges (IQR) and ranges as they were not normally distributed.

Comparisons of continuous variables across binary groups were performed using a Wilcoxon rank sum test. A Spearman's rank correlation was used to assess the relationship between two continuous variables. Categorical variables are reported with frequencies and percentages and compared across groups using a chi-squared test, or a Fisher's exact test if small numbers. A stepwise logistic regression model was used to investigate the predictors of identification of the tattooed node. All analyses were performed using the SAS statistical package, version 9.4. Significance was determined at the 5% level.
This study is registered with ClinicalTrials.gov, number NCT03640819.

Results

Between April 2018 and July 2019 a total of 110 women with breast cancer who had needle biopsy of the axillary lymph node were eligible for inclusion in the study. 82 patients underwent primary surgery and 28 received NACT. Of the 28 patients that had NACT, 6 underwent SNB before NACT and therefore were included with the 82 patients that did not receive NACT for analyses.

Patient demographics, type of breast and axillary surgery and tumour characteristics are shown in *Table 1*. All women underwent axillary surgery (SNB or ALND) and all but two women had breast surgery (breast-conserving surgery or mastectomy).The median age of the women was 59 (range 31 to 88) years. 48 of 110 (44%) underwent SNB and 62 (56%) ALND. SNB was performed using a combination of isotope and blue dye in the most patients and the sentinel lymph node was identified in 46 of the 48 (96%) who had a SNB. 61 of the 110 (55%) patients were node positive at presentation (43 of 88 (49%) in the primary surgery group and 18 of the 22 (82%) in the NACT group.

Tattooing results

Table 2 shows the results of carbon dye tattooing. The dye was injected in the node at the time of needle biopsy in 75 of 110 (68%) and at a separate visit after the results of the biopsy in 35 (32%). The median volume of dye injected was 2.0 ml (range 0.2-4.2). The dye was injected in the cortex and perinodal tissue in 102 patients and additionally in the needle tract in the initial 8 patients. The duration between tattoo

and surgery was 28 days (IQR 19-34) for primary surgery and 199 days (IQR 167-225) for NACT group.

Tattooed node was identified in 90 of 110 (82%) patients. The identification rate was 81% (39 of 48) in patients who underwent SNB and 82% (51 of 62) for ALND; p=0.89. Patients undergoing SNB where the tattooed node was not identified had ≤2 nodes removed. The identification rate was significantly higher (76 of 88, 86%) in the primary surgery group compared with NACT (14 of 22, 64%); p=0.03. Of those undergoing NACT, the identification rate was not significantly better in the patients undergoing SNB (3 of 4, 75%) compared with ALND (11 of 18, 61%); p>0.99. Of those undergoing SNB where the tattooed node was identified, the tattooed node was the sentinel node in 78% (28 of 36) of patients in the primary surgery group and 100% (3 of 3) in the NACT group; p>0.99. The presence of diffuse black staining was seen in 4 of these 31 (13%) patients were the tattooed node was the sentinel node.

The median number of tattooed nodes identified by the surgeon was 1 (range 0 to 6) and on histology was 1 (range 0 to 8) *(Figure 2)*. There was diffuse black staining of axillary tissue in 22% of patients (19 of 88) in the primary surgery group, which was significantly less than 55% (12 of 22) in the NACT group (p=0.002).

There were no allergic reactions reported but one patient reported skin discoloration in the axilla.

Other factors affecting identification rate of tattooed node

Volume of injected dye

The volume of dye used for those where the tattooed node was identified was similar to that used where the tattooed node was not identified (median 2.0 (IQR 1.2-2.9) versus median 2.0 (IQR 0.3-2.5), p=0.25). The volume of dye injected was not correlated with the number of tattooed nodes identified and removed (Spearman's correlation p=0.59) .The volume of dye injected was not significantly associated with the presence of diffuse black staining (median 2.2 (IQR 1.8-3.0) compared with the volume used for those without black staining (median 2.0 (IQR 1.2-2.6; p=0.13).

Following these analyses, we injected varying volumes of dye ex-vivo in lymph glands of different sizes *(Figure 3)* to determine the optimum volume for injection. We found that a volume of 0.2 to 0.4 ml is sufficient to stain the lymph node. Even with this small volume, the dye spills into adjacent perinodal tissue and therefore separate injection either around the node or in the tract is not required.

Timing of injection

The median time between the dye injection and surgery where the tattooed node was identified was 30 days (IQR 21-42), which was similar to the median of 36 days for those where the tattooed node was not identified (IQR 24-165); p=0.36. The timing was also not significantly different between these groups for those having NACT (median 203 (IQR 172-225) versus median 183 (IQR 163-218) respectively, p=0.78) and also for those in the primary surgery group (median 28 (IQR 16-34) versus median 26 (IQR 14-34), p=0.59). However, the median time between the dye injection and surgery was significantly longer for those with diffuse black staining (median 59; IQR 22-183) compared to those without black staining (median 29; IQR 20-34, p=0.01).

Body mass index (BMI)

The BMI for the patients where the tattooed node was identified was not significantly different to the BMI for those where the tattooed mode was not identified (median BMI 29.3 (IQR 25.0-32.7) versus median 28.9 (IQR 25.1-34.4), p=0.62).

Multivariate analysis

Logistic regression identified primary surgery or NACT as the only independent significant predictor of the identification of the tattooed node (Odds ratio 3.26; 95% CI 1.02-10.41, p=0.05).

Surgeon learning curve

Of the 20 surgeons, the median number of surgeries conducted was 4, minimum 1 and maximum 20. A waterfall plot of the percentage of cases where the tattooed node was successfully identified did not show a learning curve *(Figure 4)*. Ten surgeons were able to identify the tattooed node in all surgeries. Only 4 (20%) out of the 20 surgeons had less than a 50% identification rate; these performed 4 or less surgeries and were

the only ones not to identify the tattooed node on the very first surgery. For the remaining six surgeons with an identification rate between 63% and 89%, it didn't seem to be the first or second surgeries where they had problems identifying the nodes.

Radiologist learning curve

Thirteen radiologists participated in the study. The tattooed node was identified for all their patients for 5 radiologists.

The first failed identification of the tattooed node was the first injection for 4 radiologists, whereas it was the 3rd, 7th, 8th and 12th for the remaining 4 radiologists. Therefore, for the majority there was an apparent learning curve.

Discussion

The results with carbon dye tattooing to mark the axillary lymph gland in patients with breast cancer were encouraging from single centre studies[10-12] (sample size 20 to 75) leading us to conduct this multicentre feasibility study to evaluate the technical aspects of the technique. Using the carbon dye to mark the node and identify it during surgery, we overall found the tattooed node in 82% of patients. In clinical practice, the technique will be used to mark the involved node in patients undergoing primary surgery and sentinel node biopsy or targeted axillary dissection (TAD) after NACT. Therefore, restricting to patients undergoing SNB, we identified the tattooed node in 82% patients in primary surgery group and 75% after NACT.

The results are less favourable when compared with 93.7% identification rate in patients undergoing primary surgery by Choy et al.[10] and post NACT identification rates of 94.6% by Natsiopoulos et al.[11], and 100% by Choy et al.[10] and Park et al.[12].This can be attributed to several reasons. These studies were single-centre in contrast to our multicentre study. Additionally, the median volume of dye injected was more in our study compared to previous studies. There was background black staining of axillary tissue in 22% patients in the primary surgery group and 55% patients in the NACT group. The data suggest that diffusion of dye is more prominent with increasing time interval between injection of dye and surgery. This may have compromised the identification rate of the tattooed node. One of our aims was to evaluate the optimal volume of injection and we found no correlation of volume to

black staining of axillary tissue, identification rate of tattooed node or number of tattooed nodes identified intra-operatively. From ex vivo work, we recommend a volume of 0.2 to 0.4 ml to mark the lymph node *(Figure 4)*. The dye should not be injected around the node or in the tract to avoid diffusion into the axillary tissue and skin discoloration.

The success rate in identifying the tattooed node did not vary with body mass index. Additionally, we did not find a learning curve for surgeons or radiologists in the study. This is not surprising as all participating surgeons and radiologists were breast specialists and experienced in the technique of sentinel node biopsy and node biopsy. This is reassuring as the technique has the potential for widespread adoption in clinical practice.

This study will lead to improved understanding of site and volume of injection, and factors that may affect identification of tattooed node. Multivariate analyses showed that identification rate of tattooed node is significantly less after NACT compared with primary surgery. These results mirror the early findings of decreased sentinel node identification rate after NACT.

In clinical practice, this technique can be used alone or in combination with clip placement. Based on the ex vivo study, we recommend that 0.2 to 0.4 ml of black dye is injected in the cortex at the time of FNA or core biopsy or after the biopsy results at a separate visit (both approaches are feasible as shown in this study). Alternatively, to reduce the risk of diffusion of the dye into surrounding tissues with time, a clip can be placed in the involved node before NACT and the clipped node/clip can be tattooed before surgery for identification intra-operatively. The surgeon can stop after confirming removal of clip on specimen x-ray in case of multiple black nodes. This technique can be used in other settings such as to mark the non-palpable node in patients undergoing excision biopsy of the axillary lymph node.

There are other techniques that can be used to offer a targeted approach e.g. placing a radioactive iodine seed[16], magnetic seed (Magseed)[13], reflector (radar technology, SAVI Scout®) or radiofrequency tag (LOCalizer™) to mark the node[15]. However, these techniques are costly, require a probe that costs more than

£10000, apart from approximately £300 per patient cost for the implant and the implant can potentially migrate. Radioactive iodine seed has the disadvantages of limited availability, regulatory issues and decay in signal with time and therefore may be difficult to detect if placed in the node before NACT. Magnetic seed can interfere with MRI scan and there is an additional cost for disposable non-metal instruments during surgery. The size of the reflector (SAVI Scout®) or radiofrequency seed (LOCalizer™) is more than 10 mm which may be a limitation when marking small nodes[15].

The carbon dye technique has the advantage of low cost (around £10 for carbon dye per patient) and absence of need for special equipment. This technique will particularly be useful in countries with limited resources. However the identification rate is no different to that reported in the initial studies with blue dye guided sentinel node biopsy. We anticipate this to improve as the technique evolves in the future. Other disadvantages are black staining of the axillary tissue and dye migration in some patients although these risks can be minimised by restricting the volume of injection to 0.2 to 0.4 ml. Importantly, this technique has limited utility in patients who have skin tattoos in areas draining to the axillary nodes.

The limitations of this study are the small number of patients who underwent SNB following NACT and we did not evaluate the false negative rate of tattooed node as node-positive patients underwent ALND (as per local standard of care) rather than SNB or targeted axillary dissection. Other limitations are varying volume of dye injected and two different black dyes used in the study.

On the basis of the results of this study, it is feasible to mark the biopsied node with black dye and successfully identify it intra-operatively. Modifications of the technique, such as injecting the dye in the cortex alone, restricting the volume to 0.2 to 0.4 ml and combining with clip placement, will be expected to improve the technical ability to identify the marked node.

Acknowledgements

FUNDER: None

COLLABORATORS (in alphabetical order)

Derby and Burton-on-Trent: M Bagnall, KL Cheung, C Courtney, R Deb, M Diaz-Sotres, J Hatt, S Menon, J Robertson, J Rutledge, M Sibbering, C Rogers, A Turnbull, Y Wahedna, S William-Jones

Leicester: M Al-Attar, N Hartley, M Kaushik, F Kenny, J Krupa, K Lambert, S Pilgrim, W Sassi, S Shokuhi

Leeds: R Achuthan, M Fletcher, B Hogan, B Kim, S McKenzie, A Nielsen-Moody, P Turton

Plymouth: K Facey, S Ramzi, A Shajidevadas, E Toth

Compliance with Ethical Standards:

Funding: None.

Conflict of Interest: Amit Goyal has no conflicts of interest. Shama Puri has no conflicts of interest. Andrea Marshall has no conflicts of interest. Kalliope Valassiadou, has no conflicts of interest. Moin Hoosein has no conflicts of interest. Amtul R Carmichael has no conflicts of interest. Gabriella Erdelyi has no conflicts of interest. Nisha Sharma has no conflicts of interest. Janet Dunn has no conflicts of interest. Joanne York has no conflicts of interest.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent: Informed consent was obtained from all individual participants included in the study.

Reference List

(1) Puri S, Sharma N, Newcombe RG, Kaushik M, Al-Attar M, Pascaline S et al. Axillary tumour burden in women with one abnormal node on ultrasound compared to women with multiple abnormal nodes. *Clin Radiol* 2018; **73**(4):391-395.

(2) Donker M, van TG, Straver ME, Meijnen P, van de Velde CJ, Mansel RE et al. Radiotherapy or surgery of the axilla after a positive sentinel node in breast cancer (EORTC 10981-22023 AMAROS): a randomised, multicentre, open-label, phase 3 non-inferiority trial. *Lancet Oncol* 2014; **15**(12):1303-1310.

(3) Giuliano AE, Ballman KV, McCall L, Beitsch PD, Brennan MB, Kelemen PR et al. Effect of Axillary Dissection vs No Axillary Dissection on 10-Year Overall Survival Among Women With Invasive Breast Cancer and Sentinel Node Metastasis: The ACOSOG Z0011 (Alliance) Randomized Clinical Trial. *JAMA* 2017; **318**(10):918-926.

(4) Nathanson SD, Burke M, Slater R, Kapke A. Preoperative identification of the sentinel lymph node in breast cancer. *Ann Surg Oncol* 2007; **14**(11):3102-3110.

(5) Boughey JC, Suman VJ, Mittendorf EA, Ahrendt GM, Wilke LG, Taback B et al. Sentinel lymph node surgery after neoadjuvant chemotherapy in patients with node-positive breast cancer: the ACOSOG Z1071 (Alliance) clinical trial. *JAMA* 2013; **310**(14):1455-1461.

(6) Kuehn T, Bauerfeind I, Fehm T, Fleige B, Hausschild M, Helms G et al. Sentinel-lymph-node biopsy in patients with breast cancer before and after neoadjuvant chemotherapy (SENTINA): a prospective, multicentre cohort study. *Lancet Oncol* 2013; **14**(7):609-618.

(7) Simons JM, van Nijnatten TJA, van der Pol CC, Luiten EJT, Koppert LB, Smidt ML. Diagnostic Accuracy of Different Surgical Procedures for Axillary Staging After Neoadjuvant Systemic Therapy in Node-positive Breast Cancer: A Systematic Review and Meta-analysis. *Ann Surg* 2019; **269**(3):432-442.

(8) Hartmann S, Reimer T, Gerber B, Stubert J, Stengel B, Stachs A. Wire localization of clip-marked axillary lymph nodes in breast cancer patients treated with primary systemic therapy. *Eur J Surg Oncol* 2018; **44**(9):1307-1311.

(9) Nguyen TT, Hieken TJ, Glazebrook KN, Boughey JC. Localizing the Clipped Node in Patients with Node-Positive Breast Cancer Treated with Neoadjuvant Chemotherapy: Early Learning Experience and Challenges. *Ann Surg Oncol* 2017; **24**(10):3011-3016.

(10) Choy N, Lipson J, Porter C, Ozawa M, Kieryn A, Pal S et al. Initial results with preoperative tattooing of biopsied axillary lymph nodes and correlation to sentinel lymph nodes in breast cancer patients. *Ann Surg Oncol* 2015; **22**(2):377-382.

(11) Natsiopoulos I, Intzes S, Liappis T, Zarampoukas K, Zarampoukas T, Zacharopoulou V et al. Axillary Lymph Node Tattooing and Targeted Axillary Dissection in Breast Cancer Patients Who Presented as cN+ Before Neoadjuvant Chemotherapy and Became cN0 After Treatment. *Clin Breast Cancer* 2019; **19**(3):208-215.

(12) Park S, Koo JS, Kim GM, Sohn J, Kim SI, Cho YU et al. Feasibility of Charcoal Tattooing of Cytology-Proven Metastatic Axillary Lymph Node at Diagnosis and Sentinel Lymph Node Biopsy after Neoadjuvant Chemotherapy in Breast Cancer Patients. *Cancer Res Treat* 2018; **50**(3):801-812.

(13) Greenwood HI, Wong JM, Mukhtar RA, Alvarado MD, Price ER. Feasibility of Magnetic Seeds for Preoperative Localization of Axillary Lymph Nodes in Breast Cancer Treatment. *AJR Am J Roentgenol* 2019; **213**(4):953-957.

(14) Falcon S, Weinfurtner RJ, Mooney B, Niell BL. SAVI SCOUT(R) localization of breast lesions as a practical alternative to wires: Outcomes and suggestions for trouble-shooting. *Clin Imaging* 2018; **52**:280-286.

(15) Tayeh S, Gera R, Perry N, Michell M, Malhotra A, Mokbel K. The Use of Magnetic Seeds and Radiofrequency Identifier Tags in Breast Surgery for Non-palpable Lesions. *Anticancer Res* 2020; **40**(1):315-321.

(16) Donker M, Straver ME, Wesseling J, Loo CE, Schot M, Drukker CA et al. Marking axillary lymph nodes with radioactive iodine seeds for axillary staging after neoadjuvant systemic treatment in breast cancer patients: the MARI procedure. *Ann Surg* 2015; **261**(2):378-382.

TABLE 1 Clinicopathological characteristics of patients

Characteristic	Primary Surgery n=88 (%)	NACT n=22 (%)
Age y (median[range])	60 (31-88)	51 (34-78)
Body mass index (median[range])	28.8 (19.9-49.6)	30.7 (22.0-39.5)
Node positive on biopsy at presentation	43 (49)	18 (82)
Breast surgery		
Wide local excision	42 (48)	6 (27)
Mastectomy	45 (51)	15 (68)
No breast surgery	1 (1)	1 (5)
Axillary Surgery		
Sentinel node biopsy	44 (50)	4 (18)
Technique		
Isotope and blue dye	41 (93)	3 (75)
Isotope only	0	1 (25)
Blue dye only	3 (7)	0
Sentinel node identified		
Yes	42 (95)	4 (100)
No	2 (5)	0
No. of nodes removed with SNB, (median [range])	2 (1-4)	2 (1-3)
Axillary lymph node dissection	44 (50)	18 (82)
Pathology tumour size mm (median [range])	27 (0-135)	18 (0-102)
Multifocal or multicentric tumours	21 (24)	6 (27)
Tumour grade		
I	7 (8)	1 (5)
II	38 (44)	11 (50)
III	43 (49)	8 (36)
Not known	0	2 (9)
Tumour histology		
Invasive ductal	58 (66)	15 (68)
Invasive lobular	15 (17)	1 (5)
Other (mixed, tubular, mucinous, medullary, papillary)	15 (17)	4 (18)
Not known	0	2 (9)
Lymphovascular invasion		
Present	39 (44)	7 (32)
Absent	42 (48)	10 (45)
Uncertain or not known	7 (8)	5 (23)
ER status		
Positive	72 (82)	12 (54)
Negative	15 (17)	9 (41)
Not known	1 (1)	1 (5)
PR status		
Positive	54 (61)	8 (36)
Negative	21 (24)	6 (27)
Not known	13 (15)	8 (36)
HER2 status		

Positive	16 (18)	8 (36)
Negative	71 (81)	13 (59)
Not known	1 (1)	1 (5)

TABLE 2 Tattooing results

	Primary Surgery n=88 (%)	**NACT n=22 (%)**
Timing of injection of dye		
At the time of FNA or core biopsy	59 (67)	16 (73)
Separate visit	29 (33)	6 (27)
Volume of dye injected ml (median[range])	1.9 (0.2-4.2)	2.0 (1.0-4.2)
Site/s of injection of dye		
cortex, perinodal tissue, needle tract	6 (7)	2 (9)
cortex and perinodal tissue	82 (93)	20 (91)
Tattoo to surgery months (median[range])	0.9 (0.1-7.5)	6.5 (2.5-9.2)
Tattooed node identified	76 (86)	14 (64)
Sentinel node biopsy	36/44 (82)	3/4 (75)
Tattooed node was the sentinel node	28 (78)	3 (100)
Axillary lymph node dissection	40/44 (91)	11/18 (61)
Number of tattooed nodes (median[range])	1 (0-6)	1 (0-6)
Presence of diffuse black staining of axillary tissue at the time of surgery	19 (22)	12 (55)

FIG. 1 Surgeon removing a tattooed blue sentinel node

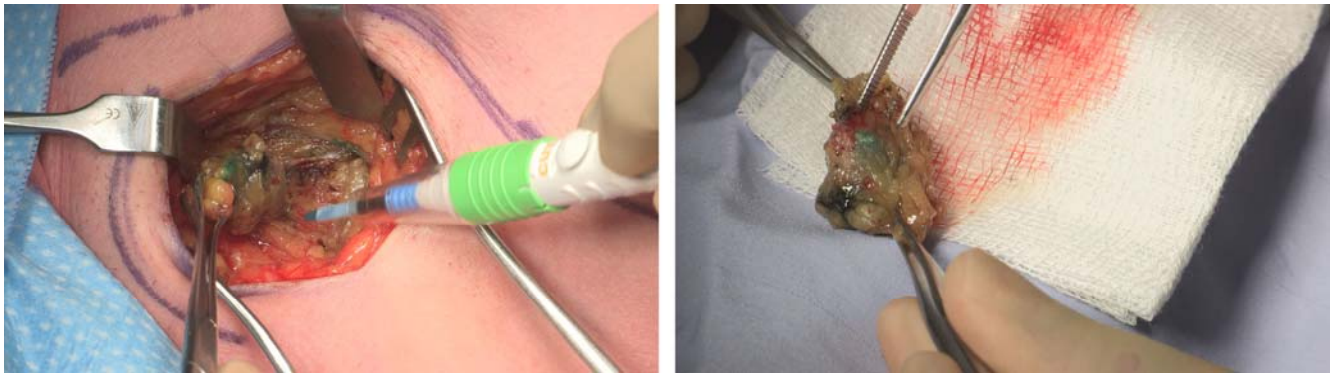


FIG. 2 Tattoo pigment in the lymph node capsule (A) and parenchyma (B) (haematoxylin-eosin x40)

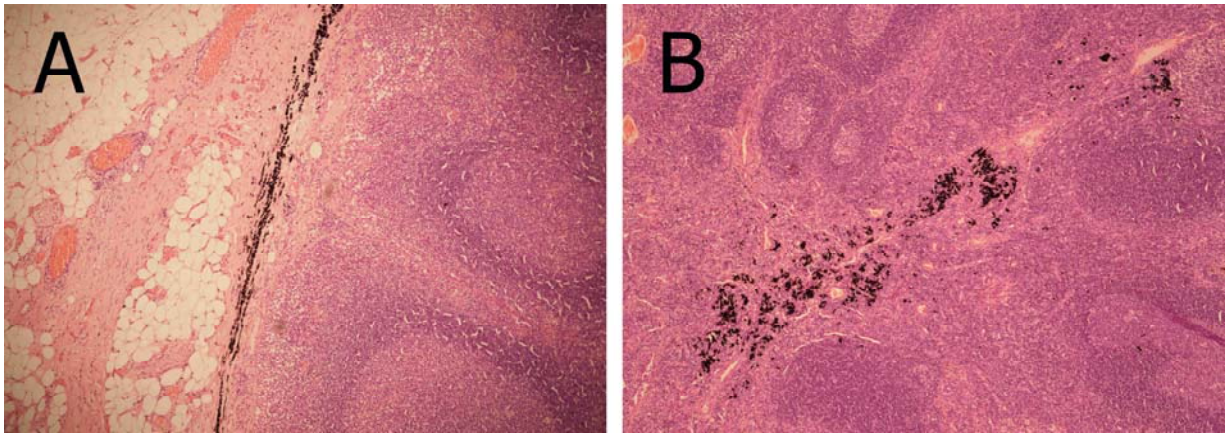


FIG. 3 Ex-vivo volume of injection and tattooing of nodes

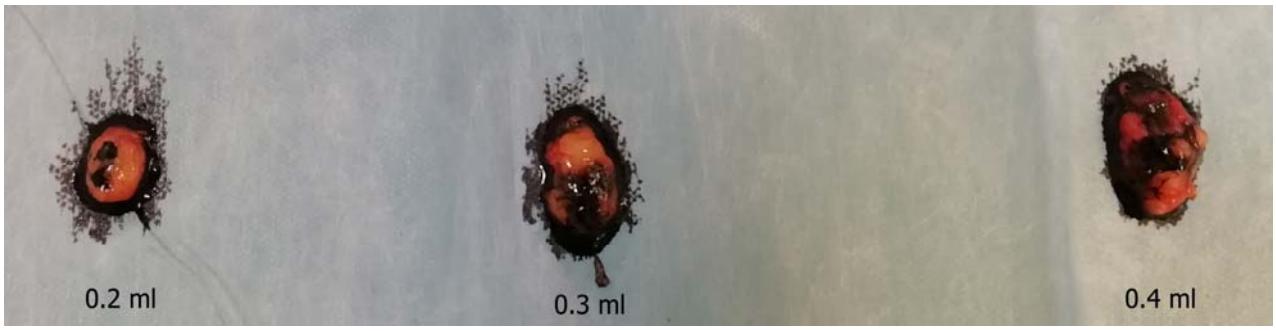
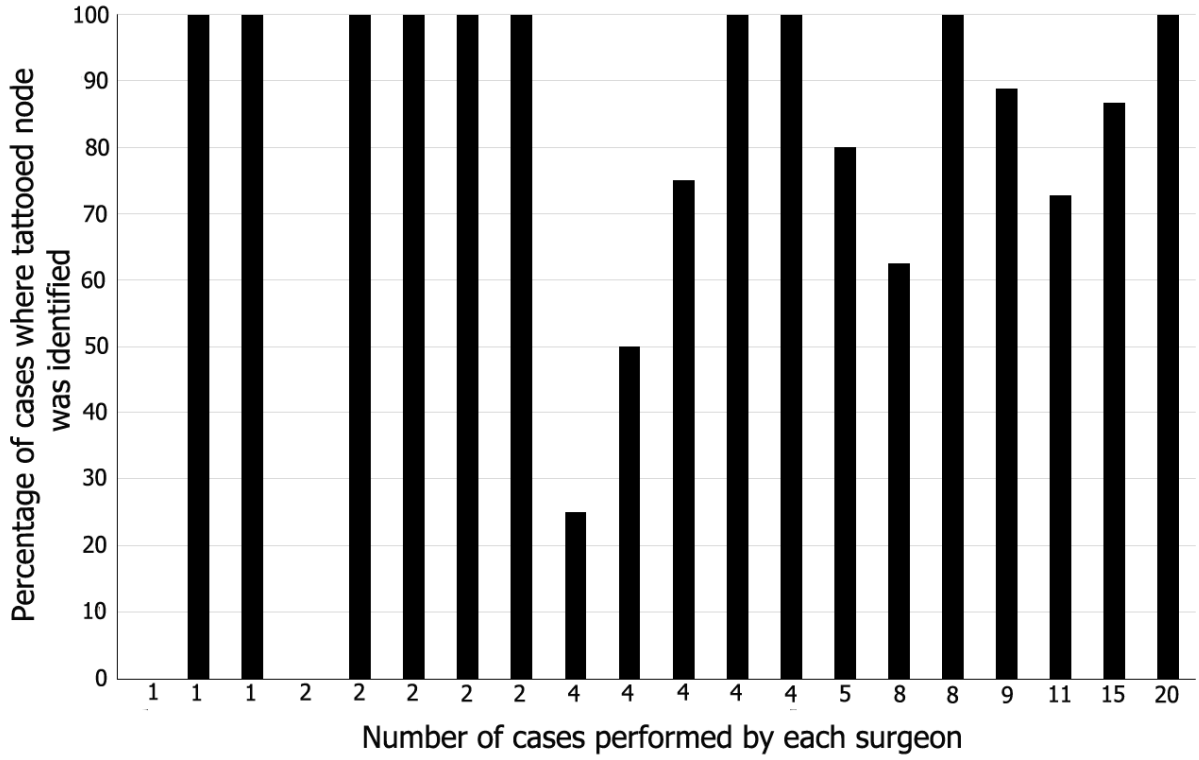


FIG. 4 Surgeon case numbers and identification of tattooed node



Two surgeons had zero percent identification rate of the tattooed node (0 of 1 total cases performed, 0 of 2 total cases performed)