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Original article

Multiple Step-section Frozen Section sentinel lymph node biopsy – A review of 717 patients

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ABSTRACT

Sentinel Lymph Node Biopsy (SLNB) is the standard of care for axillary staging in breast cancer. Multiple Step-section Frozen Section (MSFS) analysis is used in our institution for SLNB. This is performed intra-operatively by freezing sentinel lymph nodes to obtain multiple step-sections which are examined histologically for evidence of metastases. Patients whose sentinel lymph nodes contained macro-metastases proceeded to an axillary node clearance during the same operation.

717 patients over a two and a half year period had MSFS analysis. With regards to macrometastases, MSFS analysis had a sensitivity of 93.8%, a specificity of 99.3%, a positive-predictive value of 97.4% and a negative-predictive value of 98.2%.

MSFS analysis of sentinel lymph nodes is a safe and accurate procedure. It is a relatively cost-effective alternative to molecular technologies relying on DNA amplification and more accurate than standard frozen section or touch-prep cytology.

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Introduction

Sentinel Lymph Node Biopsy (SLNB) is currently a routine procedure that is the standard of care for axillary staging in early breast cancer. This reduces morbidity from unnecessary axillary lymph node dissection (ALND) in patients who are axillary node negative [1]. The American Society of Clinical Oncology 2005 guidelines recommend paraffin sections of the sentinel lymph node and staining with Haematoxylin & Eosin as the gold standard to assess SLNB [2]. However, a rapid and accurate intra-operative analysis of the sentinel lymph node (SLN) is useful as it allows the patient to avoid a second surgery and general anaesthetic as well as avoiding any delay in treatment.

Current practice for intra-operative analysis of SLNB samples varies – in a pan-European survey of 240 units, 69.7% used frozen section, 11.7% using frozen section and imprint cytology and 11% using imprint cytology alone [3]. In a 2011 review in the British Journal of Surgery, frozen section sensitivity ranges from 57 to 74% and specificity ranges from 99 to 100% [4]. In the literature, protocols for frozen section are not standardised and thus difficult to be compared.

Our institution performs intra-operative Multi Step-Section Frozen Section (MSFS) analysis of the SLN for early breast cancer. We believe that analysing the SLN with multiple sections as well as the considerable experience of our histopathology scientists and consultants have afforded us superior results compared to other reports on frozen section analysis. We thus present our experience and results of this technique in a large consecutive series of 717 patients.

Materials and methods

Patient selection

A retrospective review of all breast cancer patients who had SLNB from July 2008 to December 2010 were included in this retrospective review. The period of study was chosen to begin from

Abbreviations: MSFS, Multiple Step-section Frozen Section; ALND, axillary lymph node dissection; SLN, sentinel lymph node; WLE, wide local excision; DCIS, ductal carcinoma in-situ; RT-PCR, Reverse Transcriptase Polymerase Chain Reaction; OSNA, One Step Nucleic Acid Amplification.

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the time of reliable electronic patient records in the department of histopathology up to the start of this review.

Sentinel lymph node biopsy

The axillae of all breast cancer patients were evaluated with triple assessment – clinical and radiographic/sonographic assessment followed by core biopsy or fine needle aspiration cytology. If axillary staging on triple assessment was clear, the patient was scheduled for SLNB during the same operation for primary resection of their breast cancer (either wide local excision or mastectomy). Patent Blue V dye was used in localising the SLN – this involved sub-dermal infiltration of the ipsilateral nipple-areolar complex and subsequent breast massage over 2 min to disseminate the dye to the axilla.

All lymph nodes containing blue dye or non-blue clinically involved nodes encountered during exploration of the axilla were resected and sent for MSFS analysis. During the interim whilst waiting for results from MSFS, primary resection of the breast cancer was performed.

Histopathology

Excised SLNs were immediately conveyed to the laboratory from theatre. The peri-nodal fibro-fatty tissue was carefully removed to avoid any damage to the lymph node capsule. If the lymph node was less than 5 mm in diameter it was taken for frozen section examination without slicing. If between 5 mm and 10 mm, the lymph node was bisected along its longitudinal axis and both parts taken for frozen section examination. Lymph nodes more than 10 mm in diameter were sliced longitudinally into sections each 5 mm and all taken for frozen section examination. If two or more sentinel lymph nodes were sent, all lymph nodes were examined accordingly. The lymph node tissue was embedded in medium used for freezing the tissues (Cellpath, Hemel Hempstead, England). Sections were rapidly frozen and cut at once upon the cryostat. At least three microtome sections of 3–4 μm thickness were taken, placed onto labelled slides and rapidly stained with haematoxylin and eosin.

To speed the processing of each SLN, two scientists were employed (one involved in the stage of preparation and sectioning and the other responsible for the staining) so that multiple lymph nodes could be rapidly processed into multiple step sections on slides. MSFS slides were then submitted to the consultant histopathologist who examined each section for macrometastases (tumour deposits $\geq$2 mm in size) and micrometastases ($<$2 mm in size). Based on quality of the specimens as determined by the histopathologist or clinical suspicion from the operating surgeon, further step sections were obtained to look more closely for metastases. If the diagnosis was still in doubt, the opinion of a second histopathologist was obtained.

Any remaining nodal tissue was fixed in formalin and processed into 2 mm paraffin blocks over the next two days. The paraffin blocks and the MSFS slides were then reviewed by the original histopathologist to issue the formal report.

Immediate ALND or further treatment

Results of the MSFS were telephoned direct from the histopathologist to the operating surgeon. If the LNs contained macrometastases, the patient was treated with ALND during the same operation. If the LNs contained only micrometastases, the decision to proceed to ALND was based on the surgeon's discretion and the patient's clinical presentation. Patients whose SLNB was clear did not receive any further immediate treatment to their axilla. If the residual nodal tissue in paraffin blocks or if the MSFS slides on the second examination were found to contain metastases, the patient was recalled for a delayed ALND; otherwise, the patient received further axillary treatment according to their breast cancer histology and staging.

Analysis

A retrospective review of 717 consecutive breast cancer patients over two and a half years was undertaken. Patient details, SLNB status, residual nodal tissue status, immediate or delayed ALND status were recorded from data maintained by the department of histopathology. Data was recorded on Microsoft Excel 2007 and statistical analysis was performed on Graphpad Instat version 3.10. Accuracy, sensitivity, specificity, positive predictive value and negative predictive value of MSFS were calculated with a positive SLNB defined as having macrometastases and with the results of Paraffin Sectioning as the gold standard for diagnosis of nodal metastases.

Results

A two and a half year retrospective review was conducted of 717 consecutive patients (7 male, median age 60; range 23–89) who underwent SLNB from 15th July 2008 to 23rd December 2010. A total of 1129 lymph nodes were retrieved with an average of 1.57 LNs resected per patient (see Table 1 for full breakdown) – the minimum of zero lymph nodes was due to one patient with no lymph nodes found on MSFS or on paraffin block diagnosis.

The majority of operations consisted of 520 wide local excisions (WLE) accounting for just under two-thirds (64.7%) of positive SLNBs. With regards to other tumour characteristics, positive SLNBs were mainly identified in invasive ductal carcinomas, Grade 2 tumours or T1c tumours (i.e. 10–20 mm in size). Forty-two patients (26.9%) underwent SLNB for ductal carcinoma in-situ and one patient had a positive SLNB on MSFS (high grade DCIS). Other details of the operations performed and tumour characteristics are detailed in Table 2.

Macrometastases (see Table 3)

693 patients (96.7%) had a blue SLN identified. 156 out of the total 717 patients (21.8%) were found to have macrometastases on MSFS. However, four of the 156 patients had no further metastases detected on paraffin blocks – this was due to tumour tissue being entirely cut out during MSFS and these four patients were regarded to have micrometastases. 561 patients (78.2%) had no metastases on MSFS and 10 patients were later found to have metastatic deposits seen on paraffin block analysis.

Micrometastases (see Table 4)

MSFS also detected micrometastases in eight patients (1.11%). Paraffin blocks revealed that one of the eight patients did not have

Table 1
SLNB breakdown.

	Total SLNs per patient	Blue SLNs (metastases)	Blue SLNs (clear)	Non-blue SLNs (metastases)	Non-blue SLNs (clear)
Patients	–	149	572	17	80
LNs	1129	177	827	18	107
Min	0	0	0	0	0
Max	7	3	5	2	6
Median	1	0	5	2	6

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