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Original article

Breast cancer metastasis burden in sentinel nodes analysed using one-step nucleic acid amplification predicts axillary nodal status

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ABSTRACT

Background: In breast cancer patients undergoing sentinel lymph node biopsy (SLNB) analysis using one-step nucleic acid amplification (OSNA), clarity is required as to the risk factors for non-sentinel lymph node (NSLN) involvement upon axillary lymph node dissection (ALND). This study aims to identify these factors, including categorising by extent of sentinel node positivity: solitary positive node (solitary), multiple nodes with some positive (multiple incomplete positive), or multiple nodes all of which are positive (multiple all positive).

Methods: We conducted a cohort study using prospectively collected data on 856 SLNBs analysed using OSNA, from patients with $cT_{1\text{-}3}$ clinically node-negative invasive breast cancer. ALND was performed for 289 positive SLNBs.

Results: NSLN metastases were identified in 73 (25.3%) ALNDs. Significant factors for NSLN involvement on multivariate analysis were: SLNB macrometastases (cytokeratin-19 mRNA count >5000 copies/μl) (adj.OR = 3.01; 95% CI, 1.61–5.66; p = 0.0006), multiple all positive vs. multiple incomplete positive SLNB (adj.OR = 2.92; 95% CI, 1.38–6.19; p = 0.0050), and undergoing mastectomy (adj.OR = 1.89; 95% CI, 1.00–3.55; p = 0.0486). Amongst multiple incomplete positive SLNBs, an 8.8% NSLN risk was identified when only micrometastases were present.

Conclusion: Extent of sentinel lymph node positivity measured using OSNA predicts NSLN metastasis risk, aiding decisions surrounding axillary treatment.

Introduction

In breast cancer patients, axillary node status has been established as the single most important predictor of disease-free and overall survival [1–3]. With the advent of sentinel lymph node biopsy (SLNB) [4], and its introduction as a method of evaluating the axilla in breast cancer [5], accurate intraoperative staging of the axilla became possible without the need for the more radical axillary lymph node dissection (ALND) in those with negative SLNBs [6–8]. Current National Institute for Health and Care Excellence (NICE) guidelines recommend that patients with a positive SLNB undergo completion ALND, justified by evidence from a meta-analysis (comprising 7765 positive SLNBs) demonstrating metastases in non-sentinel lymph nodes (NSLNs) in 53% of cases [9,10]. However, the American College of Surgeons Oncology Group (ACOSOG) Z0011 trial has challenged the necessity of completion ALND, demonstrating no difference in 5-year overall or disease-free survival between SLNB positive patients randomised to either completion ALND or watchful waiting with delayed ALND if indicated [11]. Despite these findings, controversy continues to exist over the use of completion ALND, due to the Z0011 trial failing to accrue a sufficient sample size to generate adequate statistical power, the sample being unrepresentative of typical breast cancer patient populations, and limitations in the trial's methodology and radiotherapy techniques [12,13]. Current opinion remains divided over the need for completion ALND, with many authors identifying

Abbreviations: SLNB, sentinel lymph node biopsy; OSNA, one-step nucleic acid amplification; NSLN, non-sentinel lymph node; ALND, axillary lymph node dissection; WLE, wide-local excision.

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the need for further randomised-controlled trials, while advocating assessment on an individual patient basis in the interim until a consensus is reached [14,15].

One-step nucleic acid amplification (OSNA) is an established molecular technique for the rapid assessment of whole lymph nodes intraoperatively. A positive OSNA analysis on a SLNB specimen has been demonstrated to predict NSLN metastasis with similar reliability to conventional histology techniques [16,17]. A recent systematic review of OSNA accuracy as part of a NICE diagnostics guideline identified that OSNA is likely to be equally cost-effective or more cost-effective than histological assessment of sentinel nodes [18]. However, a subsequent systematic review and economic evaluation of similar data, has not corroborated the cost-effectiveness of OSNA in comparison to histological assessment [19]. It has been difficult to quantify the effect on the overall breast cancer patient treatment pathway. For SLNBs analysed histologically, there has been a great deal of research attempting to quantify risk of NSLN involvement, allowing the generation of predictive nomograms [20]. However, for patients whose SLNB analysis has been performed using OSNA, greater clarity is required as to the specific factors that quantify the risk of further axillary involvement. According to Halsted's theory, breast cancer metastasis is thought to involve step-wise progression. Applying this principle in breast cancer spread, if a negative node is also present in a positive SLNB specimen, there is unlikely to be further metastases in the ALND. Consequently this study classified positive SLNB samples according to extent of sentinel lymph node positivity: SLNBs with only one lymph node, which contains metastases (solitary), SLNBs with multiple lymph nodes, with some (but not all) containing metastases (multiple incomplete positive), and SLNBs with multiple nodes, all containing metastases (multiple all positive). This study aims to determine whether extent of sentinel lymph node positivity analysed using OSNA could guide the extent of further axillary dissection.

Materials and methods

Sample and setting

Subjects of this cohort study were female patients with cT1-3, N0, M0 primary breast cancers on tumour, nodes, metastasis (TNM) staging (following clinical and radiological assessment), who underwent SLNB with intraoperative evaluation by OSNA. All patients underwent axillary ultrasound to assess for nodal disease. Patients were excluded from the study if they had no evidence of invasion/microinvasion on primary tumour histology, if they had undergone previous non-oncological breast surgery on the ipsilateral breast, or if they had received neoadjuvant chemotherapy. Data were collected prospectively between December 2008 and December 2012, to generate a total of 856 SLNB procedures on 844 patients (12 patients underwent bilateral SLNBs for bilateral invasive breast cancers). A consort diagram has been developed to clarify sample generation (Fig. 1). The study was carried out at the Royal Surrey County Hospital (RSCH), a district general hospital and specialist tertiary centre for cancer care and pathology.

Surgery

Surgical management of the breast cancer patient was performed in accordance with NICE 2009 guidelines [9]. Preoperatively the radioisotope technetium-99m sulphur colloid is injected into the peri-areolar tissues, and a lymphoscintigram is performed to aid sentinel lymph node localisation. Intraoperatively, isosulfan blue dye is also injected into peri-areolar tissues, and the SLNB is performed with the aid of a gamma probe. Level 1 ALND was performed for all micrometastases (1+ POS and INHIBITED POS OSNA outputs), and level 3 ALND was performed for all macrometastases (2+ POS OSNA output). Primary tumour resection was either performed by wide-local excision (WLE) or total mastectomy.

Pathology processing

The OSNA procedure was performed on SLNB samples as described previously (Snook et al. 2011), using the RD100I OSNA system (Sysmex, Kobe, Japan). The molecular marker, cytokeratin-19 (CK-19) mRNA, has been demonstrated to be positive in 98.2% of breast cancers [21]. Quantification of CK-19 mRNA into three categories: >5000 copies/μl, 250–5000 copies/μl, and <250 copies/μl, generated similar categories of metastatic spread to those created in conventional histology preparations: macrometastasis (2+ POS), micrometastasis (1+ POS), and negative lymph node respectively (Tsujimoto et al. 2007). Occasionally the reaction can be inhibited when the sample is tested neat, perhaps by adipose tissue within the sample. Therefore a dilute sample is analysed in parallel, and assessed if the original sample test is negative. If the dilute sample is positive the result is classified as INHIBITED POS. These inhibited samples are also considered micrometastases, as they display a similar risk profile to 1+ POS results (see Table 1).

Primary tumour and ALND specimens were examined as per National Health Service Pathology Reporting of Breast Disease (NHSBSP) guidelines [22]. Primary tumour histopathological factors identified for this study include: histological sub-type (invasive ductal carcinoma (IDC), invasive lobular carcinoma (ILC), papillary or other), size (mm), grade (low = 1, intermediate = 2, high = 3), oestrogen receptor (ER) status (positive or negative), progesterone receptor (PR) status (positive or negative), human epidermal growth factor receptor 2 (HER2) status (positive or negative), presence of multifocality, and presence of lymphovascular invasion (LVI). For NSLNs from ALND specimens, the number examined, and the number of metastases were recorded.

Statistical analysis

Positive SLNB specimens were identified and classified according to extent of sentinel lymph node involvement. The categories used were: solitary positive node in the SLNB (solitary); multiple lymph nodes in the SLNB, of which only some are positive (multiple incomplete positive); and specimens containing multiple lymph nodes, all of which are positive (multiple all positive). Total CK-19 mRNA copy number was also calculated through the summation of CK-19 copy numbers for all positive lymph nodes. Presence or absence of metastases in the ALND sample was the dependent variable for all analyses.

Univariate analyses were performed using binary logistic regression, on the following variables: Extent of SLNB positivity, age, primary operation type (WLE/mastectomy), number of positive sentinel lymph nodes, OSNA result (macrometastases vs. micrometastases), primary tumour histopathological factors (see previous), and total CK-19 copy number. Wald tests were performed for categorical variables with more than two categories for ease of representation. A step-wise binary logistic regression using Akaike information criterion (AIC) was performed as a multivariate analysis for all factors found to be significant on univariate analyses. The adequacy of model fit was evidenced using a receiver operating characteristic (ROC) curve (Area Under the Curve (AUC) = 76.0%) and a Hosmer–Lemeshow plot.

Separate Chi-squared tests and Fisher's exact tests were performed analysing extent of SLNB positivity for samples containing macrometastasis or those with solely micrometastases. A subset