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The use of onestep nucleic acid amplification (OSNA) and tumour related factors in the treatment of axillary breast cancer: A predictive model☆

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Abstract

Aims: We aimed to determine the effectiveness of CK19 mRNA copy number and tumour related factors in predicting non-sentinel axillary nodal involvement, in order to facilitate the formulation of local treatment guidelines for axillary clearance (ANC) following intra-operative analysis of the sentinel node biopsy (SNB) using one-step nucleic acid amplification (OSNA).

Methods: Patients due to have (SNB) at our institution for breast cancer as well as patients with high grade ductal carcinoma in situ with pre-operative negative assessment of the axilla were included. Alternate slices of each node were sent for assessment by either OSNA or histopathology. Immediate ANC was performed if OSNA was positive. The CK19 mRNA nodal copy number, the total tumour load (TTL) measured by summation of mRNA copy numbers of all positive nodes, the nodal status at ANC and tumour characteristics for each patient were recorded. A model of risk probability was constructed using TTL and tumour related factors.

Results: 664 nodes were analysed from 425 patients who had SNB performed between 2011 and 2014. ANC was performed on 105 of these patients. The concordance between OSNA and histology was 91.4% and negative predictive value (NPV) was 97%.

TTL ($p = 0.003$) and LVI ($p = 0.04$) were identified as risk factors for non-sentinel nodal involvement. The risk probability model identified all patients with pN2 disease for ANC.

Conclusion: In the future a decision to perform ANC will be based on a risk stratification model based on TTL and tumour related factors.

Keywords: Breast cancer; Sentinel node analysis; One-step nucleic acid amplification assay; Axillary clearance; Axillary metastasis; Axillary staging

Introduction

Molecular assay techniques used have been shown to be an accurate and reliable tool to assess axillary sentinel lymph node status. The current most widely used molecular assay, One Step Nucleic acid Amplification (OSNA), developed by Sysmex®, utilises Cytokeratin 19 (CK19) alone as a marker.[4] OSNA has been validated in our own practice and elsewhere[1–10] and combined analysis of similar studies gives an overall negative predictive value (NPV) of 98%.[9]

Meta-analysis by Teirnan et al. reviewed some of the published literature comparing OSNA to histopathology.[11] Any differences between the two methods are accounted for by the fact that OSNA is more sensitive than histopathology in detecting micro-metastases.[11]

More than 200 centres are currently using OSNA globally with the majority of these based in Europe with centres also in Asia-Pacific region.

Both whole node and half-node analysis are used, where alternate slices of the sentinel node are processed through either standard histopathology techniques or OSNA. Both

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techniques have been validated and are included in the current UK guidelines.[12]

In addition, OSNA may also provide a rational method in terms of decision-making for omitting axillary node clearance in the presence of positive sentinel nodes,[12] as it is a quantitative technique that lends itself to the role of surrogate marker for axillary nodal involvement. In the last 2 years, the relationship between CK19 mRNA copy numbers as a molecular measure of tumour load in the sentinel lymph node and prediction of non-sentinel nodal involvement in the axilla has been under investigation. Several studies have shown that increasing tumour load in the sentinel node appears to predict the likelihood of non-sentinel node involvement based on CK19 copy number threshold values[13–21] (Supplementary Table A). Several of the studies agree that mRNA copy number thresholds higher than those attributed to a positive result by the manufacturer need to be considered as a threshold for ANC.[18,20,21,23] Cut off thresholds for detecting more than 1 additional non-sentinel node have been suggested to lie at 7700 mRNA copy number (using whole node analysis)[15] but this would result in a false negative rate of 17.4%, which is not likely to be acceptable to clinicians or patients. If TTL is considered, a 15,000 mRNA copy number has been suggested as threshold for performing ANC.[17] While this has a high NPV (85.5%), there would still be a substantial proportion of patients with axillary metastases who would fall below this level.

Histopathology assessment of the SNB is able to predict the likelihood of axillary involvement by the size of the metastases with estimated risk of 50% for metastases greater than 2 mm.[22] It has so far not been used to predict the number of involved nodes present. In contrast, summation of copy numbers for all positive nodes using OSNA, known as the total tumour load (TTL) has been strongly suggested to predict the number of nodes involved.[10,19,23–26]

Additionally, the role of tumour biology in axillary involvement cannot be ignored.[18] Factors identified as significant in the development of non-sentinel nodal include tumour grade, ratio of involved sentinel lymph nodes, and sentinel lymph node involvement pattern (ITCs vs micro-metastasis/macro-metastasis),[27] sentinel lymph node metastasis greater than 2 mm, and lympho-vascular invasion in the primary tumour.[28] Tumours less than 20 mm and without lymphatic invasion do not appear to lead to non-sentinel nodal involvement.[29] A model that includes, TTL and tumour related factors may provide a patient-specific risk assessment.

Aims

This study expands on previous work, aiming to facilitate the formulation of guidelines for ANC or equivalent treatment through a more detailed investigation of the relationship between TTL and non-sentinel nodal involvement, in consideration with tumour characteristics.

Patients and methods

The methodology has been previously described in our initial publication.[24] Briefly women due to have SNB in the Breast Unit of Royal Free Hospital in London for a pre-operative diagnosis of breast cancer as well as selected patients with high grade ductal carcinoma in situ (DCIS) were selected for study. Patients treated with neo-adjuvant treatment prior to surgery were excluded. All patients had been assessed pre-operatively through triple assessment and ultrasound, with fine needle aspiration cytology (FNAC) of the axilla when necessary, and were not found to have involved axillary nodes. All patients gave consent to have ANC if the intra-operative assessment of the sentinel node biopsy by OSNA was positive. If immediate ANC was carried out on clinical suspicions alone, were excluded from further analysis.

As previously published[24] SNB was performed using the combined technique of blue dye and radioactive isotope for node localization.[30] The harvested, freshly received, sentinel lymph nodes were cleared from extra-nodal fat tissue, weighed and measured. Lymph nodes weighing more than 600 mg were further subdivided into two samples and separate analyses were carried out. However, the final result was recorded for the whole node and conveyed to the surgeon intra-operatively.

The lymph nodes were cut into 1.5–2 mm thick parallel slices along their long axis (bread-loafing) and processed alternately so that half of the slices were used for immediate OSNA amplification and half were fixed in 10% formal saline for routine histological analysis.

The OSNA method was performed with the gene amplification detector RD100i (Sysmex®, Kobe, Japan) according to previously reported protocols based on the manufacturer's instructions.

According to previously described cut off values for OSNA, macro-metastases (++) were defined as >5000 copies/μL of CK19 mRNA, micro-metastases (+) as 250–5000 copies/μL and the sentinel node was considered as negative when the CK19 mRNA result was <250 copies/μL. This meant that isolated tumour cells would have been below the set threshold for positivity.

As previously,[22] for histology, the slices were embedded in sequence with the top cut surface of one section and the bottom surface of the next section represented. Histological assessment, was performed by two breast pathologists, and the protocol used included examining the initial H&E section then step sections taken at 200 μm until the tissue has been exhausted. This is to enable detection of metastasis greater than or equal to 0.2 mm, which may be located at one pole of the lymph node. The metastatic tumour load was measured in two dimensions and the metastases were classified according to the 7th Edition of the American