Can OSNA® CK19 copy number predict nodal status when axillary dissection is not performed?

'The OSNA® CK19 mRNA copy number in the SLN was the only independent predictor of ≥ 4 LN having metastases.'

Ohi *et al.* 2012 [1]

Introduction

For over 450 000 women each year across Europe, a diagnosis of breast cancer is the start of a difficult and stressful journey. Healthcare professionals work diligently to provide the best possible treatment and outcome for all patients. This work has focussed on improving the prognosis while minimising side-effects, and the continuing trend towards minimally invasive surgery has, rightly, been part of this focus. The Almanac Trial [2] was key to the introduction of Sentinel Lymph Node Biopsy (SLNB) into guidelines. However, it was severely impacted by the fact that analysis of Sentinel Lymph Nodes (SLNs) could only be done post-operatively by histopathology or intra-operatively by either imprint cytology or frozen section, both of which are of poor sensitivity.

One Step Nucleic Acid Amplification (OSNA®) was introduced to meet the need for accurate intra-operative analysis of SLNs following the acceptance of SLNB as the surgical gold standard. It was designed to meet the expectations of the main worldwide guidelines in detecting micro- and macrometastases by giving an indication for the size of metastatic tumour burden. Intra-operative analysis had not been previously accepted into guidelines due to the lack of sensitivity of conventional techniques. OSNA®, however, has changed this scenario and is now recommended in several European guidelines.

'Whole node analysis should be fully implemented in local clinical practice to reduce the risk of tissue allocation bias.'
NICE Guidance 2013 [3]

OSNA® is becoming increasingly established as the standardised approach for intra- or post-operative analysis of the whole sentinel node in breast cancer patients. To date, more than 200 OSNA® systems are in routine use in Europe. At the time of writing, over 95 000 patients throughout Europe have benefitted directly from the use of this technology.

Many centres are looking to introduce OSNA® on the basis of its well-established benefits:

- Accurate and standardised staging of patients
- Highly sensitive and specific method
- Data from different publications support the prediction of non-sentinel node involvement
- The aim to eliminate second surgeries
- Support of earlier access to additional essential therapies
- Lower psychological pressure for both negative and positive patients
- Significant improvement in managing patient care pathways
- Reductions in waiting lists and bed stays
- Ability to increase rate of immediate reconstructions
- Positive effect on hospital costs

'The Committee concluded that, (…) it was likely that the RD-100*i* OSNA® system was equally or more cost effective than post-operative histopathology.'
NICE Guidance 2013 [3]

OSNA® beyond informed intra-operative surgical decisions

Controversy remains concerning the Z0011 [4] and AMAROS [5] trials. The limitations of these studies are widely discussed and have recently been addressed by Jagsi *et al.* [6], Bundred *et al.* [7] and Zellars [8].

After the uncertainties surrounding these trials, the importance of the standardised OSNA® analysis has become even more relevant, and not only for surgical decisions. Many are concerned that replacing Axillary Lymph Node Dissection (ALND) with radiotherapy means that vital staging information is lost.

'The AMAROS Trial cannot answer the remaining question of which subset of clinically node-negative, sentinel node positive patients still require axillary treatments.'
Donker *et al.* 2014 [5]

As clinical opinion is evolving, the ability to make differential therapeutic decisions based on nodal tumour burden and to question the assignment of 'micrometastases' or 'macrometastases' or simply the number of involved nodes is even more important. Key to this decision is access to the most accurate and standardised SLN analysis to provide the best information about the axillary status and how to treat the patient.

'The question that needs to be asked is: if there is no benefit to axillary clearance for nodal micrometastases, what threshold of sentinel involvement should lead to further axillary treatment?'
Bundred *et al.* 2014 [7]

However, most recently, whether micrometastatic SLN has a significant impact on survival and whether patients with such minimal involvement should undergo ALND has been addressed by Shimazu [9], Ogiya *et al.* [10] and Babar *et al.* [11].

'In patients with micrometastases, 15% (20/136) had further positive NLSNs and a further 6% (8/136) had > 4 overall positive nodes (SLN + NSLD) thus requiring adjuvant supraclavicular/chest wall radiotherapy.'
Babar *et al.* 2014 [11]

Nevertheless, the accuracy of OSNA® has been clearly established [12–18]. OSNA® was developed to distinguish between 'positive' and 'negative' and between the histological definition of 'micrometastasis' and 'macrometastasis'. Currently, most OSNA® users apply the CK19 copy number concept, reflecting the nodal tumour burden, as predictive tool for further non-sentinel involvement.

This prompted the question:

Can the CK19 copy number provide staging information that would not be available in cases in which ALND is not performed?

The answer: Leading publications indicate yes.

A number of publications including Ohi *et al.* [1], Di-Filippo *et al.* [19], Peg *et al.* [20], Heilmann *et al.* [21], Williams *et al.* [22] and Deambrogio *et al.* [23] have investigated the OSNA® copy number as a standardised tool for predicting non-sentinel involvement.

Conclusions include:

- OSNA® can predict axillary node status better and independently of the number of affected SLNs [20]
- The OSNA® CK19 mRNA copy number in the SLN was the most significant predictor of non-SLN metastases [1]
- The OSNA® CK19 mRNA copy number ($\geq 1.0 \times 10^5$) in the SLN was the only independent predictor of ≥ 4 LN having metastases [1]
- OSNA® CK19 copy number seems to be a solid parameter for a reliable nomogram guiding surgical decisions intra-operatively [19]
- We suggest that the CK19 mRNA copy number could be the only parameter to consider in the intra-operative management of the axilla [23]

OSNA®
CK19

These publications provide a range of compelling evidence to use OSNA® in a manner which supports personalised surgical and non-surgical treatment of the patient. Data shows that OSNA® CK19 copy number is able to provide more standardised and accurate differentiation. OSNA® seems to be the best tool to answer questions that are still open in the event that only the SLN result is available to support clinical decisions.

'(…) tumour burden in the sentinel lymph node is the most powerful predictor of non-sentinel node positivity and in the prediction of patients who have 4 or more positive lymph nodes, a group of patients whose prognosis is significantly worse, require additional radiotherapy field treatment (to the supraclavicular fossa) and who make up $8-13\%$ of the patients in the Z0011 and AMAROS studies.'

Rayter 2015 [24]
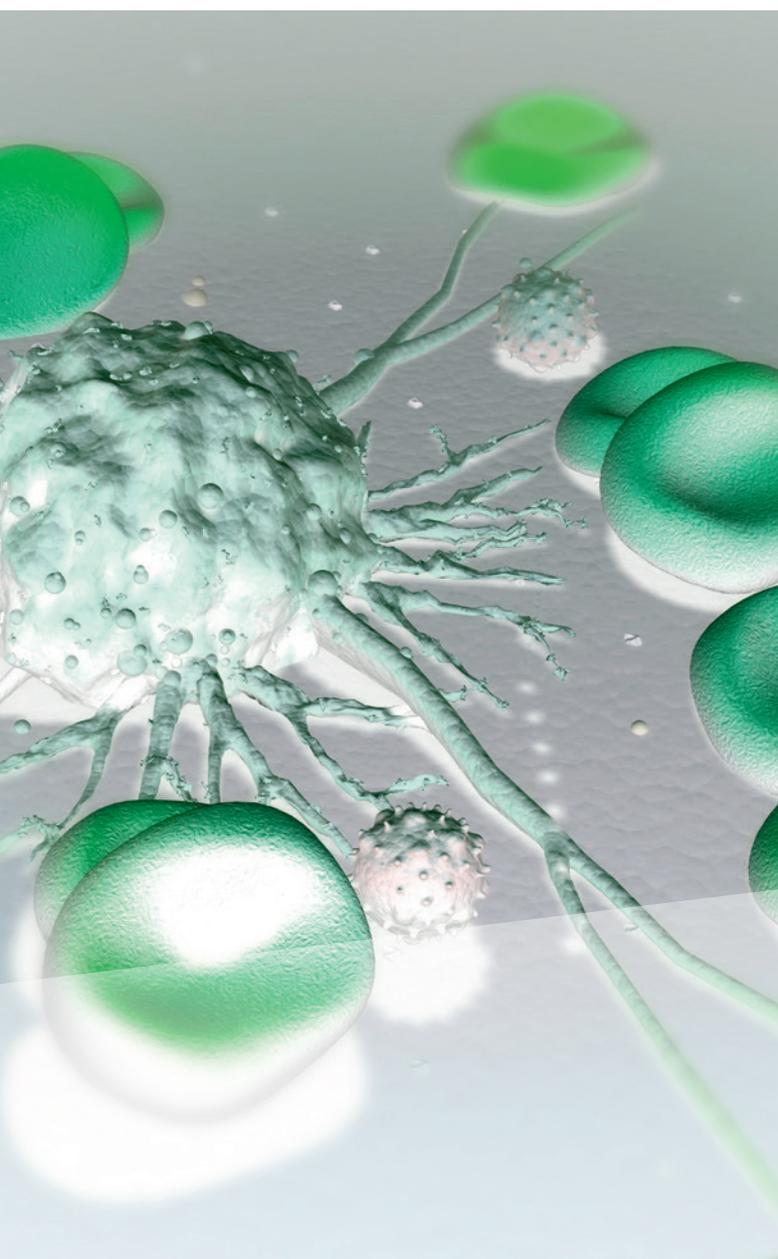
OSNA® in intra-operative setting

OSNA® can be used in both an intra- or post-operative setting. However, the widespread use of OSNA® is based in the intra-operative setting because of the many additional patient and service benefits.

Any OSNA® centre can testify that the overwhelming majority of patients want a one-step surgical procedure. Additionally, some hospitals receive patient referrals on the basis that they provide standardised whole node analysis in an intra-operative timeframe.

Patients with a negative result can be informed in the recovery room and do not need to live with the stress of waiting for a delayed post-operative result at the clinic.

The limitations of conventional histopathology such as poor sensitivity, the need for post-operative confirmation and the issue of tissue allocation bias is well known and reviewed by Cserni et al. [25]. In contrast, OSNA® shows sensitivity and specificity of approx. 97 % compared to intensive post-operative histology and does not require post-operative confirmation [12 – 18].

A whole node positive OSNA® result makes it possible for immediate surgical decisions to be taken, thereby effectively eliminating second surgeries. Patients can proceed to further therapy immediately. It also allows differential decisions to be taken on the basis of micro- or macrometastatic nodal tumour burden. If your policy is not to clear micrometastases, following an OSNA® result, you will have a standardised whole node result to support that decision.

Mathematical models have been developed to predict NSLN metastasis in patients with positive SLN. Breast cancer nomograms might include several clinicopathological variables such as tumour size, histology, tumour grade, lymphovascular invasion, hormone receptors, and the number of positive nodes [26]. OSNA® CK19 copy number results have been incorporated as a central variable in most recent nomograms, and may be used even intra-operatively in a simple combination with tumour size [19].

Conclusion

The OSNA® system is a standardised method for accurate SLN analysis, providing accurate nodal tumour burden. An increasing number of publications show that the OSNA® result has grown to become a powerful predictive tool for further axillary involvement, supporting intra-operative surgical decisions as well as subsequent adjuvant therapies. Nevertheless, the final treatment decision is dependent on the physicians' approach:

1. Priority is to select patients who can be safely spared from ALND, with high negative predictive value (NPV) and sensitivity: **low CK19 copy number cut-off** [21, 23]

2. Priority is to avoid as many unnecessary ALNDs as possible, with high specificity and high positive predictive value (PPV): **high CK19 copy number cut-off** [20, 27]

'(…) molecular techniques such as OSNA® procedure can improve breast cancer patients' management, because it allows discriminating between eligible and ineligible patients for lymphadenectomy or other axillary procedures.'
Piñero-Madrona 2014 [27]

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