

Review

Pathological validation and significance of micrometastasis in sentinel nodes in primary breast cancer

Rajendra S Rampaul*, Ahmed Miremadi†, Sarah E Pinder†, Andrew Lee† and Ian O Ellis†

*Department of Surgery and †Department of Pathology, Nottingham City Hospital, Nottingham, UK

Correspondence: Dr Ian O Ellis, FRCPath, Reader in Pathology, City Hospital, Nottingham, Hucknall Road, Nottingham NG1 5PB, UK. Tel: +44 115 969 1169; fax: +44 115 962 7768; e-mail: ian.ellis@nottingham.ac.uk

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Abstract

In embracing a multidisciplinary approach to the management of patients with sentinel node biopsy in breast cancer, the pathologist task is to screen sentinel nodes for possible metastasis. The consequences of missing sentinel node micrometastasis can directly influence treatment strategies, and this screening therefore has to be performed with more attention than usual. There is presently great diversity in the histopathological work-up of sentinel nodes, with many centres employing additional techniques such as immunohistochemistry, reverse transcription polymerase chain reaction or flow cytometry in addition to routine haematoxylin and eosin staining. In this review, we address the pathological validation and significance of micrometastasis in sentinel node biopsy in primary breast cancer.

Keywords: micrometastasis, sentinel nodes

Introduction

The pathological methods used to assess axillary lymph node disease are of paramount importance because lymph node status is a powerful prognostic factor at the time of diagnosis. It has been shown through numerous studies that routine histological examination of dissected nodes may be inadequate depending on the thoroughness of examination [1,2]. Sapir and Amromin were, in 1948, the first to demonstrate this. They showed that, in 30 patients whose lymph nodes were reported as negative by standard histopathological methods using haematoxylin and eosin (H&E), re-examination of multiple sections revealed 10 patients (33%) had metastatic disease [1]. Pickren [2], working on lymph node specimens from 199 patients, similarly found that 22% of those free of disease on routine histopathological examination had occult metastasis using serial sectioning. The presence of such metastatic disease by serial sectioning has been shown to confer a significant

adverse effect on recurrence and survival in some series. The major disadvantages, however, are the cost and labour implications in processing axillary lymph nodes through serial sectioning to search for occult disease.

Sentinel lymph node biopsy has rapidly emerged as a real surgical option in the management of primary breast cancer. The sensitivity of this method in axillary staging of breast cancer has been evaluated in numerous studies [3–10], and has been shown to depend on the pathological methods used to assess the sentinel lymph node. By reducing the number of lymph nodes to be studied, sentinel lymph node dissection makes more exhaustive histopathological examination possible. The optimal extent of the histopathological work-up is yet to be determined but, nonetheless, offers the possibility of examining lymph nodes in much greater detail without the drawbacks of cost and labour restrictions.

Pathological validation

There is presently no consensus for the optimal handling of sentinel nodes in the laboratory. Most centres have developed their own 'in-house' protocols, which are invariably tied in with the institution's research ambitions. This *ad hoc* approach unfortunately can influence interpretation of results, as the amount of material being assessed between centres would be non-uniform even if we were to ignore methodological differences.

In a number of large studies, using different histopathologic techniques, the sentinel lymph node false negative rate (defined as how often the sentinel lymph node is negative for malignancy when cancer is present in the rest of the axilla) has varied between 0 and 11%. For example, Giuliano *et al* [3] reported a false negative rate of 11% in a series of 174 patients. Their group obtained a frozen section intraoperatively and recommended taking permanent sections from at least two levels of the tissue block at 40 μm intervals for H&E staining and cytokeratin immunohistochemistry [4]. Krag *et al* [5] similarly reported a false negative rate of 11% in 405 patients. Lymph nodes were either bisected or sliced multiple times and one to three H&E sections were taken from the surface of each tissue block. Results of a more detailed examination of lymph nodes in 329 of these patients were reported by Weaver *et al* [6]. H&E stained deeper sections at 100 and 200 μm into the blocks, as well as cytokeratin immunohistochemical stains at 100 μm , were made and examined for all lymph nodes. The authors reported an overall decrease in residual axillary lymph node metastases from 13.3 to 11.1% after using deeper sections and immunohistochemical stains. None of the newly detected metastases were larger than 1 mm, and more than 60% of them had a diameter of less than 0.1 mm.

O'Hea *et al* [7], in a study of 55 patients at the Memorial Sloan-Kettering Cancer Centre, found a false negative rate of 13% using the routine pathological technique with a single section taken through each node. They reported using frozen section examination of the sentinel node for all patients with T1b or larger invasive cancers [8]. If the frozen section proved to be negative, the remainder of each sentinel lymph node is fixed and three additional sections are taken and stained by both H&E and immunohistochemistry.

Veronesi *et al* [9] reported a false negative rate of 4.7% in a study of 163 patients and, in another study from the same centre, Viale *et al* [10] reported a false negative rate of 7% in 155 patients. All sentinel lymph nodes in the latter study were bisected and frozen. Fifteen pairs of adjacent frozen sections were cut at 50 μm intervals (a total of 60 sections per lymph node). Additional pairs of sections were cut at 100 μm intervals whenever residual tissue was left. One section of each pair was stained with H&E and the other for cytokeratins. Non-sentinel lymph nodes were

examined by standard histopathological techniques. Just 35.7% of positive nodes were detected on levels taken beyond the second pair of sections, and so would be missed if only one or two central sections were examined. All metastatic foci were identified on H&E stained sections, although in three cases cytokeratin immunoreactivity was used to confirm the metastatic nature of cells. Viale *et al* [10] concluded that the intraoperative examination of axillary sentinel lymph nodes using serial sectioning is feasible and effective in predicting the axillary lymph node status.

A number of studies have tried, however, to address the question of optimal histopathologic examination of sentinel lymph nodes by comparison of different protocols performed on the same sample. Turner *et al* [4] examined 60 sentinel nodes, step sectioned at 10 levels separated by 40 μm , and stained by H&E and cytokeratin immunohistochemistry (IHC). Nine sentinel nodes (15%) showed metastases in the first two levels, but study of levels 3–10 revealed only two additional metastases (3%). In another study, Cserni [11] reported the results of serial sectioning and IHC staining of sentinel lymph nodes from 58 patients. Nodes were sectioned serially up to extinction of the blocks and every 10th to 20th level was examined. After every sixth section taken for H&E, one was taken for IHC staining. The study showed that, in 8 out of 26 (31%) positive nodes, the metastases could not be found if only one or two central sections were examined. IHC staining caused the detection of only two of these metastases. While Zhang *et al* [12] reported that taking three levels at approximately 25, 50 and 75% of the tissue blocks discovered almost all metastases, the same strategy in the study of Cserni [11] would have missed 15% of the metastatic foci. van Diest [13] recommended, in an accompanying editorial, four additional levels at 250 μm intervals with H&E and IHC staining when the first-level H&E section is negative.

A closer look at the literature interestingly reveals that the Italian group of Veronesi *et al* [9] do not 'lose' tissue to the molecular laboratory as do Giuliano *et al* [3]. The significance of interpreting results from such heterogeneous methodologies, where not all tissue is assessed histopathologically, is clearly highlighted in a recent paper by Dowlatshahi *et al* [14] who subjected the entire sentinel lymph node from 52 patients to analysis and found significant occult disease. Although the results of Dowlatshahi *et al* require prospective confirmation in larger studies, it nonetheless emphasises the need for uniformity in examining sentinel lymph nodes.

We at Nottingham City Hospital employ the MRC ALMANAC protocol to assess all SNB. These nodes are cut into slices about 3 mm thick, taken perpendicular to the long axis to maximise the assessment of the marginal sinus, with one node per cassette. We have found in our experi-

ence that the majority of nodes can be completely embedded in one cassette. Larger nodes have alternate slices embedded and may require more than one cassette. Very large obviously involved nodes have one section taken. This approach is consistent with recommendations of the National Health Service Breast Screening Programme [15].

Some authors have advocated intraoperative frozen sections or imprint cytology of axillary lymph nodes. Conventional frozen sections, we believe, have an unacceptably high false negative rate of 10–30% [16–19]. More intensive intraoperative assessment with serial sections and immunohistochemistry has been described, but this is time consuming and labour intensive. A frozen section might be appropriate in selected cases; for example, if the node is macroscopically abnormal and this is confirmed histologically to be metastatic carcinoma, further axillary surgery can be performed immediately. Some studies have found low false negative rates of 2–3% with intraoperative imprint cytology [16,20], but not all have been able to achieve this level of accuracy [18]. Most breast carcinomas can be diagnosed preoperatively, and we extremely rarely perform frozen section diagnosis of the primary tumour. Our feeling is that frozen section and imprint cytology assessment of axillary nodes is also inappropriate.

Reverse transcription polymerase chain reaction (PCR) is even more sensitive than immunohistochemistry at detecting metastatic tumour [21,22]. Two types of method can be used to detect tumour cells with molecular techniques. Firstly, a genetic defect such as chromosomal rearrangement or mutation can be identified. The problem with this method is that no genetic defect is seen in all breast carcinomas. The second method is based on a molecular marker that is transcribed by tumour cells, but not by the adjacent tissue. To obtain a marker that has this specificity and is expressed in the majority of tumours is difficult. It may be necessary to use a panel of markers. A major problem with PCR is a high false positive rate due to the sensitivity of the method. It is also not possible with PCR to determine whether the DNA comes from a viable cell. An advantage of H&E sections and IHC is that the morphology of the cells can be examined and malignancy confirmed. A major unresolved question is whether carcinoma detected only by PCR is of prognostic significance.

Clinical significance of micrometastasis

There is no universally agreed definition of micrometastasis. Huvos *et al* defined micrometastasis as metastatic foci less than 2 mm in diameter [23] and this is still widely used. In fact, the fifth edition of the AJCC staging manual uses the same definition; however, the arbitrary cutoff point varies between 0.2 and 2 mm in various studies.

The detection rate of micrometastases in axillary lymph nodes has been reported to range from 9 to 46% [14,24].

Studies have used serial sectioning with or without immunohistochemical stains for the detection of micrometastatic foci and, as described, these methods have been shown to have a definite impact on detection rates.

Despite all this, the clinical significance of micrometastasis is yet to be determined. Nodal micrometastases appear to have a small but significant adverse effect on distant recurrence and survival in some studies, but not in all [16]. It is imperative that the prognostic significance of nodal micrometastases be determined, as there are important implications for systemic adjuvant chemotherapy and hormonal therapy. The International (Ludwig) Breast Cancer Study [25], one of the largest micrometastases studies performed to date, showed that both disease free interval and overall survival were significantly affected by the presence of micrometastatic disease at 5 and 6 years of follow-up. At 6 years median follow-up, DFS was reduced from 71% in the node negative group to 53% in micrometastatic node positive patients ($P=0.0008$). The overall survival was 80% in the node negative group and 70% in the node positive group ($P=0.0009$) [26]. Rosen *et al* found that patients had poorer disease free interval and overall survival at 10 years with nodal metastasis smaller than 2 mm. However, this described a group with T1 tumours, whereas no survival disadvantage as such was found in T2 cancer [27]. Present-day data of sentinel lymph node micrometastases do not possess sufficient follow-up to observe any survival effects as some retrospective studies have shown, and therefore the prognostic significance of such occult metastasis at this time is unknown.

Conclusion

The traditional 'Halsted' view of breast cancer was that there was a logical sequential pattern of spread of the carcinoma, and this was used to justify radical surgery. Fisher *et al* [28] and other workers have more recently proposed that carcinoma of the breast is often disseminated at the time of diagnosis, and that nodal metastases are merely a marker of systemic disease. There is a trend towards less radical surgery to both the breast and the axilla associated with this philosophical change. Axillary sentinel lymph node biopsy is thus a reversion to the 'Halsted' philosophy, while at the same time continuing the trend towards less radical surgery. In reality, the truth probably lies somewhere between the 'Halsted' and 'Fisher' models. Two central questions particularly require answers from clinical trials: whether patients with a negative sentinel axillary lymph node(s) can safely avoid further axillary surgery, and the clinical significance of micrometastases. Of critical importance to both these questions is the way in which the pathologist should assess the node biopsy, and thus how negative and positive nodes are defined. The cost in time, equipment and consumables needs to be compared for sentinel node biopsy and other forms of axillary surgery. Comparison of the effectiveness of sentinel node biopsy

with axillary node sampling also needs to be addressed, and this forms part of the UK ALMANAC study.

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