Many patients survive breast cancer. Until newer, probably molecular, indicators are determined, axillary lymph node status remains the key parameter that determines both disease-free and overall survival in patients with breast cancer. Approximately 70% of women with operable breast cancer will have negative axillary lymph nodes. Of those patients with T1N0 tumours (ie tumours less than 20mm in diameter and with negative lymph nodes) 70% will have an excellent prognosis with surgery alone. Thirty percent however, will develop metastatic disease and die within 10 years. It has been suggested that this subgroup of node negative patients who do develop metastases may have clinically and pathologically ‘occult’ metastases in their axillary lymph nodes. While certainly not the entire story, in retrospective studies of histologically negative axillary lymph nodes, up to 25% of cases have shown metastatic disease on further, more detailed assessment of the nodes. Metastases are missed because they are not actually included in the initial haematoxylin and eosin stained section of the node, or sometimes they are present, but are not identified by the pathologist for one reason or another, such as with metastatic lobular carcinoma, when the tumour cells are scattered and resemble surrounding lymphocytes.

There have been a large number of protocols aimed at more thorough pathological examination of axillary lymph nodes for metastatic tumour deposits. These have mostly involved cutting more deeply into the nodes. This has ranged from one extra section, to taking extra sections at every 100µm right through the node, to taking one further level at 500µm or various combinations of those. A range of immunohistochemical stains for keratins and other epithelial markers has also been used to try to highlight (metastatic) epithelial cells in axillary lymph nodes to make the cells more readily seen by the pathologist. The more thoroughly a node is analysed, the more likely secondary tumours are to be found. Cutting levels through lymph nodes and performing immunohistochemical stains is time consuming and expensive and the amount of effort applied has to be proportional to the expected yield.

Size of axillary node metastases

As the natural history of breast cancer has changed – or more correctly, has been brought forward with the introduction of screening mammography – the median tumour size at diagnosis has decreased, and so too has the incidence of metastases giving a reduced disease-free survival both at five years and 10 years of follow-up. It will be interesting to see what influence deposits less than 0.5mm across have at 15 and 20 years.

Sentinel lymph nodes

The sentinel lymph node is the first node that drains the tumour. Using radioisotopes and coloured dyes, sentinel axillary lymph nodes are being identified with increasing accuracy. As sentinel nodes are more likely to contain metastases than non-sentinel nodes, detailed and accurate assessment of these nodes is now performed routinely. Formerly-occult secondaries have been found in 12-29% of sentinel node biopsy specimens from patients with T1 tumours (those less than 20mm across).

If the sentinel node is involved, should further dissection of the remainder of the axilla occur? Yes, until a lot more information is known about the significance of both sentinel nodes and axillary micrometastases. One study showed that non-sentinel lymph node involvement was significantly less for patients in whom the sentinel node metastasis measured less than 1mm across (16 vs 36%, p = 0.02). While evaluating lymph nodes from an axillary dissection specimen with deeper levels and immunohistochemistry is a relatively time consuming and expensive exercise, detailed examination of sentinel nodes is not much to ask. The only question is how to best examine them.

As a start, sentinel lymph nodes should be submitted for histology in their entirety. They should then be cut into approximately 2mm thick slices and embedded. Cutting through the node in this way often means that less sectioning will be required later at histology. Some groups recommend intraoperative assessment of sentinel lymph nodes. This is because approximately 25% of patients can then proceed to an immediate axillary dissection, thus avoiding a second operation. With frozen section, the reliability has been reported to be about 65%. However, there is the not insignificant concern that substantial amounts of tissue may be lost while facing into the node, and that smaller metastases may be missed. With intraoperative imprint cytology, multiple cut surfaces can be examined quickly, without loss of tissue, but there is a significantly higher chance of indeterminant results.

Deciding how far apart to have the step sections through the node and at how many levels to have immunohistochemical stains for epithelial cells largely depends on the size of the micrometastasis that you are concerned about. If you are concerned about 2mm metastases, the sectioning would be
further apart than if you were concerned about 0.2mm metastases. In a study we performed of 208 cases of breast cancer which had negative axillary lymph nodes on the initial haematoxylin and eosin assessment, occult metastases were found in 25% of cases. Each block was sectioned at four levels, each separated by 100µm and each level was stained with H and E, MNF116 an anticytokeratin antibody and the antibody BC2, reactive with MUC1 epithelial mucin core protein. If only the first (at 0µm) and fourth (at 300µm) levels were analysed, which would have represented a considerable saving in labour, 96% of the metastases would have been detected. Eleven percent of the metastases were present as small, scattered deposits and a further 55% were less than 0.5mm in diameter. The remaining 34% were greater than 0.5mm in diameter.

The Australia and New Zealand guidelines for sentinel node biopsy recommend that if the initial haematoxylin and eosin stained section is negative, that four levels be cut at 500µm intervals and that an anticytokeratin antibody be used on the first level. While approximately 25% of our cases would have been missed if the 0.5mm step sectioning had been used, that only occult metastases 0.5mm in diameter or greater affected survival, means that missing the smaller occult metastases may not have mattered clinically, at least in terms of 10 year survival analysis. Thorough assessment of one or two sentinel nodes, the results of which will determine whether or not subsequent surgery is performed, is certainly warranted. A prospective study of sentinel node occult metastases in 200 patients with breast cancer examined the entire lymph node at 0.25mm intervals (including with immunohistochemistry) and showed occult metastases in 25% of cases. The mean size for the largest H and E-detected metastases was 0.75mm. These values appear quite large, especially when compared with the 1mm mean size on H and E, and 0.1mm size on immunohistochemistry, as described by Turner et al.

**Location of metastases in nodes**

There has been discussion with respect to the different prognostic significance of occult metastases in various locations within the node. Carter et al considered malignant cells in the extracapsular sinus of the lymph node to be an example of passive transportation and without prognostic significance, unlike so-called active migration of tumour cells into lymph node tissue proper. Hartveit et al however, were more concerned about micrometastases in the capsular lymphatics than those in the substance of the node. Those in the substance of the node, they found, were associated with a better prognosis, which was similar to that of patients with node negative disease. As well as being essentially the first port of call for metastatic disease, the sentinel node, in effect, is also a gate-keeper for the tumour cells. If the tumour cells are not retained there for any length of time, the ‘sentinel’ function is lost. If tumour cells are ‘passively transported’ along lymphatics, including sentinel node subcapsular lymphatics, the opportunity for an immune attack or containment of the metastatic tumour cells in the sentinel node may be lost. Large numbers of cases would be required to clarify this issue.

**Lobular carcinoma**

Invasive lobular carcinoma generally shows a histological pattern of single cell invasion, rather than the glands and tubules characteristic of invasive ductal carcinoma. If invasive lobular carcinoma is associated with axillary lymph node metastases, the pattern of invasion is similar to single cells. These scattered single cells can be very hard to distinguish from surrounding lymphocytes in lymph nodes and so the false negative rate for metastatic invasive lobular cancer is quite high. In our series, 25% of invasive ductal carcinomas had occult metastases compared with 38% of invasive lobular carcinomas. The lower rate (6%) for cancers of special type probably reflects their usually less aggressive clinical course. As the rate for occult metastases is so high with invasive lobular carcinomas, all histologically negative axillary nodes from such cases, whether they are sentinel nodes or not, should have as a minimum, immunohistochemical staining of at least one further section. de Mascarel and Trojanii found occult metastases in 41% of 89 cases of invasive lobular cancer. However, while disease-free survival was reduced in their patients with invasive ductal carcinoma, no effect was seen in those with invasive lobular carcinoma, similar to our findings. Many factors, as well as axillary lymph node status, affect long-term survival in patients with breast cancer.

**Bone marrow metastases**

The most common site for breast cancer metastases is bone marrow and up to 80% of patients with metastatic breast cancer will have bone marrow involvement. Also, as many as 40% of patients with primary, operable breast cancer, are thought to have tumour cells present in their bone marrow. The presence of occult metastases in the bone marrow has been shown to be unrelated to the presence of lymph node metastases, although that may be a reflection of how extensively the lymph nodes are examined. Bone marrow micrometastases were associated with distant metastases (<0.001) but not with local recurrence (p = 0.77). While further prospective studies are required, it may be prudent to consider bone marrow aspiration in patients with negative sentinel lymph nodes, to document if metastatic disease is present. However, as some clinical trials have shown, a survival advantage in women receiving adjuvant treatment, regardless of their nodal status’ detection of occult metastases either in the axilla or the bone marrow may be less important.

**Further studies**

Three large prospective studies are underway which will provide further very useful information about axillary lymph node metastases. The National Surgical Adjuvant Breast and Bowel Project (NSABP) B-32 trial plans to accrue 4,000 patients with breast cancer. The Minimally Invasive Molecular Staging of Breast Cancer Study (MIMS) will study 1,130 women with breast cancer. Real-time RT-PCR analysis of sentinel nodes, axillary lymph nodes, bone marrow and peripheral blood will be examined. It is hoped that in these studies, very detailed analysis of the axillary lymph nodes will be undertaken, so that the true prognostic importance of micrometastases can be determined.

**References**


