Editorial

Histopathological workup of sentinel lymph nodes: how much is enough?

The sentinel lymph node biopsy is rapidly gaining popularity as a staging procedure for several solid tumours such as melanoma,[1 2] vulvar carcinoma,[3] penile cancer,[4] colorectal cancer,[5] neuroendocrine skin cancer,[6] squamous cell cancer of the scrotum,[7] and particularly breast cancer.[8–10] It enables selective targeting of the first tumour draining lymph node, where the initial metastases will form. Conceptually, a negative sentinel node predicts the absence of tumour metastases in the other regional lymph nodes with high degree of accuracy. If this hypothesis were true, regional lymph node dissection could be avoided in the case of a negative sentinel node. Apart from the obvious savings in the overall costs of surgical treatment, this would also prevent the many side effects of complete lymph node dissection.

Evaluations of the sensitivity and specificity of the sentinel node procedure are still ongoing for different tumour types and different node regions. However, in The Netherlands, and also in other countries, the sentinel node biopsy is increasingly used as a definite staging procedure in stage I/II breast cancer. Only in case of a tumour positive sentinel node on histopathological investigation is axillary lymph node dissection performed. For melanoma, systemic dissemination investigation is done only in case of a positive sentinel node, and when no distant metastases are found, regional lymph node dissection is performed subsequently.

The task of the pathologist is to screen sentinel nodes for possible metastases. In view of the consequences of missing sentinel node micrometastases (omission of lymph node dissection may lead to untreatable local tumour outgrowth in tumour bearing lymph nodes that have been left behind) there is a general feeling that this screening has to be done with more attention than usual, but how much is enough? In this editorial I shall attempt to provide some guidelines on this, after reviewing theoretical considerations and assessing the results of recent studies that have provided information on the tumour load of sentinel nodes. It has to be borne in mind that accurate staging by the pathologist can only be fully realised when the surgical technique is impeccable. Although it is beyond the scope of my article to discuss this, the best surgical results seem to be achieved by combining the blue dye and radioactive tracer techniques and performing preoperative lymphoscintigraphy.

Finding tumour cells in sentinel nodes—theoretical considerations

Suppose we want to find a single tumour cell in a sentinel node. An average sentinel node is about 1 cm in size. Normally, it would be halved before embedding, leaving two pieces of 0.5 cm. When cutting 4 μm thick sections, a total of 1250 sections could be produced from this sentinel node. Assuming an average tumour cell diameter of 15 μm, this tumour cell would be in four to five sections. Taking a single random section, the chance of finding this tumour cell would be 4–5/1250 = 0.3–0.4%. When we take random multiple sections, theoretically up to 1246 sections may be necessary to find the tumour cell. Taking sections at regular intervals is much more efficient, since we would always find the tumour cell at a step interval of 12 μm (every fourth section), which would mean 312 sections. This is of course still too much work, so a 100% sensitivity cannot be achieved, for practical reasons. Fortunately, there will usually be more than a single cell. The chance of finding one tumour cell will then increase, although not linearly as some tumour cells may be in the same section. For instance, preliminary sampling experiments indicate that when 10 sections are systematically taken (1 in 128) the chance of a positive section in case of a single, five, and 10 tumour cells is 3.7%, 15.5%, and 24.1%, respectively.

Often, there will be groups of tumour cells. A group of 10 average tumour cells will have a diameter of about 32 μm. Such a group will always be found with a step size of 36 μm, or every 9th section, which would mean 139 sections for an average sentinel node. A group of 20 average tumour cells will have an average diameter of about 41 μm. Such a group will always be found with a step size of 44 μm, or every 11th section, which would mean 114 sections for an average sentinel node. These examples make it clear that, assuming a random distribution of tumour cells through the lymph node, it is easier to find one of a certain number of single cells than a group of an equal number of tumour cells. In several studies, metastases with a diameter smaller than 2 mm have been regarded as micrometastases. In an average sentinel node, a group of tumour cells with a diameter of 2 mm would always be found with three step sections at regular intervals. In the setting of the sentinel node, a metastasis of 2 mm in diameter is relatively large.

The above assumes a random distribution of tumour cells through the sentinel node. It has been suggested that there may a preferential entry of tumour cells through the contralateral side of the hilum of lymph nodes.[11] This would have important consequences, as bisecting the long axis of the sentinel node from outer capsule to hilum would produce two cut surfaces from the midline region where the first single tumour cells or small groups of tumour cells would be readily visible. Experimental evidence for this theory is, however, lacking, and in practice it is quite difficult to identify the hilum.

Histopathological work up—how much is enough?

In practice, there will often be mixture of single cells and cells in groups. As the average number and distribution of tumour cells in sentinel nodes of different organs is not known, it is quite difficult to estimate the cost–efficiency of different ways of sentinel node investigation. How much work should we spent on a single sentinel node for what yield? There is a general consensus that sentinel nodes deserve more attention than usual, including step sections and immunohistochemistry (IHC). In different cancers, step haematoxylin and eosin (H&E) sections and IHC increase the percentages of metastases found in lymph

nodes in general by about 10% and 20%, respectively (for an overview see Van Diest *et al*[12]). There is, however, no consensus between different studies as to how many step sections are needed, and what the step size should be. Recent studies have provided breast cancer sentinel node data that are useful in arriving at evidence based guidelines in this respect, including one in this issue of the *Journal of Clinical Pathology*.[13]

Turner *et al* examined 60 sentinel nodes by step H&E sections and cytokeratin IHC at 10 levels separated by 40 μm.[11] Levels 1 and 2 yielded additional micrometastases in nine sentinel nodes (15%), but in levels 3–10 only two further metastases were found (3%). They therefore recommended that only two levels should be studied, separated by 40 μm. However, their sentinel node slices were 2–3 mm thick, so even with 10 levels at 40 μm, only 400 μm is investigated, which accounts for no more than 13–20% of the sentinel node slices. Although they attempted to cut the sentinel nodes through the hilum, this seems to be insufficient, and may explain the disappointing yield of this procedure. Rather than taking many sections at small intervals, it may be more efficient to take fewer sections at larger step intervals. In the study by Cserni,[13] sentinel nodes were serially sectioned and every 10th to 20th level was examined by H&E and/or immunohistochemistry. A central cross section through the sentinel node would have failed to detect metastases in eight of 26 lymph nodes (31%), leading to a false negative sentinel node status in six of 21 patients (29%). The proportion of metastases found increased from 69% with only a central cross section to 77% with five further steps, to 81% with 10 steps, and to 96% with 15 steps. Only at 45 steps, was 100% sensitivity found. From his figure 1 it can be seen that the smallest tumour deposit was about 25–50 μm in size, so the above theoretical scenario may be somewhat conservative. Cserni[13] states that with a three level approach at 25%, 50%, and 75% of the block, metastases would have been missed in 15% of patients. A previous non-sentinel node study of Zhang *et al* found almost all metastases with such a three level approach.[14] Our own sentinel node protocol[8 10 12 15] prescribes step sectioning at four further levels with an interval of 250 μm, with H&E and immunohistochemistry when the level 1 H&E section is negative (five levels in total). As we perform frozen section analysis routinely, which leads to some loss of material, this ensures sampling through a significant part of the sentinel node. In practice, this has proven to be an acceptable workload. In a series of 105 sentinel nodes, 10 (9.5%) were only positive in the final three levels (unpublished results). This clearly shows the need for extensive sampling when level 1 is negative. We do not attempt to cut through the hilum, as in our hands this is impractical.

There is still a need for larger studies on cancers from different organs with complete serial sectioning and IHC of sentinel nodes, as this would provide invaluable information on the distribution of tumour cells in these nodes. Until such results become available, we can use the information from the above studies to arrive at guidelines for sentinel node investigation.

Additional techniques for finding sentinel node metastases

Histochemical stains such as PAS and Schmorl for adenocarcinoma and melanoma cells, respectively, have insufficient sensitivity and specificify and cannot be recommended. Imprint cytology is under study as an additional method for the (intra- or postoperative) detection of lymph node metastases of prostate[16] and breast cancer.[15 17 18] So far, it is not yet clear that the sensitivity and specificity of imprints are high enough to be recommended for routine use.

Several non-morphological methods have been proposed for detection of sentinel node metastases (for an overview see Van Diest *et al*[12]). Flow cytometry has too low a sensitivity. The reverse transcriptase polymerase chain reaction (RT-PCR) has very high sensitivity as a single cell can be detected among 10^6–10^7 normal cells. It remains to be proven that such a sensitivity is clinically useful, and false positive results (for example, owing to contamination or benign inclusions) have been described.[19] RT-PCR cannot therefore yet be recommended for routine use.

Recommendations

Based on the above, the following recommendations can be made for sentinel node investigation. If desired, frozen section analysis can safely be performed on adenocarcinoma and squamous cell carcinoma sentinel nodes, while taking certain precautions.[15 21] For melanoma, this is probably not the case. In that case the sentinel node should be fixed in neutral buffered formaldehyde, lamellated according to its size, and completely embedded. Sentinel nodes smaller than 0.5 cm are processed and paraffin embedded intact, those between 0.5 and 1 cm are halved, and those larger than 1 cm are lamellated into pieces of approximately 0.5 cm in size. It is efficient to embed these different slices in the same block. One initial 4 μm thick H&E stained section is made per block. When negative, four further step ribbons are cut at an interval of 250 μm. One section from each ribbon is stained with H&E, and one is used for IHC (a section from the level 1 ribbon is also used for IHC). For adenocarcinomas, the CAM5.2 antibody (Becton Dickinson) can be recommended. In general, no background staining is seen with this antibody and its sensitivity is about 100%. Caveats are epithelial and mesothelial inclusions, which can be especially difficult to interpret in IHC sections; this is the reason for always making an H&E stained section. Sometimes staining of sinus lining cells may be observed, but these are easily recognised morphologically. For squamous cell carcinoma metastases, AE1/3 (Boehringer Mannheim) is a reliable antibody. It has high specificity and sensitivity. For melanoma metastases, the S100 and HMB45 antibodies (Dako) are used in combination. S100 is very sensitive for melanoma because almost all melanomas are S100 positive, but it is not specific as it also stains dendritic cells. HMB45 is very specific but it is only positive in a proportion of melanomas (low sensitivity). It may be difficult to discriminate melanoma cells from capsule naevus cells. The latter cells, however, clearly lie within the lymph node capsule, and show no atypia or mitoses. In order to avoid false negative and false positive IHC results, appropriate positive and negative controls should be applied. Although IHC increases the costs of consumables for the sentinel node investigation, to examine well stained IHC sections for small tumour deposits is much faster for the pathologist than lengthy scrutiny of H&E sections, and represents a saving of the pathologist's time.

Even this intensive protocol will miss some micrometastases, as explained above. However, the question arises as to whether a higher sensitivity will ever have clinical significance, because second echelon breast cancer metastases are almost never found when the true sentinel node is negative by extensive histopathological investigation with step sections and immunohistochemistry.[20] At the same time, more intensive pathological evaluation of the sentinel node will reveal micrometastases that would normally not have been detected. Particularly in breast cancer, finding these micrometastases turns a patient into "lymph node

positive" (upstaging) and usually means that the patient will receive adjuvant treatment. This is questionable, as there is hardly any difference in prognosis between patients with micrometastases and "really" lymph node negative patients. For colonic cancer and melanoma, the situation may, however, be different. Large clinical trials are required to assess the value of adjuvant treatment in patients with sentinel node micrometastases.

Conclusions

Being a reasonable compromise between sensitivity on the one hand and time and money spent on the other, the above recommendations may serve as guidelines for sentinel node workup for different kinds of tumours. IHC is a standard technique that is available in every pathology laboratory. Although the histopathological workup of the sentinel node requires more time and dedication from the pathologist, it should be borne in mind that there will be fewer axillary lymph node dissections specimens to handle, which saves time. However, for the quality of future sentinel node trials it is crucial that the sentinel nodes (and non-sentinel nodes) are processed and examined according to precisely formulated protocols and that a proper budget be reserved for such examination. The extra money spent on such intensive but definitely worthwhile workup should be no problem, as overall the sentinel node procedure will probably save costs.[21] Further studies are necessary to establish the role of sophisticated non-morphological techniques for detecting sentinel node metastases, such as RT-PCR.

PAUL J VAN DIEST

Department of Pathology, Free University Hospital,
Amsterdam, The Netherlands

1 Pijpers R, Borgstein PJ, Meijer S, *et al*. Sentinel node biopsy in melanoma patients: dynamic lymphoscintigraphy followed by intraoperative gamma probe and vital blue guidance. *World J Surg* 1997;**21**:788–92.

2 Veen H van der, Hoekstra OS, Paul MA, *et al*. Gamma probe-guided sentinel node biopsy to select patients with melanoma for lymphadenectomy. *Br J Surg* 1994;**81**:1769–70.

3 Decesare SL, Fiorica JV, Roberts WS, *et al*. A pilot study utilizing intraoperative lymphoscintigraphy for identification of the sentinel node in vulvar cancer. *Gynecol Oncol* 1997;**66**:425–8.

4 Pettaway CA, Pisters LL, Dinney CP, *et al*. Sentinel lymph node dissection for penile cancer: the MD Anderson Cancer Center experience. *J Urol* 1995;**154**:1999–2003.

5 Lechner P. Probe-guided surgery of colorectal carcinoma. *Acta Med Austriaca* 1997;**24**:68–72.

6 Javaheri S, Cruse CW, Stadelman WK, *et al*. Sentinel node excision for the diagnosis of metastatic neuroendocrine carcinoma of the skin: a case report. *Ann Plast Surg* 1997;**39**:299–302.

7 Lowe FC. Squamous-cell carcinoma of the scrotum. *Urol Clin North Am* 1992;**19**:397–405.

8 Borgstein PJ, Pijpers R, Comans EFI, *et al*. Sentinel lymph node biopsy in breast cancer: guidelines and pitfalls of lymphoscintigraphy and gamma probe detection. *J Am Coll Surg* 1998;**186**:275–83.

9 Meijer S, Pijpers R, Borgstein PJ, *et al*. The sentinel node procedure: standard intervention for surgical treatment of breast cancer. *Ned Tijdschr Geneeskd* 1998;**142**:2235–7.

10 Pijpers R, Meijer S, Hoekstra OS, *et al*. Impact of lymphoscintigraphy on sentinel node identification with Technetium-99m-colloidal albumin in breast cancer. *J Nucl Med* 1997;**38**:366–8.

11 Turner RR, Ollila DW, Stern S, *et al*. Optimal histopathologic examination of the sentinel lymph node for breast carcinoma staging. *Am J Surg Pathol* 1999;**23**:263–7.

12 Diest PJ van, Peterse HL, Borgstein PJ, *et al*. Pathologic investigation of sentinel lymph nodes. *Eur J Nuclear Med* 1999;**26**:S43–9.

13 Cserni G. Metastases in axillary sentinel lymph nodes in breast cancer as detected by intensive histopathological workup. *J Clin Pathol* 1999;**52**:922–4.

14 Zhang PJ, Reisner RM, Nangia R, *et al*. Effectiveness of multiple level sectioning in detecting axillary nodal micrometastasis in breast cancer. A retrospective study with immunohistochemical analysis. *Arch Pathol Lab Med* 1998;**122**:687–90.

15 Diest PJ van, Torrenga H, Borgstein PJ, *et al*. Reliability of intra-operative frozen section and imprint cytological investigation of sentinel lymph nodes in breast cancer. *Histopathology* 1999;**35**:14–18.

16 Gentry JF. Pelvic lymph node metastases in prostatic carcinoma. The value of touch imprint cytology. *Am J Surg Pathol* 1986;**10**:718–27.

17 Hadjiminas DJ, Burke M. Intraoperative assessment of nodal status in the selection of patients with breast cancer for axillary clearance. *Br J Surg* 1994;**81**:1615–16.

18 Fisher CJ, Boyle S, Burke M, *et al*. Intraoperative assessment of nodal status in the selection of patients with breast cancer for axillary clearance. *Br J Surg* 1993;**80**:457–58.

19 Bostick PJ, Chatterjee S, Chi DD, *et al*. Limitations of specific reverse-transcriptase polymerase chain reaction markers in the detection of metastases in the lymph nodes and blood of breast cancer patients. *J Clin Oncol* 1998;**16**:2632–40.

20 Turner RR, Ollila DW, Krasne DL, *et al*. Histopathologic validation of the sentinel lymph node hypothesis for breast carcinoma. *Ann Surg* 1997;**226**:271–8.

21 Flett MM, Going JJ, Stanton PD, *et al*. Sentinel node localization in patients with breast cancer. *Br J Surg* 1998;**85**:991–3.