

Axillary staging of breast cancer and the sentinel node

G Cserni

Abstract

Pathological aspects of axillary nodal staging of breast cancer and in particular sentinel lymph node (SLN) biopsy are reviewed. SLN biopsy seems an almost ideal staging procedure because it has both high accuracy and a low false negative rate. It may also allow a cost effective use of more sensitive methods of metastasis detection. However, the biological relevance of metastases detected only by modern tools remains to be elucidated. This review focuses on standard axillary staging and the histopathological investigation of SLNs, with emphasis on the intraoperative setting. Future trends including ancillary studies, quality control issues, prediction of non-SLN involvement, and suggestions concerning the minimum requirements for the histology of axillary SLNs are also discussed.

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Nodal status is the single most important prognostic factor in breast carcinoma,¹⁻³ and it has a major influence when decisions are made about adjuvant systemic treatment. Alternative modes of assessment of the axillary nodal status include physical examination⁴⁻⁹ and imaging techniques,¹⁰⁻¹⁸ but none has equalled the "gold standard" of histology¹⁹ of lymph nodes recovered from axillary dissection (AD) specimens. It has been stated that a minimum of 10 nodes should be assessed for accurate staging of the axilla.²⁰⁻²³

However, the histological assessment of axillary lymph nodes is not a standardised procedure, and is influenced by several factors. For example, it is dependent on the extent of surgery. Complete AD²⁴⁻²⁶ yields more nodes than level I and II or level I dissections.²⁷⁻²⁹ Axillary "four node" sampling selects nodes by location and consistency, and significantly reduces the number of nodes recovered^{30 31}; it is considered an adequate form of axillary staging by some,^{32 33} but further axillary treatment (surgical or irradiation) is required if positive nodes are found.

Macroscopic assessment of the AD specimen by the pathologist also influences the number of nodes taken for histology. Anatomical and surgical factors may contribute to differences in the numbers of lymph nodes examined,^{34 35} but the main factor seems to be the ability of the pathologist to retrieve the nodes from the axillary fat.³⁶ Although we were able to increase the median number of

recovered lymph nodes from 10 to 22 in our audit study, this did not influence the proportion of node positive cases.³⁶ Fat clearing techniques may increase lymph node yield further, but do not influence staging fundamentally,^{37 38} and this is why these costly methods are not considered essential.³⁹ Both our audit study and the clearing studies cited above indicate that very small nodes, which are time consuming to retrieve, seldom affect nodal stage, and should not necessarily be recovered. In fact, as few as six nodes may give an accurate staging in a large proportion of cases.⁴⁰

The histological assessment of axillary lymph nodes is probably most affected by the methods of microscopic investigation applied. Examining a single central cross-section was advised against as early as 1961,⁴¹ but is still routine in many laboratories. Multiple level sectioning and/or immunohistochemistry (IHC) may result in nodes previously regarded as negative being reclassified as positive in 10-30% of patients.⁴²⁻⁴⁸ However, the biological relevance of these occult, previously undetected metastases is controversial; some studies have concluded that they represent no survival disadvantage,^{41 42 48-50} whereas others have concluded the converse.^{43 44 46 47 51 52} One study⁴⁵ also highlights the role of the individual pathologist as a factor influencing the histopathological evaluation of lymph nodes, because 46 (3.8%) of 1203 axillas originally considered negative were found to be positive on a centralised review of the slides.

Although axillary staging has been based on AD, there have been rational claims that this procedure has no therapeutic benefit in node negative patients, who are nevertheless at risk of its side effects, most notably lymphoedema, and to a lesser extent neuronal damage.⁵³⁻⁵⁵ Such complications are said to occur in 8-15% of the patients undergoing AD. Because the median tumour size at the time of first detection has decreased⁵⁶ as a result of screening programmes, the proportion of node negative (pN0) tumours has increased, and thus fewer patients may now require AD on a therapeutic basis. This has brought the sentinel lymph node (SLN) concept to the forefront of changes in the practice of axillary nodal staging, with important implications for both surgeons and histopathologists.

The theory of SLNs was first formulated for penile carcinoma.⁵⁷ It implies that lymph nodes draining any one site have a hierarchical organisation through which lymph flows in a systematic order. Metastasis from a tumour drained by these lymph nodes will be first arrested by

Bács-Kiskun County
Teaching Hospital,
Department of
Pathology, H-6000
Kecskemét, Nyíri út 38,
Hungary
G Cserni

Correspondence to:
Dr Cserni
cserni@freemail.c3.hu

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the most proximal node or nodes in this orderly arrangement. These nodes (or node) are the SLNs and are predictive of the likelihood of involvement of other members of the local nodal network. Therefore, in theory, the identification of SLNs and the evaluation of their metastatic status can be used to determine the extent of nodal dissection required. Cutaneous malignant melanomas were the first tumours where the introduction of SLN biopsy altered the staging and management schemes after several studies reinforced the results of the initial publication of Morton and colleagues.⁵⁸ Breast cancer was the second type of tumour widely studied, after isotope guided⁵⁹ and vital blue dye guided⁶⁰ techniques of SLN biopsy had been described. Other tumours that have been assessed for the feasibility of SLN biopsy include Merkel cell carcinomas of the skin, thyroid neoplasms, and vulvar, oral, head and neck, and colorectal carcinomas. It has been proposed that the SLN theory applies to all solid neoplasms.⁶¹ However, technical limitations at some anatomical sites and tumour incidence mean that cutaneous malignant melanoma and breast carcinomas are the two largest groups of tumours studied to date.

In breast cancer, feasibility studies⁵²⁻⁶⁸ confirm that with either vital blue dyes (the most commonly applied are isosulfane blue in the USA and patent blue in Europe) or 99m-Tc labelled colloids (usually sulphur colloids in the USA, and human colloidal albumin in Europe) and a hand held γ probe, often preceded by lymphoscintigraphic imaging, or with a combination of these two methods, one or a few specific SLNs can be identified and removed. These nodes are the most likely sites of metastases, their negative status correctly predicts the negative status of the axilla in over 90% of cases and the false negative rate of SLNs is low (0–11% in larger studies, being lower after the completion of a learning phase). These features might allow AD to be restricted to SLN positive patients.

SLNs pose a challenge to the diagnostic histopathologist. This review summarises the current literature and formulates guidelines for pathologists dealing with SLNs from patients with breast cancer.

Macroscopy of sentinel lymph nodes

The macroscopic examination of the SLNs does not differ from that of other isolated nodes sent for pathology. Record size, consistency, and any special features, such as the presence of macrometastasis or fatty change. Whenever a vital blue dye is used, the colour of the node might be an important clue, as may be the presence of one or two blue stained lymphatics leading to the SLN. These features might help in the recognition of the rare greyish anthracotic nodes that can be identified falsely as SLNs if only a dye technique is used.⁶⁹ If a radiolabelled colloid is used for the identification of the SLN, documentation of its radioactivity is also important, although in most cases this is not the duty of the pathology staff.

Some institutions require special radiation safety procedures, such as the wearing of film

badges or the storage of the nodes for 48 hours (six half lives of 99m-Tc) before processing,⁷⁰ but most authorities state that the exposure and hazards to the pathology staff are minimal during dissection and microscopic assessment, and safety measures should mainly apply to the disposal of waste material, which should be stored until it becomes non-radioactive^{71 72}; radiation monitoring is recommended for pregnant staff members,⁷² who are advised not to work with such material.

The extent of histopathological examination of the sentinel nodes

As stated above, a widely used routine method of assessing an axillary lymph node is to take one haematoxylin and eosin (HE) stained section from the central plane of the node. The NHS breast screening programme recommends taking up to four separate blocks from each node, depending on its size.

The literature varies considerably concerning the extent of the reported histopathological investigation of SLNs. Some authors perform the same "routine" procedure for SLNs and non-SLNs,⁷³⁻⁸¹ which probably means one section for each lymph node in most cases, or may in a minority of cases mean a three to four level assessment by HE staining. Other authors document more detailed investigation of SLNs, with serial or step sectioning and/or IHC of epithelial markers (mostly cytokeratins (CKs)).⁸²⁻⁹⁰ The variety of the SLN assessment techniques probably results from the fact that the biological relevance of the extremely small micrometastases detected by more detailed histology has not been established. In feasibility studies, even conventional histology is sufficient to show that SLNs are the most likely sites of lymphogenic metastases, because the number of cases with metastases confined to SLNs is relatively high, even with normal HE staining.

On the other hand, it has been claimed that the assessment of SLNs at multiple levels with IHC results in improved staging.⁸² Standard (probably one cut surface) examination of the axillary lymph nodes of over 100 patients yielded 13% fewer node positive patients than the number detected by more detailed histology of the SLN, which included six to eight levels of the nodes immunostained for CKs if HE negative. The more detailed histology also resulted in a significantly higher incidence of micrometastases⁹¹: 38.2% *v* 10.3%. It has also been shown that the same detailed histology does not increase the detection rate of metastases in non-SLNs that are negative on HE if the SLN is also negative.^{92 93} Several later studies have revealed an increased rate in the detection of epithelial neoplastic cells lodged in lymph nodes with a more detailed histopathological investigation. Table 1 summarises these studies. The table only includes those Medline identifiable studies that give a full description of the histopathology protocol and allow a comparison with standard histology. The demonstration of micrometastases in SLNs may be clinically important,^{97 98} and might influence decisions regarding systemic treatment.^{99 100}

Table 1 Overview of studies comparing the detailed histopathology of sentinel lymph nodes (SLNs) and standard assessment

Reference	Number of patients	Number (%) [*] upstaged	Details
Cserni (1999) ⁹⁰	58	6 (24%)	Mean of 49 level HE and multiple level CK IHC if negative <i>v</i> central cross section
Cserni (1999) ⁹⁰	58	3 (13.6%)	As above <i>v</i> 3 level HE at 25%, 50%, and 75% height of the lymph node tissue block
Czerniecki <i>et al</i> (1999) ⁸⁸	41	3 (10.3%)	4 level CK IHC <i>v</i> 2 HE faces of bivalved nodes
Dowlathshahi <i>et al</i> (1999) ⁸⁹	52	24 (52.2%)	Serial sections and CK IHC at 0.25 mm intervals <i>v</i> HE at 2 mm intervals
Jannink <i>et al</i> (1998) ⁸⁷	19	3 (23.1%)	Serial sections at 0.5 mm intervals <i>v</i> single HE
Kelley <i>et al</i> (1999) ⁹⁴	28	2 (10.5%)	4 level HE and 2 level CK and EMA IHC <i>v</i> 1 level HE
Pendas <i>et al</i> (1999) ⁹⁵	478	41 (10.6%)	Bivalved or multiple sectioned CK IHC <i>v</i> HE of same levels
Torrença <i>et al</i> (2000) ⁹⁶	250	9 (4.1%) ^{**}	5 level HE at 0.25 mm intervals <i>v</i> 1 level HE
Torrença <i>et al</i> (2000) ⁹⁶	250	19 (8.3%) ^{**}	5 level CK at 0.25 mm intervals <i>v</i> 1 level HE
Torrença <i>et al</i> (2000) ⁹⁶	250	14 (6.3%) ^{**}	5 level CK at 0.25 mm intervals <i>v</i> 1 level CK

CK IHC differs from study to study, and the antibodies AE1/3, MNF116, CK8/18 and CAM 5.2 were all used.

^{*}Cases with metastases detected only on a more detailed evaluation divided by the sum of the numbers of cases finally found negative and those upstaged by detailed histology, expressed as a percentage.

^{**}Numbers refer to SLNs and not patients; the total number of SLNs recovered from the 250 patients was 315 (PJ van Diest, 2000, personal communication). EMA, epithelial membrane antigen; CK, cytokeratin; HE, haematoxylin and eosin; IHC, immunohistochemistry.

Table 1 highlights the variations in the methods of processing the SLNs and points to a tremendous variation in the percentage of “occult” metastases discovered by means of more detailed investigations. From these data, it is difficult to draw conclusions of value for everyday practice. It seems clear, however, that a “one HE level approach” for negative SLNs is inadequate, and more detailed sampling is warranted. The depth of these details remains to be elucidated, and reports like those listed in table 1 might help in formulating best practice guidelines in this respect.

A mathematical model has been constructed for the optimum investigation of SLNs.¹⁰¹ The model suggests that slicing the SLNs at 2 mm intervals and then taking sections at 0.25 mm intervals for HE and CK analysis is a cost effective method of detecting metastases down to 0.1 mm. This model has already been tested⁸⁹ (table 1). The number of cases with intranodal tumour cells detected by the more detailed approach of using CK IHC at 0.25 mm intervals (24 new metastases detected in 46 patients considered negative after HE staining of the SLNs at 2 mm intervals) far exceeds the 10–15% proportion of node negative breast cancer patients who die of their disease. If only larger tumour cell “colonies” (defined as a group of over 20–30 malignant cells) are considered, the number of metastases detected by IHC at 0.25 mm intervals more closely matches the rate of node negative patients succumbing to their disease (12 new metastases instead of 24). Accordingly, paucicellular metastases may not have the same prognosis as larger ones.¹⁰² A definition and distinction of clinically meaningful micrometastases is clearly needed, but the necessary data are still lacking.¹⁰³

One weakness of the mathematical model described above is that it presumes that spherical metastases are randomly distributed within nodes. However, the Santa Monica group suggests that metastases are most commonly found in sections incorporating the hilum of the SLN.¹⁰⁴ This may prove a useful hypothesis, although finding the hilum is more difficult in practice than in theory. It has also been argued that no experimental evidence supports this hypothesis.¹⁰⁵ The use of a vital blue dye in the identification of SLNs may allow visualisation of the junction between the lymphatic vessel

draining the tumour and the SLN. This area is the most likely site of metastasis.¹⁰⁶ Therefore, the search for metastases may centre more on the area including the point of inflow of the blue stained lymphatic vessel and the hilum of the SLN. Unfortunately, the painstaking study relating to the testing of the mathematical model⁸⁹ did not consider the issue of the sites of the metastases inside the SLN.

Intraoperative assessment of sentinel lymph nodes

Because one of the main purposes of SLN biopsy is to obviate the need for AD in patients not requiring this procedure for treatment, the intraoperative assessment of SLNs is especially important. Both imprint cytology and frozen sections have advantages and drawbacks in this context.

Imprint cytology offers a cheap, easy, and fast way of assessing the nodal status. Tumour cells adhere to the slide when the touch preparation is made and neoplastic cells are usually easily detected with conventional stains such as HE or Diff-Quik. However, some cases cause difficulties in interpretation, because activated endothelial cells, follicle centre cells, and epithelioid histiocytes may present as atypical cells. Such cells may lead to the “suspicious” (C3 or C4) diagnostic category, an equivalent of the deferred diagnosis in frozen section evaluation and encountered more often. Low volume metastases, metastases from lobular or low grade ductal carcinomas, and metastases with a diffuse unicellular infiltrating pattern may remain undetected by conventional stains, and the application of rapid CK IHC might be of value in this setting.¹⁰⁷ The imprint usually adequately represents the cut surface from which it is taken, but because some metastases are not located at the level of initial cutting, taking the imprint from multiple levels can increase the rate of detection of metastases (own departmental database, 1999). The sampling rules mentioned at the end of the previous section should also apply here. The method requires a pathologist trained in cytopathology to achieve an acceptable degree of sensitivity, and to avoid false positives.^{108 109} Scraping and then smearing the cut surface might be a suitable alternative to touch imprints.¹¹⁰

Frozen sectioning gives a tissue diagnosis and the number of unresolved (deferred) cases is lower than with imprint cytology. However, the method is more expensive, requires technical staff, takes more time, and causes artefacts in the tissues. It can be combined with rapid CK IHC,¹¹¹ and this may increase the rate of detection of micrometastases, just as in permanent sections. The role of IHC may diminish if serial sections are made from frozen tissues, because its function is reduced to elucidating the nature of a few suspicious cells seen on HE.¹¹² Serial sections on frozen material have been challenged because of their high cost and time requirements, but the users of the method have argued that most of the metastases are seen in the first few slides and this may allow a faster intraoperative decision. A further argument was that the operation protocol involves the SLN biopsy as a first step, followed by the removal of the tumour, which allows more time for the pathologist. It is likely, however, that extensive investigations of SLNs on frozen sections will not become popular. A theoretical objection to freezing may be the loss of interpretable tissue, but because histopathology is a sampling related investigation in all circumstances, 100% sensitivity cannot be expected even with permanent sections. Through the addition of IHC, artefacts might be overcome because positive immunostaining for CKs might resolve problems caused by tissue distortion resulting from freezing. Frozen sectioning has been advised against in most breast lesions, in favour of preoperative diagnosis, and there seems to be a general consensus on not freezing breast lesions < 1 cm^{113 114} because diagnosis might be compromised by tissue loss and artefacts. These considerations must naturally be taken into account, but the detection of a metastasis within a lymph node, even if it is a micrometastasis, should not be compromised by the frozen sectioning procedure, especially if IHC is used in negative or doubtful cases.

Care must be taken in the evaluation of CK stains in both frozen sections and imprint cytology, because some non-neoplastic cells might also stain positive; these include interdigitating dendritic reticulum cells and benign epithelial inclusions. To avoid false positivity, immunostains should always be assessed in the knowledge of possible errors and, whenever possible, in the knowledge of the primary tumour cytological characteristics. They should be reported only by experienced, fully trained pathologists.

Table 2 compares the results of the two methods of intraoperative assessment. There is a large variation in the reported ranges of sensitivity and false negativity. This is partly the result of the differences in the methodology involved in the final histology. As expected, the highest accuracy rates and lowest false negative rates are seen in studies in which intraoperative assessment of one level and final HE histology of the same level are compared.^{110 121}

Although imprint cytology may seem inferior to frozen sectioning in sensitivity and negative predictive value in a single cut surface investigation with standard stains,¹¹⁷ sampling from multiple levels and the addition of CK IHC may minimise this. Both methods seem adequate in the intraoperative assessment of SLNs,¹²⁰ but several undetected metastases should be expected in both, and permanent sections should complement all negative intraoperative investigations.

Further perspectives. Analysis of the metastases in sentinel nodes

Multiple feasibility studies suggest that SLN biopsy is an ideal staging procedure for patients with early breast cancer. It is self evident that the removal of negative lymph nodes from the axilla is illogical, because it offers no therapeutic advantage, but has a non-negligible potential morbidity. On this basis, several institutions have introduced SLN biopsy with no AD for SLN negative patients and selective AD for

Table 2 Results on intraoperative assessment of SLNs

Method	Ref.	N	Levels	Stains	Final histology	TP	TN	FP	FN	Acc.	Sens.	Spec.	PPV	NPV	FNR	FRR
FS	79	107	3 consecutive	HE	3 HE	32	57	0	18	83%	64%	100%	100%	76%	36%	24%
FS	77	56	NI	HE	HE	NI	NI	0	3	95%	NI	NI	NI	NI	NI	NI
FS	115	96	3 (each side)	HE	HE	24	68	0	4	96%	86%	100%	100%	94%	14%	6%
FS	116	47*	NI	HE	HE	10	36	0	1	98%	91%	100%	100%	97%	9%	3%
FS	117	54	2	HE	HE more + IHC	21	31	0	2	96%	91%	100%	100%	94%	9%	6%
		74*	2	HE	HE more + IHC	27	43	0	4	95%	87%	100%	100%	91%	13%	9%
FS	118	28	1 (2)	HE	IHC	6	17	0	5	82%	55%	100%	100%	77%	45%	23%
FS	119	62	≥ 1	HE	HE + IHC same	19	34	0	9	85%	68%	100%	100%	79%	32%	21%
IC + FS	109, 120	278	1	DQ	HE same level	53	206	0	19	93%	74%	100%	100%	92%	26%	8%
		278	1	DQ	HE more + IHC	53	167	0	58	79%	48%	100%	100%	74%	52%	26%
IC + FS	119	38	≥ 1	MG + IHC/HE	HE + IHC same	3	25	0	10	92%	77%	100%	100%	89%	23%	11%
IC	117	45	2	DQ	HE more + IHC	14	23	0	8	82%	64%	100%	100%	74%	36%	26%
		*59	2	DQ	HE more + IHC	16	33	0	10	83%	62%	100%	100%	77%	38%	23%
IC	119	38	1	MG + IHC	HE + IHC same	6	25	0	7	82%	46%	100%	100%	78%	54%	22%
IC	107	381**	2	DQ	HE more + IHC	15	254	1	35	88%	30%	100%	94%	88%	70%	12%
IC	121	124*	1	HE	HE same level	22	101	0	1	99%	96%	100%	100%	99%	5%	1%
IC	110	55	2	HE	HE same level	14	40	0	1	98%	93%	100%	100%	98%	7%	2%
IC	122	25	1	RAL	NI	4	19	0	2	92%	66%	100%	100%	90%	33%	10%
IC	123	161**	2	IHC	HE more + IHC	30	126	0	5	97%	86%	100%	100%	96%	14%	4%

Levels refers to the levels sampled during intraoperative assessment.

Stains refers to the stains used for intraoperative assessment.

Final histology refers to the final histology that served as a basis for comparison.

*On an SLN and **on a grossly negative SLN (and not patient) basis.

Acc., accuracy (overall predictive value); DQ, Diff-Quik; FN, false negatives; FNR, false negative rate (false negatives/all positives); FP, false positives; FRR, false re-assurance rate (false negatives/false and true negatives); FS, frozen section; HE, haematoxylin and eosin; IC, imprint cytology; IHC, immunohistochemistry to epithelial markers; MG, May-Giemsa; N, number of patients; NI: no information; NPV, negative predictive value; PPV, positive predictive value; RAL, rapid cytological stain RAL 555; Sens., sensitivity; Spec., specificity; TN, true negatives; TP, true positives.

those with a positive SLN, mostly within clinical trials. Feasibility studies with completion AD as control and cases with selective AD have highlighted that many patients have metastases limited to the SLNs. These patients could also be candidates for the omission of AD. SLN metastasis size and tumour size seem to be the major predictors of further non-SLN involvement.¹²⁴⁻¹²⁷ Patients with small tumours (≤ 2 cm) and micrometastasis (≤ 2 mm) to SLNs are very unlikely to have non-SLN involvement. Our own study has reached the same conclusions, but suggests that the location of the SLN metastases in the sinuses or in the parenchyma may be used instead of their size in the predictive model of non-SLN involvement (own departmental database, 1999). Thus, measurement of the micrometastases might be a further task of the pathologist if these preliminary results are confirmed by larger studies. A step in this direction might be the clinical trial sponsored by the American College of Surgeons, which aims to elucidate the biological meaning of axillary micrometastases, and randomises patients with positive SLNs either to completion AD or to no further treatment of the axilla.¹²⁸ However, the encouraging results discussed above must be treated with caution. Even small SLN metastases (< 1 mm²) detected by IHC can be associated with non-SLN involvement, and small tumours (pT1a; ≤ 0.5 cm) metastatic to the SLN might have metastasis beyond this node.¹²⁹ We have also encountered some cases with minimal SLN involvement (a few cells) and non-SLN metastasis, and over 20% of our cases with tumoral involvement of SLNs ≤ 2 mm had non-SLN involvement too (own departmental database, 1999). Considering these facts, it seems that predictive models of non-SLN involvement have the same limitations as predictive models of axillary involvement in general.

Molecular analysis of sentinel lymph nodes

As mentioned above, intensive histology has been shown to demonstrate more neoplastic cells in SLNs than standard histology, and this has led to the investigation of methods that are potentially even more sensitive.

Noguchi *et al* assessed the value of the detection of CK-19 and MUC-1 mRNA by the reverse transcriptase polymerase chain reaction (RT-PCR) and found that this was positive in a breast cancer cell line, 23 primary breast carcinomas, and 10 histologically positive lymph nodes.¹³⁰ They detected positivity with these markers in lymph nodes that were negative on histology in five and three cases, respectively. Their dilution studies indicated that the CK-19 RT-PCR was more sensitive, and they continued the investigation on a patient basis with halves of histologically negative nodes of a patient pooled as one sample. They found that seven of 48 (15%) histologically node negative patients (node negative on the basis of a single HE slide from a half node) were RT-PCR positive. On the other hand, they also noted that one of 42 RT-PCR negative patients had a

small metastasis confirmed by histology. Their explanation for this failure was the dilution effect caused by the pooling of negative lymph nodes, which seems unlikely. However, halving of the lymph nodes may be an alternative explanation¹⁰⁶⁻¹³¹ because metastases are not randomly distributed in lymph nodes. The 15% rate of detection of occult metastases by RT-PCR in the cited study¹³⁰ is not higher than the detection rate reached by serial sectioning and IHC (table 1). Other studies have questioned the specificity of both MUC-1 and CK-19 because their mRNAs are expressed in lymph nodes of patients without cancer.¹³²⁻¹³⁴ Carcinoembryonic antigen (CEA) and mammaglobin are potential candidates for multiple marker RT-PCR,¹³⁴ and have been tested at the H Lee Moffitt Cancer Center¹³⁵; 40 (40%) of 102 histologically negative SLNs were found positive with at least one of the markers, and 11 (11%) were positive with both markers. However, of 168 histologically positive SLNs, 10 (6%) tested negative to both markers, including two SLNs with massive metastatic deposits. The Santa Monica group has also reported results with a triple marker RT-PCR method.¹³⁶ The three markers used in their study included C-Met (also known as hepatocyte growth factor), $\beta 1 \rightarrow 4$ GalNAc-T (a carbohydrate transferase), and P97 (a cell surface glycoprotein also known as melanotransferrin); none of these markers is specific for breast cancer or breast epithelium, but their expression has been described in malignant breast tissues. Of 57 SLNs, 17 were shown to harbour metastases by their protocol involving the use of the multiple level HE and IHC stains described above.⁸² Of the remaining 40 negative nodes, 17 (43%) proved to be positive to all three markers used, and 31 (78%) tested positive to at least one of them. Only one histologically positive node was negative for all markers.

It seems clear from these results that the lack of a specific single marker is currently the major limitation of RT-PCR technology. One step that aims to overcome this problem is the use of multiple markers, but again the interpretation of single marker positivity remains controversial. Just as in the case of the IHC study cited,⁸⁹ high rates of conversion to positive are at odds with clinical observations, in particular the lower percentage (10-15%) of node negative patients who succumb to their disease. Specificity and sensitivity issues must be considered together in the light of some positive SLNs testing negative in most studies, because such errors are unlikely to be the result merely of sampling errors. Until these issues are resolved the clinical importance of RT-PCR positivity is questionable.

Although one study has demonstrated a recurrence free survival advantage for patients with CEA RT-PCR and histology negative lymph nodes over those who have RT-PCR positive lymph nodes negative on histology and those who have metastases detected with both technologies, the authors did not indicate the extent of histology applied in their protocol, and a major problem in the concept of the study was the combination of patients with

gastrointestinal cancer and patients with breast cancer.¹³⁷

Ancillary studies of SLNs such as those involving RT-PCR should at the moment be regarded as research tools in search of improved markers.

Quality control issues

Some other quality control issues must be considered. Several studies have been based on the suggestion of Borgstein and colleagues¹³⁸ that the breast parenchyma and the overlying skin share their lymphatics because they are embryologically related, and hence that peritumoral and intradermal or periareolar injections of the tracing agents are equivalent.^{83 139 140} Nevertheless, there are also contradictory data, primarily the non-visualisation of the internal mammary draining paths if the 99m-Tc-labelled colloid is not given intraparenchymally.⁶⁸ A recent study has demonstrated spatial and sequential mismatches in node labelling in a small percentage of cases if the radiolabelled tracer is given peritumorally or intradermally.¹⁴¹ These observations suggest that intradermal or periareolar techniques may identify SLNs in a large proportion of cases, but may also miss them in a few, and that the two injection techniques are therefore complementary rather than interchangeable. If the protocol involves a blue dye injected peritumorally, the pathologist might be able to verify successful peritumoral injection.¹⁴² Our own feasibility study has shown a few cases in which a palpable non-malignant lump in the breast misled the surgeon, and the injection was not given peritumorally as intended. Until lymphatic drainage pathways from the breast parenchyma are completely understood, such a failure of dye administration should perhaps exclude the patient from the peritumoral protocol.

The analysis of false negative SLNs has revealed some primary tumour characteristics that could offer an explanation for the false results. These include multifocality, large size of the tumour or a previous biopsy cavity, and extensive peritumoral vascular invasion.^{77 78 86 143-145} The pathologist is responsible for reporting these assessable features correctly, because in the future they might become factors indicating an AD even in the event of a negative SLN biopsy.

Conclusions

The SLN theory has led to a revolution in the staging of solid neoplasms, especially malignant melanoma and breast cancer. Although SLN biopsy is not yet the standard of care,^{128 146} it has every chance of becoming so in patients with early breast cancer. Multiple clinical trials have been initiated to clarify the rates of recurrence and survival of patients undergoing SLN status based selective AD,¹⁴⁷ including the ALMANAC (axillary lymphatic mapping against nodal axillary clearance) trial in the UK, in which the complications, quality of life, costs (primary outcome measures), and axillary recurrence rates (secondary end point) of the SLN biopsy are compared with those of the

current standard of care (sampling or clearance) at the given institution. Early results of the first trials will emerge soon. The H Lee Moffitt Cancer Center—for example, reports no axillary recurrence after a mean follow up of 20 months in the 368 patients with breast cancer treated without AD selected from 514 patients undergoing SLN biopsy.⁶⁸ Thus, the SLN biopsy may shortly become the standard of care in many countries, and already is in some. Pathologists must therefore be prepared to meet the challenges of SLN biopsy.

Although there are many unanswered questions at the moment, it appears wise to break down the approach to SLNs into two settings.¹⁴⁸ The research setting requires a well defined protocol for the processing of SLNs, which may depend largely upon the questions posed by the study. The non-research setting leaves the development of the SLN processing protocol to the pathologist alone. It is clear that single level HE assessment is inadequate, and a minimum requirement might be HE stained slides from at least three distinct levels, which is the standard in some institutions,¹⁴⁹ including many histopathology laboratories in the UK. The inclusion of CK IHC in the protocol of HE negative nodes is supported by more and more data, and the approach applied by Giuliano and colleagues⁸² seems reasonable (six to eight levels of the bivalved nodes, of which two are stained with HE). A similar approach (three to five levels with HE, with CK IHC if these prove negative) was suggested by a recent review, and also appears acceptable.¹⁵⁰ One or other of these protocols^{82 150} is strongly recommended for the histopathological assessment of SLNs. One must accept that because histopathology is based on sampling, 100% sensitivity cannot be expected. Further enhancement of systems for the detection of neoplastic cells is better reserved for the research setting. The intraoperative assessment of SLNs is important, and both imprint cytology and frozen sectioning offer an adequate alternative or complementary approach to this. The choice must be based on the available institutional resources. The choice of the area sampled in the intraoperative, permanent, or ancillary setting might reduce the costs of the investigation. If a blue dye tracer is used, the search for metastases should focus more on the point of inflow of the blue lymphatic, where it can be identified. Confirming that blue dye has been injected correctly in relation to the site of the tumour might become a part of the quality assurance issues in the SLN biopsy protocol if the protocol requires peritumoral or supratumoral injection of the tracer.

Note added in proof

Data from our departmental database mentioned in the “Analysis of the metastases in sentinel nodes” section have now been published.¹⁵¹

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