
2. SIGNIFICANCE OF SENTINEL NODE MICROMETASTASIS

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The emergence of sentinel lymph node (SLN) biopsy as an alternative method to axillary dissection for staging the axilla in breast cancer patients has brought the value of axillary dissection into close scrutiny. In addition, it has stimulated the assessment of the biologic implications of micrometastatic disease to lymph nodes. Historical review of SLN technology demonstrates that micrometastatic disease in SLNs may be critical in the evaluation of false-negative SLNs. Furthermore, the presence of micrometastatic disease in the lymph nodes may carry prognostic significance for the overall behavior of the malignancy.

Historical Perspective

False-Negative Evaluation

In the initial trial of SLN mapping for melanoma, 10 (4.1%) of 243 patients developed recurrent disease in the basin from which the initial SLN was removed. These lymph nodes were evaluated after blue dye localization with routine hematoxylin-eosin (H&E) stains on bivalved SLNs. Within 18 months of the evaluation, these patients recurred. However, upon multiple recuts of the SLNs with evaluation by S-100 stains, 7 of 10 patients' sentinel nodes were detected to

have micrometastases.¹ This observation raised the significant question as to whether the false-negative evaluations could have been precluded if the micrometastases had been detected upon initial evaluation. Several of the initial trials evaluating SLNs in breast cancer have similarly avoided the detection of micrometastases by cytokeratin analysis or multiple sections of the SLN. The avoidance of these tests was based on the presumption that the detection of small micrometastatic disease would not alter the management of a patient with breast cancer. Therefore, these studies have demonstrated false-negative rates between 5% and 11%.^{2,3} A significant observation demonstrated that 3 (11%) of 27 patients, in whom cytokeratin immunohistochemistry (IHC) detected micrometastatic cells in SLNs, also had additional metastases detected in the remaining lymph nodes on complete axillary dissection. Therefore, we conclude that careful evaluation of SLNs for micrometastatic disease obviates the false-negative assessment of the axilla in breast carcinoma, a historical lesson learned with melanoma.

Prognostic Significance of Micrometastases in Melanoma

A seminal observation made by Heller et al⁴ demonstrated that

30% of histologically negative lymph nodes harbored metastatic melanoma cells detected by growth in tissue culture. The subsequent evaluation by the same group, using reverse-transcription polymerase chain reaction (RT-PCR) for tyrosinase in melanoma, demonstrated expression of the enzyme in 30% of histologically negative lymph nodes.⁵ Shivers et al⁶ have demonstrated that melanoma patients with RT-PCR-negative SLNs have a recurrence rate of less than 1% at five years following initial surgery. This finding makes RT-PCR results the single most important prognostic indicator of survival in melanoma.

The historical lessons learned with SLN mapping in melanoma should not be repeated with breast cancer (ie, detection of false-negative SLNs in breast cancer, as with melanoma, can be minimized by IHC staining of SLNs). Furthermore, the prognostic significance of micrometastatic SLN involvement may prove, as with melanoma, to have profound consequences in determining which patients are at risk for distant metastatic disease. Several studies to date have demonstrated that micrometastatic disease in breast cancer may indeed have therapeutic significance for breast cancer patients. This paper examines the evidence to date and reviews these findings.

Direct Evidence Supporting the Significance of Micrometastatic Disease

An accumulating body of evidence supports the hypothesis that the presence of otherwise occult or clandestine micrometastatic nodal disease has a significant effect on recurrence rates and overall survival (OS) for breast cancer patients (Table).⁷⁻¹⁵ Whereas the world's literature contains numerous conflicting observations with regard to the significance of micrometastatic disease, most of the carefully performed, larger analyses (>300 patients) demonstrate a negative impact for this histologic finding.

The most thorough study to date was performed by the International (Ludwig) Breast Cancer Study Group.⁷ This trial re-reviewed find-

ings of 921 breast cancer patients without evidence of distant metastatic disease and classified them as having node-negative disease on routine pathologic evaluation. Almost all were resected at six levels with six 3-mm sections cut at each level, two of which were stained with H&E.⁸ Only parenchymal node metastases were recorded as micrometastases. Serial sectioning identified occult micrometastatic deposits in 83 (9%) of 921 patients, with more than one affected node detected in 13 (15.6%) of 83 cases. The effect of finding previously occult micrometastatic disease on survival in this study was clear: at both five and six years of follow-up, both disease-free survival (DFS) rates and OS rates were significantly affected. At a median follow-up of six years, DFS was reduced from 71% in the node-negative group to 53% in the node-positive group ($P=0.0008$) and OS was reduced from 86% in the node-negative group to 70% in the

node-positive group ($P=0.0009$). Interesting biologic correlates observed in this trial included an association between occult micrometastatic disease and larger tumors ($P=0.02$), histologic grade ($P=0.05$), vascular invasion ($P<0.0001$), and age <50 years ($P<0.0001$).^{7,8}

A second retrospective trial re-examining nodal tissue previously classified as negative was performed by Hainsworth et al.¹² This study examined the value of IHC staining in 343 patients previously assessed as having node-negative breast cancer. Using antimucin monoclonal antibodies, occult metastases were detected in 41 patients (12%). Most occult micrometastases were <2 mm in size and were confirmed by H&E using serial sections. At a median follow-up of 79 months, the presence of occult micrometastases in two or more nodes reduced the DFS from 84% to 54% ($P<0.01$) and OS from 85% to

Previous Reports of Micrometastatic Lymph Node Disease: The Largest Trials

Author	Number of Patients	Technique	% Micrometastatic (M) or Occult (%)	Disease-free Survival	Overall Survival
Ludwig Breast Cancer Study ^{7,8}	921	SS	9 (O)	0.003	0.002
de Mascarel et al ⁹	1,680	SMS	7 (M)	0.008	0.036
	129	IHC	10 (M)	0.01	NS
Wilkinson et al ¹⁰	525	SS	17 (O)	NS	NS
Friedman et al ¹¹	1,153	SMS	23 (M)	RR = 1.7	
Hainsworth et al ¹²	343	IHC	12 (O)	0.01	0.05
Fisher et al ¹³	566	Survey	8 (M)	NS	
Clayton and Hopkins ¹⁴	399	Survey	15 (M)	NS	
Trojani et al ¹⁵	150	IHC	14 (M)	0.0025	0.02

SS = serial section
 SMS = serial macroscopic section
 IHC = immunohistochemistry
 NS = not statistically significant
 RR = relative risk

70% ($P<0.01$). This study and one by Trojani et al¹⁵ of 162 patients with node-negative breast cancer both demonstrated the beneficial impact of IHC staining in detecting micrometastases as well as the negative impact of micrometastases on DFS and OS rates.

Although studies re-examining nodal tissues that were previously declared negative for the presence of occult disease are informative, another approach to this problem has been to perform prospective analyses attempting to identify all occult nodal disease on the initial review. In a study by de Mascarel et al,⁹ 1,680 patients were subjected to a standard method of node sectioning (serial macroscopic section, SMS). This method of serial sectioning is designed to sample nodes more effectively by cutting each node into slices 1.5 mm in thickness, each of which is assessed. In this study, micrometastases were defined as lesions <0.5 mm. Of 1,680 patients, 336 were identified as node-positive, with 120 having a single micrometastasis (<0.5 mm). With median follow-up of seven years, both the DFS ($P=0.005$) and OS ($P=0.0369$) were significantly reduced in those patients with a single micrometastasis compared to those declared node-negative.

In a similar trial by Friedman et al,¹¹ 1,153 breast cancer patients underwent prospective SMS nodal analysis. This study examined the impact of the anatomic location of micrometastases (parenchymal vs peripheral sinus). Of these patients, 637 (55%) were found to be

node-negative, 41 (3.5%) were found to have a single focus in the parenchyma, and 205 (8%) were found to harbor one peripheral sinus micrometastasis. Again, this trial found an increase in relative risk associated with presence of parenchymal micrometastasis (RR = 1.7) or peripheral sinus micrometastasis (RR = 1.7, $P=0.05$)

Conflicting Evidence Suggesting No Impact of Micrometastatic Disease

Many of the initial reports concerning the effect of nodal micrometastasis on breast cancer were negative studies unable to reach statistical significance. Unfortunately, however, many of these studies were performed without resectioning nodes previously classified as node-negative, or they involved sample sizes that were too small. Instead, several of the studies simply identified the presence of random micrometastases in nodes assessed by standard pathologic techniques (such as one H&E-stained section analyzed per node). This sort of minimal analysis, avoiding meticulous and serial sectioning methods with or without the use of IHC staining, yields an accurate numerator (number of micrometastases) without a correct denominator (number of nodes without micrometastasis). One example of this form of analysis is found in a trial reported in 1978 by Fisher et al.¹³ In this prospective trial, one section from each node of 566 patients was examined by routine H&E staining for the presence

of metastatic nodal disease. If a nodal metastasis was identified and was <2 mm, it was classified as a micrometastasis. It is easy to see how this sort of classification scheme measures a completely different set of biologic data from methods designed to identify occult micrometastatic disease. In this study, 8% of patients were found to have micrometastases, according to the investigators' definition. This classification resulted in no effect on OS, although treatment failure rates in this group approached failure rates found in patients with micrometastases. The authors concluded that their sample sizes were likely too small to detect significant differences using their experimental approach. They further predicted that a sample size of 1,400 would need to identify a 10% difference in OS with a confidence interval of 95%.

Another similarly performed study by Clayton and Hopkins¹⁴ reported in 1993 re-examined 399 node-positive patients for the presence of micrometastases <2 mm in size. Of these patients, 62 were classified as harboring micrometastasis, yet when survival rates were compared with those of node-negative patients, no differences in OS were identified. Again, no attempt at serial sectioning was made in this retrospective study.

The majority of older studies of micrometastatic disease inadequately addressed the issue and therefore can be dismissed. However, a study by Wilkinson et al¹⁰ appears to have carefully assessed the significance of micrometastatic disease, although

the sample size was likely too small. In this study, 525 cases of node-negative breast cancer with five or more years of follow-up were selected for further analysis. Negative nodes were resected in serial fashion and inspected using routine H&E histologic methods. On average, 24 sections were examined from each lymph node, and 84 (17%) cases of occult metastases were found. In addition, five cases of "overlooked" micrometastases were detected on reevaluation of the original pathologic slides. Despite finding these occult lesions, no significant difference in OS could be demonstrated. There was, however, a reduced survivorship ($P=0.003$) associated with micrometastases that were overlooked on initial pathologic review. The results of this study are curious and contradict the findings of numerous other positive studies. It is difficult to explain why this fairly large study failed to show a disadvantage to occult micrometastases, whereas smaller trials have found significant differences. This discrepancy is particularly concerning because the presence of occult micrometastases was linked to lymphovascular invasion in the primary tumors ($P<0.0005$). Therefore, we concluded that a lack of survival significance may be related to the relatively small sample size and lack of incorporation of IHC techniques to improve the detection of micrometastases. In other words, analysis of large numbers of serial sections is inadequate to determine the ultimate significance of micrometastasis unless more patients are studied or more sensitive methods are used to detect these lesions.¹⁶

Micrometastatic Disease Linked to Sentinel Lymph Node Biopsy

Although micrometastatic disease is biologically important, it appears to be an uncommonly observed event unless special pathologic sectioning or staining techniques are used. These techniques have generally been considered too labor-intensive and cost-ineffective for routine implementation. Recently, however, with the introduction of SLN biopsy, it has become feasible to incorporate some of the more sensitive pathologic methods into routine clinical practice. For example, although it is standard practice to analyze one or two sections from each sampled axillary lymph node by H&E staining, it is now possible to perform serial sectioning of a few SLNs for the same cost. In addition, the use of IHC staining may now be incorporated to enhance the detection rate of otherwise occult micrometastases.

Although numerous well-designed retrospective studies have suggested the negative impact for micrometastatic diseases, the significance of micrometastatic cancer in the SLN is as yet undetermined due to a lack of meaningful follow-up. However, as previously described with melanoma, there may be significant impact on prognosis and enhanced ability to preclude false-negative evaluations by careful analysis of the SLN. Whereas the routine use of SLN biopsy was incorporated into in our practice in

1994, we believe that will likely require a five-year median follow-up time of sufficient numbers of patients to adequately address this important issue. In light of the number of cases of micrometastatic disease detected thus far using the SLN biopsy technique, it is clear that the significance of these findings has to be determined in prospective, randomized trials. Current trials underway, such as the American College of Surgeons Breast Lymphatic Mapping Trial and the Moffitt Cancer Center/Department of Defense Trial, will attempt to address the significance of micrometastatic disease detected in the SLN.

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