

EVALUATION OF SENTINEL LYMPH NODES

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Axillary lymph nodes are the most common sites of metastasis in breast carcinoma and their status is the most important factor in the prognosis of invasive breast cancer. Metastatic involvement of axillary lymph nodes generally follows an orderly and progressive pattern from levels I and/or II, and then on to the level III nodes. Level III nodes are rarely involved (1-3%) in the absence of nodal disease at levels I and II.

There is no consensus on the extent of axillary dissection needed to gain accurate prognostic information; the recommendations of surgeons range from axillary sampling to total axillary clearance. Mathieson et al. found that removing a minimum of 10 lymph nodes would accurately stage the axilla. This minimum number was confirmed by subsequent studies. Similarly, the National Surgical Adjuvant Breast Project (NSABP) reported that when 6-10 level-1 lymph nodes are negative for metastatic tumor, evaluation of additional lymph nodes does not have a significant effect on lymph node staging.

In addition to the number of lymph nodes removed, the extent of pathologic evaluation is critical in staging of axilla. After gross identification of lymph nodes, sections are taken for histologic evaluation. In many institutions the evaluation of an axillary lymph node consists of dividing the node in halves, preferably at the hilum; if the gross evaluation is negative for metastatic disease, one section is submitted for histologic evaluation.

Several different methods have been proposed to increase the detection rate of axillary metastases. Serial subgrossing and histologic step-sectioning of each lymph node have been proposed, and this intensive evaluation has been shown to upstage nodal status in a significant number of patients. Some investigators suggested routine use of immunohistochemical (IHC) assay to detect metastatic carcinoma cells. In more recent years, molecular biologic techniques have been used to detect even small numbers of metastatic tumor cells. Unfortunately, in most clinical settings, such detailed histologic evaluation and application of expensive techniques for the entire axilla are prohibitively expensive.

Sentinel lymph node (SLN) biopsy is an attractive alternative for accurate staging of axillary lymph nodes, because the SLN is presumed to be the first node that receives metastatic tumor cells. If the SLN is negative for metastasis, the other axillary lymph nodes are unlikely to contain tumor. Published studies also suggest that the absence of metastatic tumor cells in the SLN accurately predicts in 95-100% of cases the absence metastasis in the remaining axilla. Furthermore, since the SLN specimen consists of either

a single or a couple of lymph nodes, focused and detailed histopathologic evaluation and additional testing such as IHC assay is feasible in most clinical settings. Unfortunately, no well-defined methodological approach to histopathologic evaluation of the sentinel lymph nodes has been developed.

Lymph nodes are usually round or reniform organs of variable size. The lymphnode is encapsulated in dense fibrous connective tissue (lymph node capsule) from which trabeculae extend into the node. Afferent lymphatic vessels divide into several branches outside the lymph node capsule, then enter the lymph node in the subcapsular sinus. Around the entire circumferential perimeter of the lymph node, lymph drains into the subcapsular sinus, which in turn drains via a series of interconnecting channels into efferent lymphatic vessels at the hilum of the node. Theoretically, therefore, tumor cells circulating in lymphatic system first drain in the subcapsular sinuses of the lymph nodes

The sentinel lymph nodes should be identified, and each placed into a separate container, and transported immediately to the pathology laboratory. Since the radioactivity level is quite low by the time the specimen is transported to the pathology laboratory, it is not necessary to isolate it. It can be grossed in a fresh state using a routine pathology laboratory set-up.

After measurement, the specimen should be carefully examined and the number of lymph nodes present should be documented. The size, color, and texture of each node must be recorded. The presence of blue dye should be noted. Cserni has suggested that by following the blue dye intensity in a given node, it is possible to identify the nodal area most likely to contain metastatic tumor. In our experience the intensity of the blue dye shows marked variation from case to case.

The relationship of sentinel lymph node and surrounding adipose tissue should be noted. Extranodal tumor extension of breast cancer is an important prognostic factor that is usually marked by adherence of the adipose tissue. To assess the area size of extranodal extension, perinodal adipose tissue should be included in the histologic evaluation. If a node is smaller than 3 mm, it should be left uncut, and fixed intact. A small nick of the capsule may help the fixative to penetrate and ensure proper fixation. Lymph nodes that measure 3-5 mm should be sectioned serially at 3 – 4 mm intervals at a perpendicular-to-long axis to reveal as much of the lymph node's parenchymal surface as possible. Sections should be made with a sharp, clean scalpel to avoid crush artifact and

contamination. Crushed lymphocytes or histiocytes may mimic carcinoma on sections that are suboptimally fixed or processed. Contamination is especially troublesome when tissue is processed for molecular assay techniques. Even benign breast tissue contamination may cause false-positive results. The cut surfaces must be carefully examined and described.

Definition of Micrometastases: Any metastatic deposit not found on gross inspection and palpation but identified on light-microscopic evaluation could be broadly defined as micrometastasis. In the literature, the term micrometastasis is used to describe small metastatic deposits, but the definition of "small" varies in each study. Originally, Huvos et al. described micrometastasis as a single metastatic focus of less than 2 mm. Others used 1.3 mm or 0.5 mm as the upper limits of size. For staging purposes, the American Joint Committee on Cancer defines micrometastasis as a single metastatic focus 0.2-2.0 mm.

Some authors use the term occult metastases interchangeably with micrometastasis. Occult metastases are metastatic deposits that were not observed during the initial routine histopathologic evaluation but became apparent at deeper levels or on IHC evaluation. Although occult does not necessarily denote a size, occult metastases are usually quite small. Another term used in the literature is clandestine metastasis. A term introduced by Friedman et al. to describe small tumor emboli in the sinuses.

Detection Methods of Micrometastases: Except for some cases of lobular carcinoma, most of the "usual" metastases are readily detectable on routine hematoxylin and eosin (H&E)-stained sections when tumor cells are present on that particular section. When the metastatic tumor focus is small, however, the probability of the tumor cells presence on a representative histologic nodal section is directly related to the number of sections evaluated. Meyer calculated the probability of finding micrometastases of specific sizes on serial sections of sentinel lymph nodes. His data indicated that if a lymph node is sectioned at 2-mm intervals and embedded in toto for microsectioning, and if one H&E section of each level is examined, the probabilities of finding a 2 mm and 1 mm metastasis are 1.0 and 0.51, respectively. According to Meyer's data, examination of 16 microscopic levels would virtually detect all metastases 0.15 mm and larger in lymph nodes that are serially sectioned at 2 mm intervals.

Similarly, Friedman et al. calculated that statistically certain detection of a single tumor cell in a 5-mm node would require examination of 250 slides. A variety of other methods have been described in the literature to detect occult metastases.

1. Additional Sectioning with H&E Stain. The simplest approach is to obtain additional sections for H&E staining from the paraffin blocks. The number of additional sections, thickness of each section and the distance between each section can vary. Depending on these factors, examination of additional deeper levels may mean a few additional sections or serial sectioning through the

entire paraffin block, the latter approach may yield hundreds of additional slides.

2. IHC Stains for Epithelial Markers. IHC has been used to increase the sensitivity of detection of micrometastases. A number of different polyclonal and monoclonal antibodies against epithelial antigens (cytokeratins and other proteins such as epithelial membrane antigen) have been used. Metastases have been reported in 9–33% of patients whose lymph nodes were believed to be negative on initial examination. The percentage is generally higher in studies in which IHC was performed on deeper levels instead of on de-stained original H&E slides. These reports of higher detection rates on IHC performed on additional levels further illustrate the significance of serial sectioning. Unfortunately, no antigen specific to breast carcinoma has been identified. All antibodies that have been used for IHC can react with both benign and malignant epithelial cells. Therefore, pathologists should be cautious when they evaluate IHC. To diagnose metastasis, the cells should have morphologic features of carcinoma in addition to IHC positivity. In fact, the ability of simultaneous evaluation of morphologic features and IHC reactivity is the main advantage of IHC techniques over molecular biologic techniques.

3. Molecular Diagnostic Techniques. During the last decade some innovative molecular diagnostic techniques have been used to increase the likelihood of detecting breast cancer metastasis. In reverse transcriptase-polymerase chain reaction (RT-PCR). Tumor cells are not visualized, but mRNA related to breast carcinoma cells is demonstrated by gel electrophoresis. RT-PCR is a sensitive method capable of detecting one metastatic cell in a population of 10⁶ normal lymphoid cells. The markers used for this purpose include cytokeratins and MUC-1. Depending on the markers used 15–40% of lymph nodes believed to be negative on light-microscopic evaluation of H&E and IHC stains showed positive results with RT-PCR. Because the technique is so sensitive, false positivity due to contamination from normal breast is a serious concern, and the specimen must be handled with special care. Cross reactivity with homologous genes has been observed as another source of false-positive results. The biologic and clinical significance of RT-PCR-positive and light-microscopy negative lymph nodes is not known. Clearly, additional studies are needed to refine the techniques and determine the clinical significance of their results.

Localization of metastasis in sentinel lymph nodes has not been extensively studied. Cserni suggested that nodal metastases are most likely to occur adjacent to the entire site of the vital dye into the node. In 72% of sentinel lymph nodes with metastatic tumor, the metastases were found surrounding the lymphatic drainage entry site identified by blue staining. This interesting observation should be considered in gross evaluations of sentinel lymph nodes, especially when some of the tissue is harvested for nonmorphologic studies such as RT-PCR. The likelihood of occult metastases has been shown to be higher in sentinel lymph nodes than in non-sentinel lymph nodes. Weaver et al. identified metastatic tumor in 15.9% of sentinel lymph nodes and 4.2% of non-sentinel lymph

nodes after evaluation multiple deeper levels and using IHC means. Similarly, Turner et al. showed the conversion rate (from negative to positive) of sentinel lymph nodes to be significantly higher than that of non-sentinel lymph nodes when both sentinel and non-sentinel lymph nodes were examined at deeper levels and IHC was used in the same manner. These findings give further evidence that sentinel lymph node is the most likely node to harbor metastatic tumor cells. The size of metastatic foci varies in sentinel lymph nodes. The SLN's tumor burden directly correlates with frequency of non-sentinel lymph node metastasis. If SLN metastases are apparent on initial examination (most of these tumors are larger than 1 mm), the likelihood of metastases in non-sentinel lymph nodes is greater than that of non-sentinel lymph nodes from patients whose SLN metastatic foci were identified on deeper levels and/or by IHC assays. The frequency of non-sentinel lymph node metastases in women with immunohistochemically detected SLN metastases may be between that of women with negative SLN and women with large foci of metastatic carcinoma SLN. Circulating tumor cells can be demonstrated in lymph nodes, bone marrow, and even in parenchymal organs. Although the presence of tumor cells is required, it is not adequate for establishing a metastatic focus. Development of metastasis is a complex process that requires multiple steps. The biologic significance of identifying a single malignant cells or small clusters of malignant cells in a lymph node has been the subject to discussion in the literature. Some authors hypothesized that tumor clusters in a lymph node have biologic and prognostic significance only at a certain size, and that the presence of fewer than 10 tumor cells is not prognostically significant. The appropriate designations of these clusters need further study.

Differential Diagnosis Of Metastatic Carcinoma

1. Sinus Histiocytosis: This is marked by distention of sinusoids by histiocytes with finely granular and vacuolated cytoplasm. In size and shape, the nuclei resemble those of lobular carcinoma cells. When the histiocytes show syncytial arrangement, their histologic appearance closely mimics that of metastatic lobular carcinoma cells. When histiocytes show intracytoplasmic vacuoles, they mimic signet ring cell carcinoma. Since the histiocytes have no epithelial markers, IHC for cytokeratin or epithelial membrane antigen is helpful to distinguish them from metastatic carcinoma cells. Metastatic carcinoma cells usually show strong positivity for cytokeratins. Sometimes histiocytes, especially those with cytoplasmic vacuoles, have faint cytoplasmic positivity for cytokeratins, but this type of faint staining should not be confused with strong epithelial staining. Since the histiocytes show positive staining for lysozyme, chymotrypsin, and α_1 -antitrypsin, these markers may be used to confirm the histiocytic origin of the cells in question when epithelial markers are not conclusive.

Dendritic histiocytes, which are elongated histiocytes, are part of the normal cells in the sinuses of lymph nodes and may show faint cytoplasmic positivity for cytokeratins. The typical cytoplasmic elongations of these cells should be helpful in discriminating them from metastatic carcinoma cells.

2. Nevus Cell Aggregates: Collections of cells that morphologically resemble cutaneous nevus cells may be found in the capsules of lymph nodes in various anatomic locations, including the axilla. Nevus cell aggregates may have a nodular or diffuse configuration in the lymph node capsule. The cells have oval nuclei with finely granular chromatin. Their morphologic features are identical to those of cells typically found in cutaneous nevi. Fine brown intracytoplasmic pigment can be identified in a minority of cases. Typically, nevus cell aggregates are easily identified by their morphologic appearance. If there is a question, IHC for epithelial markers may be performed. Nevus cell aggregates are negative for epithelial markers and show positivity for S-100 protein.

3. Benign Glandular Inclusions: Heterotopic benign glandular inclusions derived from skin appendices or breast may be found in axillary lymph nodes. Histologically, these inclusions are characterized by well-formed glandular structures lined by squamous, columnar, or apocrine epithelium. Unlike mesothelial inclusions found in abdominal and pelvic lymph nodes, inclusions in axillary lymph nodes are located in the lymph node capsule or in perinodal tissue. In most cases, these inclusions are easily distinguished from metastatic carcinoma. However, the lining epithelium may undergo a variety of metaplastic and hyperplastic changes that may mimic metastatic carcinoma. Comparison of morphologic features of the primary tumor and glands in the lymph node is essential to arriving at the correct diagnosis.

If the primary tumor is a well-differentiated carcinoma, especially tubular carcinoma, the distinction between metastatic carcinoma and heterotopic glandular inclusions may be difficult. Since the benign glandular inclusions usually have myoepithelial cells around the glands, IHC demonstration of myoepithelium may be helpful. Benign glandular inclusions are an important source of false-positive results when non-morphometric methods such as RT-PCR are used to evaluate sentinel lymph nodes.

4. Displacement of Benign Epithelium / Carcinoma into Lymph Nodes: The mechanical force of needle biopsies of breast may disrupt and displace both benign and malignant breast epithelium into lymph nodes. Carter et al. described 15 cases of displacement of breast epithelium and tumor cells in subcapsular sinus of axillary lymph nodes following breast biopsy and lymph node dissection. In all these cases, the epithelial clusters were associated with hemosiderin-laden macrophages, foreign body-type multinucleated giant cells, and red blood cells.

Carter et al. suggested that the epithelial clusters most likely represent artifactual displacement of cells due to previous surgical manipulation rather than true metastases. The biologic behavior of malignant cells imported into lymph nodes after mechanical force is not known. Since it is difficult to know their clinical significance, these cells should be considered to have unknown biologic significance until further clinical outcome data become available.

5. Extramedullary hematopoiesis in lymph nodes: The presence of immature hematopoietic cells and megakaryocytes in lymph nodes may mimic metastatic carcinoma. IHC stains for keratin should be helpful in this differential diagnosis since the hematopoietic cells are negative for keratin.

Intraoperative Evaluation Of Sentinel Lymph Nodes

If SLN biopsy and axillary lymph node dissection are planned as a single procedure, the intraoperative evaluation of the SLN must be rapid and accurate, if metastasis is identified in the SLN during surgery, complete axillary node dissection can be performed in the same setting, avoiding the cost and operative morbidity of second surgery. Table 1 outlines our guidelines for intraoperative evaluation of SLNs currently. However, this proposal is based purely on our own experience and as further clinical follow-up information becomes available it is subject to further modifications.

The different methods proposed for intraoperative evaluation of SLN are: Frozen Section, Cytologic preparations (touch imprints), and rapid immunohistochemical assay.

1. Frozen Section

Frozen sections have been used to identify metastatic deposits of breast cancer in many studies, with variable results, and a sensitivity of 85–95%. In some studies only one representative frozen section was used; others used multiple sections or the entire node. Metastases larger than 2 mm can be identified successfully in 95–98% of cases. However, the identification of micrometastases (metastases smaller than 2 mm) has been problematic, with a reported accuracy rate of less than 30%. The main limitations of frozen section include technical difficulties of obtaining complete sections, especially in lymph nodes with fatty replacement; and loss of morphologic details because the freezing artifacts may obliterate cytologic details that may be essential for differentiating small clusters or single tumor cells from atypical lymphohistiocytic cells.

2. Cytologic Preparations

Intraoperative cytologic examination has been shown to be a simple, reliable, and accurate technique for evaluating many surgical pathology specimens including lymph nodes. Different techniques including fine-needle aspiration, scrape preparations, and touch imprints have been used. Cytologic preparations provide clear cytologic details without artifacts of freezing.

The diagnostic value of cytologic preparations in examining sentinel lymph nodes has been investigated by several research groups. Touch imprint cytology, the most common method, consists of simple touching of the lymph node surface onto a glass slide. The main principle of this technique is that tumor cells, if present, will adhere to the slide and can be identified by their morphologic differences against the background of lymphocytes. The touch-imprint is reported to be 80–90% accurate in identifying macrometastases in SLN. However, the accuracy rate for identification of micrometas-

tases is low. In addition, the false-positive cases reported in the literature, pose a greater problem than false-negative cases. The main reason for a false-negative result is either technical or interpretive. For a variety of technical reasons, tumor cells may not adhere to slides, or touching on the slide may cause variable patterns of cell distribution on the slide. Some clusters may have monolayer cells, others may have multilayers. The variation in cell thickness may cause a fixation artifact that may lead to errors in interpretation and, in addition, it is possible that a sampling error might occur since the area of the lymph node containing a micrometastasis might not adequately sampled. Experience in the evaluation of touch imprints is essential to avoid such pitfalls.

3. Intraoperative Immunohistochemical Assay

Intraoperative IHC assay using antibodies against epithelial antigens may identify microscopic foci of metastases on frozen section and cytologic preparations of SLN. Although some researchers found that the addition of intraoperative IHC improves the sensitivity of identifying micrometastases, others showed that the addition of IHC was helpful only for confirming the metastatic nature of atypical cells observed on frozen sections or on cytology and did not significantly increase the accuracy of intraoperative evaluation of SLN.

Pathology Report of SLN

1. Number of sentinel lymph nodes identified
2. Presence or absence of metastasis
3. Size of metastasis
4. Presence of extranodal extension
5. Mode of identification of metastasis (H&E or IHC or both)

Summary

Evaluation of the sentinel lymph node is an exciting technique that allows identification of one or several lymph nodes that received lymphatic drainage from the primary tumor. Although the technique is still being assessed in various clinical trials before it can be validated for routine clinical use, high accuracy rates of predicting final lymph node status have been reported.

The extent of pathologic evaluation of SLN is not clearly described in the literature. Pathologists are encouraged to examine SLN intensively at multiple deeper levels and with IHC means. The diagnosis of metastatic carcinoma should be reserved only for patients whose nodes clearly contain tumor cells. The presence of a single keratin-positive cell or clusters of tumor cells with features suggestive of displacement should be considered as having undetermined clinical significance.

A number of large prospective clinical trials are in progress to answer the questions related to SLN evaluation technique. Until the results are known, SLN procedure and pathologic evaluation with nonstandard techniques should be considered as experimental.

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