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review

The intraoperative examination of axillary sentinel nodes

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Routine histological examination of axillary sentinel nodes predicts the nonsentinel axillary node status and may allow to spare axillary clearing in patients with breast cancer. To avoid the need for two separate surgical sessions the results of sentinel node examination should be known intraoperatively. Routine frozen section examination of sentinel nodes, however, is liable to yield false-negative results. An extensive intraoperative examination of frozen sentinel nodes which would attain a sensitivity comparable to that obtained by routine histological analysis has been therefore devised. The frozen sentinel nodes are subserially sectioned at 50 μm intervals. For each level, one section is stained with hematoxylin and eosin (H&E) and the other immunostained for cytokeratins using a rapid immunocytochemical assay. Immunocytochemistry did not increase the sensitivity of the examination. The general concordance between sentinel and axillary node status was 96.7 %; the negative predictive value of intraoperative sentinel node examination was 94.1 %. The intraoperative examination of axillary sentinel nodes is effective in predicting the axillary node status of breast cancer patients and it may be instrumental in making the decision to spare axillary clearing.

Key words: breast neoplasms - surgery; lymph node excision; axilla; lymph nodes - pathology; intraoperative period; immunohistochemistry

Introduction

The axillary sentinel node biopsy (i.e. the biopsy of the first node draining the lymph from the tumour) in patients with small primary breast carcinomas and clinically uninvolved axillary nodes is rapidly gaining wide diffusion.[1-4] Several investigations have documented that this procedure has a very high (96 to 100 %) negative predictive value with respect to the status of the remaining nonsentinel axillary nodes, and may avoid unnecessary axillary lymph node dissection (ALND) in patients with negative sentinel nodes.[5-7]

Axillary lymph node status is the most powerful prognostic parameter in breast carcinoma patients, and dictates the choice of the post-surgical adjuvant treatment. Because it is not possible to assess the node status clinically or by imaging techniques, ALND

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with the removal of at least part of the axillary nodes has represented till now the treatment of choice in ablative or conservative breast surgery.

ALND, however, may lead to several complications, like oedema, numbness, pain and weakness of the arm. Furthermore, mammographic screenings and women's consciousness have resulted in an ever increasing prevalence of patients being treated with ALND for very small primary tumours. Accordingly, these patients are less likely to have already developed axillary node metastases. Indeed, ALND will yield uninvolved lymph nodes in approximately 65-70 % of patients with small (pT1) primary tumours.[5-7]

Also, it should be taken into account that approximately 25 % of the patients who have undergone ALND and are without histopathological evidence of axillary metastases (pN0) will experience disease progression. This is most likely due to an inaccurate sampling or examination of all the nodes removed by ALND with the missing of (micro)metastatic foci. Indeed, the re-examination of axillary nodes by serial sectioning or use of more sophisticated techniques (e.g. immunocytochemistry for cytokeratins) allows to identify the missed metastases and to up-stage a similar percentage of patients.

The SNB may spare ALND in patients with uninvolved sentinel nodes, and at the same time enables the pathologists to extensively examine individual (or very few) lymph nodes by serial sectioning and use of immunocytochemical techniques. This results in the highest likelihood of detecting even the smallest metastatic deposits with a more correct staging of the disease. Indeed, the prevalence of sentinel lymph node metastases in patients with small (pT1) primaries is much higher than expected, almost reaching 35 % of the cases. In approximately 40 % of these patients, the sentinel nodes are the only involved axillary nodes, thus reinforcing the validity of the SNB concept.[8-10]

Though the short follow-up period does not allow to evaluate the clinical course of the disease in patients with uninvolved sentinel nodes, it is likely that the SNB procedure will allow to identify true pN0 patients with a very favourable prognosis.

The histopathological examination of axillary sentinel nodes

To be effective in the management of breast carcinoma patients, SNB must rely upon an extremely careful and extensive histological examination of the sentinel nodes, which must be entirely and serially sectioned at reduced intervals. Both, computer simulations and the current practice have documented that to identify small micrometastatic foci (2 mm in size or less) the sentinel nodes must be sectioned at 50-200 microns intervals, with the examination of up to 60 or more sections per node.[7,11]

The histological examination of the axillary sentinel nodes may be performed on permanent sections of formalin-fixed, paraffin-embedded tissue, or intraoperatively on frozen sections. This latter procedure has the advantage of enabling to complete the surgical treatment of the primary tumour and of the ALND in a single session. Again, even in the case of an intraoperative examination of the sentinel nodes, the nodes must be entirely and serially sectioned. Indeed, the examination of few frozen sections of only a moiety of the node (as routinely done for other purposes) will lead to an unacceptably high number of false negative diagnoses.

To overcome this drawback, we have devised a procedure for the extensive intraoperative examination of the axillary sentinel nodes.[7] The nodes are bisected and both moieties are frozen in isopentane chilled by liquid nitrogen. Fifteen pairs of frozen sections are then cut at 50 microns intervals from each moiety. Whenever lymph node tissue is left,

additional pairs of sections are cut at 100 micron intervals, until the complete examination of the node. One section of each pair is routinely stained with haematoxylin and eosin (H&E), while the other is kept unstained for the possible use of immunocytochemical reactions for cytokeratins. These are done to assess the malignant nature of suspicious cells identified in the corresponding H&E-stained sections.

In our case, the general concordance between the intraoperative examination of sentinel nodes and axillary-node status was 96.77 %, the true false-negative rate 3.22 %, the sensitivity 93.3 % and the negative predictive value 94.1 %. The time required for such an extensive examination of the sentinel nodes is approximately 40 minutes, which are normally spent by the surgeon to complete ablative or conservative mastectomy (having done the SNB first).

Immunocytochemistry and molecular biology in the examination of axillary sentinel nodes

Many reports have emphasised the role of immunocytochemistry in the accurate identification of (micro)metastases in the sentinel nodes and have recommended to perform immunoreactions for specific epithelial markers (cytokeratins) in all sentinel nodes.[12-14] It must be considered, however, that the use of immunocytochemistry does not overcome the need for an extensive sectioning of the node, which must be entirely sampled. To keep the time required and the costs of the examination of the sentinel nodes as low as possible, the use of immunocytochemistry may be confined to those cases which cannot be confidently diagnosed on purely morphological grounds. This holds particularly true for single-cell metastases commonly occurring in invasive lobular carcinomas. The percentage of cases subjected to immunocytochemistry depends upon the training and expertise of the examining pathologist on the one side, and upon the quality of the tissue sections on the other.

Even more recently, the possible role of the amplification by PCR of specific mRNA molecules to detect sentinel node metastases has been exploited.[15,16] In these procedures, RNA molecules are extracted from fresh/frozen lymph nodes, and complementary DNA is synthesised by reverse transcription. Epithelial-specific markers (cytokeratin 19, CEA, MUC1, maspin, mammaglobin, etc) are then amplified by PCR. These techniques are effective in identifying a single metastatic cell among 1,000,000 normal lymphoid cells in *in vitro* experiments. *In vivo* results, however, have been less impressive thus far. Indeed, the sensitivity of these techniques often does not reach the expected 100 % of cases known to harbour metastases (most likely due to the problems with the sampling procedures). Even more important, however, is their low specificity, with several false-positive results when the techniques are applied to uninvolved lymph nodes or to lymph nodes from patients without any neoplastic disease.

The clinical implications of micrometastases in axillary sentinel nodes

A most debated issue in the SNB procedure is related to the clinical implications of the occurrence of micrometastases in the sentinel nodes. It has been formerly discussed whether these small metastases have an impact on patients' survival, and many investigations -but not all- on the subject have documented a prognostic implication of the axillary micrometastases in the patients with long-term follow-up.

In the contest of the SNB, however, it is important to assess whether the detection of micrometastatic disease in the sentinel nodes is predictive of the occurrence of additional

metastases in the nonsentinel axillary nodes. Contrary to the findings of other groups[17,18], in our experience, approximately 25% of 150 patients with micrometastases in the axillary sentinel nodes harbour additional axillary metastases. In 75% of these cases, the additional metastases are larger than 2 mm, and their prognostic value is undisputed. Accordingly, we suggest that patients with micrometastatic disease in the sentinel nodes undergo ALND, unless they are enrolled in randomized clinical trials specifically designed to address the question of the proper surgical management of the axilla.

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