Ancillary studies (Non-core)

Immunohistochemistry (IHC)

It is important to document the immunohistochemical antibody(s) used for assessment and the result(s) of immunohistochemistry (IHC) stains in the report. The routine application of IHC to assess the presence of carcinoma in lymph nodes is not recommended. The pathologist may use IHC to evaluate cells that are suspicious but not diagnostic of carcinoma in routine haematoxylin and eosin (H&E)-stained sections, especially for lymph nodes obtained post-neoadjuvant therapies. IHC for broad spectrum cytokeratins (CKs), such as AE1/AE3, as well as other CKs (CK7, pancytokeratin, OSCAR, CK19) are suitable. The pattern of CK reactivity of the primary invasive carcinoma (e.g., CK7-negative but CK20-positive primary mammary carcinoma with apocrine morphology) may guide the choice of the IHC panel most suitable to evaluate suspicious cells in lymph nodes.

Some non-epithelial cells (such as dendritic reticulum cells or lymphoid cells) may show non-specific uptake of CKs. Keratin debris including anucleate keratin squames may also yield staining that is specific for keratin but should not be interpreted as carcinoma cells. Diagnostic interpretation mandates careful correlation of morphologic and IHC findings. In problematic cases, comparison with the morphology and IHC profile of the primary invasive carcinoma is advised, whenever possible.

When the IHC work-up demonstrates axillary lymph node metastases from an extramammary primary site (e.g., müllerian carcinoma, melanoma), this finding needs to be clearly stated in the report and the pN classification for breast carcinoma does not apply.

Although it is standard practice to assess estrogen receptor (ER), progesterone receptor (PR) and HER2 receptor status of the primary invasive carcinoma in the breast, occasionally it might be necessary to assess receptor status of nodal metastatic carcinoma. In such cases, the same guidelines for interpretation and reporting of ER, PR and HER2 status of primary invasive carcinoma should be used.

Molecular techniques

All lymph node macrometastases must be identified histologically. The use of loop-mediated isothermal amplification (LAMP), as a quantitative mRNA amplification technique, is approved as an alternative method only for the evaluation of lymph nodes that are negative by gross examination. This test requires that the entire lymph node tissue (or nearly the entire lymph node tissue) be submitted for LAMP analysis, preventing histologic examination. Consequently, any lymph node suspicious for metastatic carcinoma at gross examination should *not* be submitted for quantitative molecular metastasis analysis.

It is important to specify the results of one-step nucleic acid amplification in the report. One-step nucleic acid amplification is a commercially available LAMP-based assay for the detection of mRNA (*CK19*) associated with breast carcinoma. It is used to deduce the presence of epithelial cells in the lymph node and estimate the volume of disease.[1] The RD-100i OSNA system, a one-step nucleic acid amplification-based test for the detection and quantification of *CK19* mRNA, was formally approved by the United Kingdom's National Institute for Health and Cancer Excellence (NICE) in August 2013.[2] Analysis of the whole lymph node using the RD-100i OSNA system may be used for detecting sentinel lymph node metastases in clinically node-negative patients with early (T1-T2) invasive breast carcinoma who undergo sentinel lymph node biopsy and are candidates for axillary lymph node dissection. Histologic examination has high specificity but may miss minute deposits of carcinoma, while the one-step nucleic acid amplification assay eliminates tissue sampling bias as the whole node is analysed. The one-step nucleic acid amplification assay has a rapid turnaround time

and is less resource intensive than histology. When compared to alternate slice histology, the RD-100i OSNA has a 96% agreement. Quantification of *CK19* mRNA using one-step nucleic acid amplification correlates with the extent of carcinoma in the lymph nodes.

RD-100i one-step nucleic acid amplification is calibrated so that it may ignore ITCs (defined as *CK19* mRNA copy numbers between 100 and 250/microlitre (μL)) but can detect micrometastases (translated to *CK19* mRNA copy numbers between 250 and 5000/μL) and it may also detect macrometastases (interpreted as such if *CK19* mRNA copy numbers exceed 5000/μL). As the copy numbers are proportional to the number of cells expressing CK19 and therefore to the volume of nodal involvement, greater copy numbers reflect greater volume, and although these are not measurable in metric units due to the non-microscopic nature of the assay, results are extrapolated as micrometastases or macrometastases. Therefore, the coding of such results as pN0(mol+) (the category defined in the Union for International Cancer Control (UICC) TNM Classification of malignant tumours[3]) would be inaccurate; pN1mi(mol+) and pN1(mol+), although not defined in the UICC TNM Classification, would be the most appropriate labels for such types of nodal involvement as they refer both to the extrapolated size and the non-microscopic detection. One-step nucleic acid amplification finds application in some countries (such as Spain, France, Italy, Japan and Australia) especially in settings when rapid intraoperative assessment of lymph node status is required to expedite patient care.

False positive and false negative results may occur with quantitative molecular tests. In rare cases, a positive result may be “false positive”, biologically speaking, because it is secondary to the presence of benign mammary epithelium in a lymph node, due to displacement during prior procedure(s), ectopic intranodal benign mammary glands or skin adnexa, or endosalpingiosis/benign müllerian inclusions within the lymph nodes. Rarely, secondary involvement of an axillary lymph node by a CK19-positive carcinoma that is primary at an extramammary site might also possibly yield a false-positive result. At present, the clinical significance and management implications of a positive quantitative molecular result in the setting of a histologically negative lymph node are unknown.

References

1 Cserni G (2012). Intraoperative analysis of sentinel lymph nodes in breast cancer by one-step nucleic acid amplification. *J Clin Pathol* 65(3):193-199.

2 National Institute for Health and Care Excellence (2013). *Intraoperative tests (RD-100i OSNA system and Metasin test) for detecting sentinel lymph node metastases in breast cancer*. Available from: https://www.nice.org.uk/guidance/dg8/chapter/1-Recommendations (Accessed 1st June 2020).

3 Brierley JD, Gospodarowicz MK and Wittekind C (eds) (2016). *Union for International Cancer Control. TNM Classification of Malignant Tumours, 8th Edition*, Wiley, USA.