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Current Perspective

An updated European Organisation for Research and Treatment of Cancer (EORTC) protocol for pathological evaluation of sentinel lymph nodes for melanoma

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Abstract The sentinel lymph node (SLN) biopsy is a highly accurate staging procedure and the most important prognostic factor in melanoma patients. The European Organisation for Research and Treatment of Cancer (EORTC) Melanoma Group aimed to design an updated evolved SLN protocol for the histopathological workup and reporting. We herein recommend extending the distance between steps according to the short axis dimension of the lymph node and optimise both conventional sectioning and staining procedures including immunohistochemistry. We also provide guidance on the description of the spatial localisation of melanoma deposits in a SLN. The histopathological features to be reported include the following: presence or absence of the metastasis, the intranodal location of the metastasis

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Recommendations

(subcapsular, parenchymal, combined, extensive confluent and extensive multifocal), the number of the metastatic deposits (1, 2–5, 6–10, 11–20 and >20), the maximum dimension of the largest metastasis (indicating its site) and the presence of extracapsular extension and of naevus cells. This updated EORTC protocol is expected to clarify and simplify the existing procedures, ensuring a reasonable workload for the laboratory and for the pathologists resulting in cost saving with no loss, and possible increase, in accuracy.

1. Introduction

Sentinel lymph node (SLN) biopsy for melanoma introduced in the early 90s [1] has become an established procedure and is likely to remain so even after the Multicenter Selective Lymphadenectomy Trial-2 (MSLT-2) [2] suggested a lack of impact on melanoma-specific survival for completion lymphadenectomy. In light of the evolving landscape of adjuvant therapy, its value is now mainly as a key staging procedure to accurately define prognosis, provide more consistent grouping in clinical trials [3] and enable oncologists to assess whether a patient is eligible for systemic therapy.

A wide divergence of protocols for the histopathological handling of SLNs has developed, and this has not been resolved [3–20]. Furthermore, pathological reporting protocols are not harmonised, extensive protocols have a significant impact on laboratory workload and in addition to this, there are problems of interpretation resulting in an apparently high number of errors detected in a pathological review of clinical trials. The quality of the report depends on having an excellent quality of dissection, sectioning and staining and immunohistochemistry. The technical variability is the main culprit in interpretation errors.

The European Organisation for Research and Treatment of Cancer (EORTC) protocol established in 2003 [12] is a widely used method for the assessment of melanoma burden in SLN. The EORTC Melanoma Pathology Group is now proposing an improvement thereof by the assessment of a larger proportion of the SLN with the greatest efficiency. We noted that twice as many metastases were found by increasing the number of steps; however, this still leaves a considerable proportion of most lymph nodes unexamined, especially in those SLNs that have a more rounded shape. Because roundness correlates with a relative increase in length of the shortest axis, a larger volume of a nodal tissue parallel to the bisection plane remains unexamined compared with ellipsoid SLN using the current procedure.

The most important objective of the different protocols has been to achieve maximum likelihood of detecting metastases. Extending the distance between steps in the sectioning protocol was undertaken so that the previously neglected part of broad lymph nodes was then assessed. In a preliminary assessment, steps were increased up to 300 μm, but an increased detection rate was not seen. This supports Cochran's original hypothesis that metastases are concentrated in the central plane through the hilum and the longest dimension of the node [7]. However, wider steps could still be appropriate when the dissection of the lymph node has resulted in an asymmetric bisection of the node. In deciding that the procedure should identify metastases down to 0.1 mm, we opted for a minimum thickness of step of 0.05 mm (50 μm).

There is insufficient evidence to suggest that the EORTC protocol for the pathological evaluation of SLNs for melanoma should be fundamentally changed at this stage. It was decided to focus on the clarification and simplification of the existing procedures with the objective to reduce the technical workload thereby making it more sustainable in a routine diagnostic setting. We also decided to optimise both conventional sectioning and staining procedures including immunohistochemistry and provide guidance on the description of the spatial localisation of melanoma deposits in a SLN.

The following changes to the existing protocol are proposed:

2. Sectioning protocol

The SLN is bivalved, so revealing the largest surface area of the lymph node in two pieces for sectioning. The maximum dimension is taken from the cut surface. Both pieces are placed face down in one cassette, except when the lymph nodes are too large, in which case each piece is placed in a separate cassette.

If in error the bivalving of a more rounded node produces markedly unequal pieces, the largest of them can be further sliced or bivalved producing three pieces in total. Alternatively the sectioning steps can be extended up to 400 μm. The sectioning protocol is determined at the grossing stage by the pathologist. This in turn is based on an estimate of the length of the short axis of the node. The estimation is determined by palpation of the node between finger and thumb with

adjacent ruler. This enables an approximation of this dimension which can be made virtually instantaneously. The steps between sections is determined by the formula given in Fig. 1 and is written on the cassette to convey to the sectioning technician. This incremental increase in steps is intended to ensure that a larger proportion of the SLN is assessed microscopically.

(a) In the sectioning protocol, two sections are cut at each step, except for step 2 where three extra sections are taken and retained unstained.
(b) At each level, a section is stained for S100 protein.
(c) Only at step 2 is a section stained for H&E.
(d) One spare section is taken at all levels, except for step 2 where three spare sections are taken.

Figs. 1 and 2 illustrate the sectioning and staining protocol as above described.

In comparison with the previous protocol [12], the net result of these changes is a reduction of the total number of sections, a decrease of staining by five H&Es and two

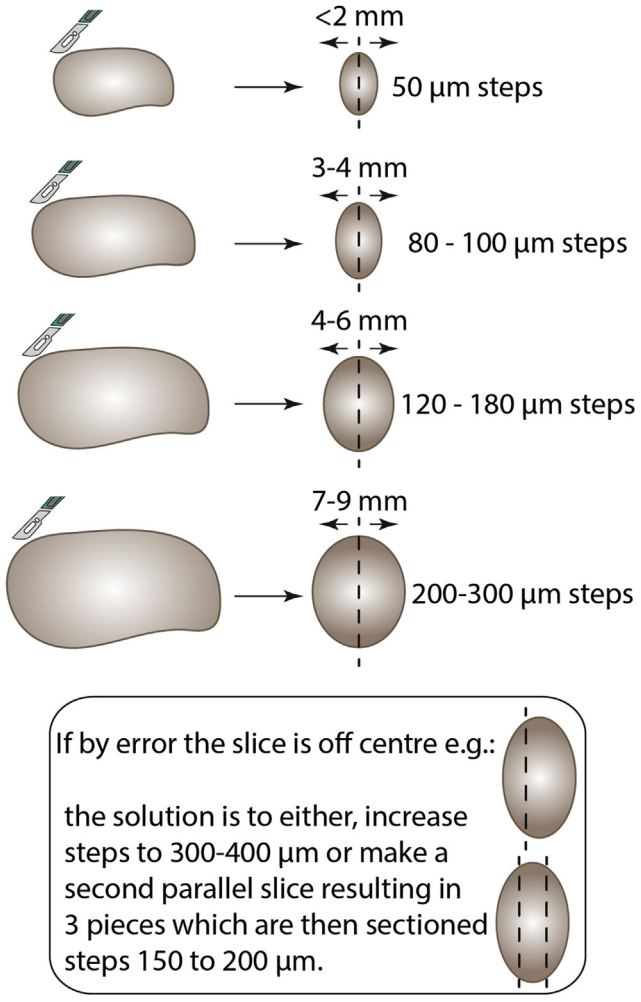

Fig. 1. Revised EORTC protocol depicting sectioning of sentinel lymph node in melanoma. EORTC, European Organisation for Research and Treatment of Cancer.

Section	Staining
1	S100
2	Spare
50/300 µm step (+50/300 µm)	
3	H&E
4	S100
5	Spare
6	Spare
7	Spare
50/300 µm step (+100/600 µm)	
8	S100
9	Spare
50/300 µm step (+150/900 µm)	
10	S100
11	Spare
50/300 µm step (+200/1200 µm)	
12	S100
13	Spare
50/300 µm step (+250/1500 µm)	
14	S100
15	Spare

*50 µm for the small lymph nodes of 5 mm or less, 300 µm in lymph nodes of more rounded configuration, i.e. with a short axis length of 9 mm or more.

Fig. 2. Revised EORTC protocol for staining sentinel lymph node in melanoma. EORTC, European Organisation for Research and Treatment of Cancer.

immunostains. The thickness of each section should be 3 µm. The unstained sections are retained at each level so that they can be used for further H&E or immunostains if needed.

3. Immunohistochemical staining

We have confidence in S100 protein staining for efficient recognition of melanoma metastases. Diaminobenzidine is the preferred chromogen because the red chromogen aminoethylcarbazole is not always well-localised.

S100 protein has a high sensitivity for melanoma detection because it is expressed in almost all primary and metastatic melanomas [21,22] but with a relatively low melanoma specificity because S100 protein expression can also be observed in nodal naevus cells, dendritic reticular cells, Schwann cells and adipocytes. This can possibly result in a confusing background staining. However, the morphological features of the other cells potentially staining with S100 in lymph nodes are so distinctive the problem is largely theoretical. Short

experience is sufficient to produce high confidence and accuracy.

HMB-45 is a relatively specific marker for melanocytes but lacks sensitivity because approximately 70–76% of melanoma are HMB-45 positive [21,22]. This stain might result in missed metastases.

Although more sensitive than HMB-45, Melan A is considered not sufficiently specific [22] and not quite as sensitive as S100 protein because it is not positive in all melanomas. It can also stain macrophages/melanophages, whereas S100 protein and HMB45 do not as a rule.

Currently, SOX-10 is under evaluation as an alternative for S100 protein because it is more specific, but its sensitivity is not well-established [23,24].

Panmelanocytic cocktails may facilitate the interpretation of challenging cases, and improvement in detection rate has been reported [25].

4. Technical general comments

The interpretation is very much dependent on the technical production of thin (3 μm) flat, complete and well-stained sections without artifact. In our experience, this is best achieved following using an extended fixation (24 h), Harris' haematoxylin and S100 protein using an automated staining platform.

5. Guidance on histopathological interpretation

Nodal naevus cells present the most important problem in SLN interpretation (Fig. 3). Naevus cells can be seen in 10–20% of SLNs for melanoma [6,25,26]. When present, they are almost always located in the fibrous tissue of the capsule or trabeculae within the lymph node but can also be seen in a paralymphatic site outside the capsule. Perilymphatic naevus cells may be seen bulging into the lymphatic space but are still surrounded by endothelium.

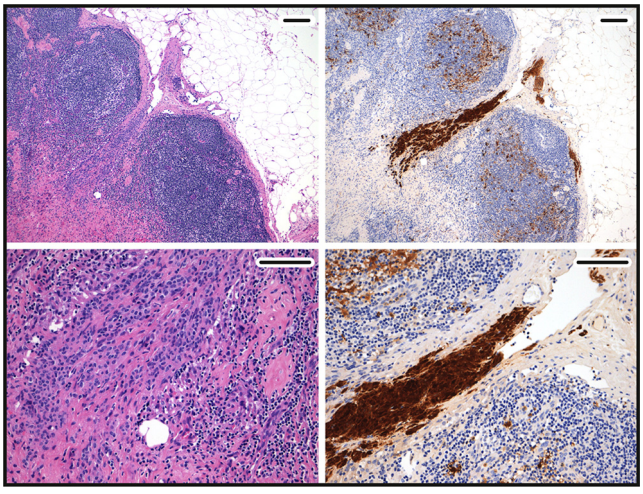

Fig. 3. Nodal naevus cells located in the capsule and tracking down the nodal trabeculae in the capsule.

Occasional collections of naevus cells in the capsule can be quite large and appear to bulge in continuity into the subcapsular space. Naevus cells from Spitz naevi, deep penetrating naevi or blue naevi may be seen rarely in sentinel lymph nodes and may not be so closely related to the fibrous tissue of the capsule or trabeculae. In these cases, a review of the primary cutaneous lesion is essential as it can help incorrect classification of the primary and putative metastasis. Indeed, a review of the primary pathology by the pathologists interpreting SLNs is desirable, although not always achievable.

The features of melanoma metastasis in SLNs can be of predictive value for the involvement of other lymph nodes. Tumour burden and the localisation is associated with distant metastasis-free and overall survival. One of these features is the pattern of distribution of the metastases within the sentinel node (Fig. 4). We recommend that melanoma metastases in a SLN can be classified as follows: subcapsular, parenchymal, combined (subcapsular and parenchymal), extensive confluent and extensive multifocal.

The 8th Edition of the American Joint Cancer Committee underscores the good prognosis of small metastases confined to the subcapsular zone [27] but also quotes 'poor reproducibility of microanatomic location in one study' [28]. In that study, however, an accurate and detailed definition of the various patterns was not given in advance to the participants. The subcapsular metastases should have a smooth parenchymal aspect rather than an irregular border or budding [29] to distinguish them from a subcapsular metastasis with parenchymal extension. According to such strict definition, a subcapsular site has not been associated with further non-sentinel nodes involved on completion lymphadenectomy [30]. The extensive multifocal microanatomical location is characterised by multiple metastatic melanoma foci spreading across more than 70% of lymph node area in the most representative slide. A number of deposits covering less than 70% qualifies as parenchymal. These distinctions are clearly arbitrary for the convenience of descriptive pattern classification.

The question whether paratrabecular metastases are equivalent to subcapsular metastases has been raised, and we recommend to classify paratrabecular metastases as parenchymal but add further specification. Whether paratrabecular site has a specific significance will be clarified in future studies.

In addition, we now recommend documenting extracapsular extension as a separate feature, not as a part of an extensive pattern, as previously described [30].

Although not currently used for staging, tumour burden is clearly important and is likely to be included in the future prognostic models in patients with the regional metastatic disease. The maximum diameter of the largest aggregate in the SLN (1.0 mm versus

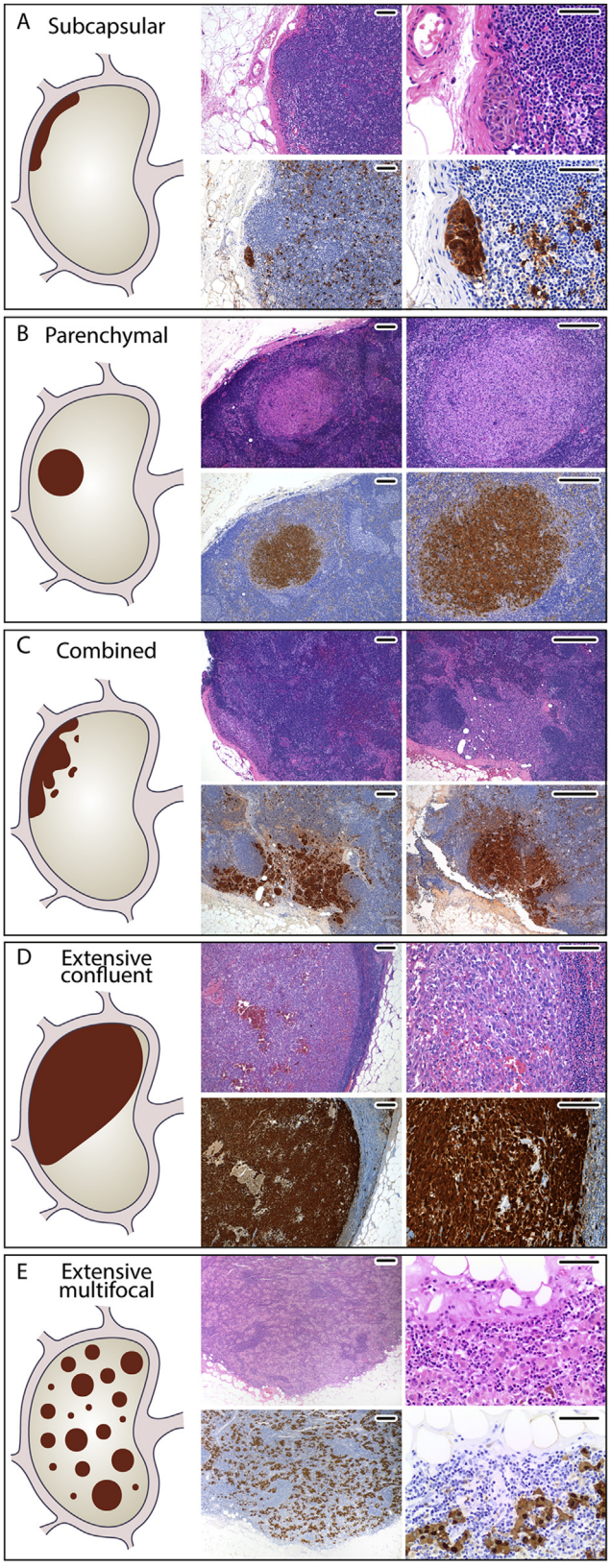

Fig. 4. Topography of metastatic deposits within the sentinel lymph node: subcapsular, parenchymal, combined, extensive confluent and extensive multifocal. Immunostain with S100 protein is shown.

>1.0 mm) has already been included as an additional inclusion criterion for participation in adjuvant clinical trials [31,32]. A range of procedures can be used to estimate quantitatively tumour burden in the SLN, such as the maximal number of metastatic foci within the node, the maximum size of the largest metastasis, the maximum depth from the capsule of the tumour deposit (tumour penetrative depth) and the percentage cross-sectional area of the SLN involved by the tumour [3,14,16,30,33–39]. Some of these involve calculations at several levels [17,40].

The maximum dimension of the largest metastasis has prognostic significance, but care must be taken to measure only those metastases with confluent neoplastic cells. The maximum dimension of the largest deposit may be a sufficiently accurate estimate of tumour burden except when the pattern of metastasis is in the form of numerous scattered small metastases. For this reason, we recommend that in addition to measuring the maximum dimension of the largest metastatic deposit (and indicating its site) and the pattern of distribution of metastases, all deposits should be counted in the most representative slides as 1, 2–5, 6–10, 11–20 or over 20. If single cells or paucicellular clusters of melanoma cells are identified, they are referred to according to their size and site as for larger metastasis.

Starz measured the depth of metastases from the capsule as another way of assessing prognosis. We feel that the assessment of metastatic pattern using the subcapsular or parenchymal site is a simpler and quicker way to achieve the same objective, but Starz method is a reasonable alternative [10,14].

The extracapsular extension is often quoted as an important predictor of poor clinical outcome and has been classified as focal (≤2 mm) or extensive (>2 mm) [34]. Rao *et al.* [41] have found that nodal extracapsular extension has a significant adverse effect on relapse-free survival but not on overall survival. Crookes *et al.* [42] have confirmed the significant independent adverse prognostic impact of extranodal spread on patterns of recurrence and survival in advanced stage melanoma patients. However, in our experience, extracapsular extension in SLNs is a rare event because it is almost always associated with extensive involvement of a large lymph node which would usually be palpable clinically and therefore not usually excised as a sentinel lymph node. Extracapsular extension, meaning invasion extending through the fibrous tissue of the capsule and into surrounding adipose tissue with or without lymphatic involvement, if present, is clearly a bad prognostic sign but is so uncommon in this context, it is not often useful. What may be confused with extracapsular extension is melanoma in afferent lymphatics or those lymphatics traversing the capsule. Tumour in afferent lymphatics without node involvement is reported as a positive SLN. This is uncommon and is usually associated with minimal or subcapsular or combined pattern of

metastases in the adjacent lymph node. When intralymphatic tumour cells are present but tumour is not seen infiltrating through the fibrous tissue of the capsule, we classify this pattern according to the intranodal pattern and have found that extracapsular lymphatic permeation in this context is not a worse predictive sign.

Regarding the non-SLNs, we recommend that each lymph node is bivalved, and from each cut-surface, two sections are stained, one stained with H&E and one with S100 protein. We recommend to stain with S100 protein since recognition of single or small clusters of melanoma cells in H&E stained sections can be difficult and, therefore, result in missed metastases.

6. Recommended reporting

It is recommended that the following histopathological features are included in the pathology report:

1. Metastases: present or absent
2. Intranodal location: subcapsular, parenchymal, combined (subcapsular and parenchymal), extensive confluent and extensive multifocal
3. Number of metastases: 1, 2–5, 6–10, 11–20 and > 20
4. Maximum dimension of the largest metastatic deposit (measured in millimeters to the nearest 0.1 mm) and indicating its site
5. Extracapsular extension: present or absent
6. Presence of naevus cells (capsular and/or trabecular)

7. Future studies

With the establishment of SLN biopsy or melanoma as a standard procedure in melanoma management, the protocol for pathological handling and reporting needs to be made as succinct and simple as is compatible with optimum management of the patients. Whether the current procedure is sufficient or excessive needs to be confirmed. Our suggestions are therefore an interim recommendation. One of the benefits foreseen in this updated EORTC protocol is the reduction in technical workload resulting in cost saving with no loss, and possible increase, in accuracy.

Future studies will be designed to clarify which patients may benefit from the SLN procedure, validate the accuracy of the current protocol and to establish which of the numerous assessments that can be performed on the primary melanoma are the most useful.

In addition, it is not clear whether multiple metastases with the same tumour burden as a single or the small number of larger metastases have the same, worse or better prognosis and requires an in-depth analysis.

Conflict of interest statement

None declared.

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