

# Sentinel lymph node investigation in melanoma: detailed analysis of the yield from step sectioning and immunohistochemistry

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**Aims:** To evaluate in detail the extent to which step sectioning and immunohistochemical examination of sentinel lymph nodes (SLNs) in patients with melanoma reveal additional node positive patients, to arrive at a sensitive yet workable protocol for histopathological SLN examination.

**Methods:** The study comprised 29 patients with one or more positive SLN after a successful SLN procedure for clinical stage I/II melanoma. SLNs were lamellated into pieces of approximately 0.5 cm in size. One initial haematoxylin and eosin (H&E) stained central cross section was made for each block. When negative, four step ribbons were cut at intervals of 250  $\mu$ m. One section from each ribbon was stained with H&E, and one was used for immunohistochemistry (IHC).

**Results:** When taking the cumulative total of detected metastases at level 5 as 100%, the percentage of SLN positive patients increased from 79%, 83%, 83%, 90% to 93% in the H&E sections through levels 1–5, and with IHC these values were 83%, 86%, 90%, 97%, and 100%, respectively. One of six patients in whom metastases were detected at levels 2–5 only had metastases in the subsequent additional lymph node dissection.

**Conclusions:** Multiple level sectioning of SLNs (five levels at 250  $\mu$ m intervals) and the use of IHC detects additional metastases up to the last level in melanoma SLNs. Although more levels of sectioning might increase the yield even further, this protocol ensures a reasonable workload for the pathologist with an acceptable sensitivity when compared with the published literature.

Since the early 1990s a simple method has been available to detect and retrieve the first tumour draining lymph node, the so called sentinel lymph node (SLN). This is done with a radioactive tracer and blue dye, which are both injected around the tumour.<sup>1–3</sup> During surgery, guided by the blue staining of the draining tissues, the radioactivity, and the preoperatively made lymphoscintigraphy, the SLN is removed and examined meticulously by the pathologist. When the SLN is identified successfully, the absence of tumour in this node predicts with a high degree of accuracy the absence of metastases in the remaining additional lymph nodes.<sup>4–7</sup> The concept of the SLN as the first lymph node that drains a primary tumour has been validated extensively in the past few years. The SLN procedure had become the standard procedure for melanoma in many centres.

“A compromise must be found between workload and sensitivity by limiting the degree of step sectioning and immunohistochemistry on multiple levels”

The reliability of the SLN procedure as an accurate staging procedure is dependent on the ability to identify the true SLN and the extent of histopathological examination of the SLN. Both are instrumental in limiting the false negative rate of the SLN procedure. Most metastatic deposits are thought to be found along the midplane of the node; therefore bisecting the node through the hilar plane, as recently described by Roberts and Cochran, may optimise the finding of metastases.<sup>8</sup>

Multiple level sectioning has been shown to increase the detection rate of SLN metastases in several studies.<sup>9–10</sup> However, complete serial sectioning would result in an unacceptable workload for the pathologist. Therefore, a compromise

must be found between workload and sensitivity by limiting the degree of step sectioning and immunohistochemistry (IHC) on multiple levels. To date, no consensus exists on the most (cost)effective protocol for pathological analysis of SLNs. In fact, only a few studies have reported on the precise yield of each additional level of step sectioning and immunohistochemistry in breast<sup>8</sup> and vulvar cancer,<sup>10</sup> but no such study has yet been published for melanoma.

The traditional protocol used in our hospital includes step sectioning at 250  $\mu$ m, as for other cancers. Although this protocol has yielded good clinical results for melanoma, it is not strictly evidence based, but rather experience based. Therefore, our study was performed to evaluate in detail the yield of multiple levels and the use of IHC for detecting metastases in SLNs, to arrive at an evidence based protocol for optimal SLN investigation.

## MATERIAL AND METHODS

From a total of 189 consecutive patients with clinical stage I/II melanoma who underwent a successful SLN procedure from to January 1998 to September 2002, we identified 29 patients in whom one or more SLN proved to be positive. The mean age of the patients was 52 years (range, 35–79), and the mean Breslow thickness was 2.78 mm (range, 0.69–12).

We evaluated the extent of SLN processing required before these patients were found to be positive. All patients who were initially negative by haematoxylin and eosin (H&E) on the first SLN section subsequently underwent standard multiple sectioning at 250  $\mu$ m intervals and staining with

**Abbreviations:** H&E, haematoxylin and eosin; IHC, immunohistochemistry; SLN, sentinel lymph node

both H&E and IHC. We assessed at which additional level patients who were initially found to be negative were subsequently converted to being SLN positive.

### Sentinel node biopsy

The day before surgery, 40 MBq of  $^{99m}\text{Tc}$  colloidal albumin was injected in two to four depots peritumorally. Lymphoscintigraphy was done to detect the presence, location, and number of focal accumulations.

Just before surgery, 0.5 ml of 2.5% patent blue solution (Guerbet, Aulnay-sous Bois, France) was injected intracutaneously just around the scar on the site of the primary tumour or, when the tumour was still in situ, around the tumour. During surgery, focal tracer accumulations were localised using a handheld  $\gamma$  probe (c-track; Carewise, Morgan Hill, California, USA). All hot and blue nodes were removed as SLNs. Furthermore, all radioactive nodes were biopsied until less than 10% of residual radioactivity, compared with the activity of the hottest SLN, remained in the lymph node basin. All patients underwent regional lymph node dissection after a positive SLN was detected.

### Tissue processing

SLNs smaller than 0.5 cm were processed intact, those between 0.5 and 1 cm were halved, and SLNs larger than 1 cm were lamellated into pieces of approximately 0.5 cm in size. They were fixed in neutral buffered formaldehyde and embedded completely. One initial 4  $\mu\text{m}$  thick H&E stained section was made from each block. When negative, an additional section was done at the first level for IHC and four step ribbons were cut at an interval of 250  $\mu\text{m}$ . From these ribbons one section was stained with H&E, one was used for IHC with S100, and one for HMB45 or Melan-A IHC (Dako A/D, Glostrup, Denmark). All slides were examined by the same pathologist (PJvD). All SLNs containing any cell compatible with melanoma cell morphology (and immunophenotype) were considered metastasis positive.

### RESULTS

Of the 29 patients eventually found to have positive SLNs, 23 were positive on the first SLN section stained with H&E. Interestingly, both patients with more than one positive SLN were found to have SLN metastases on the first H&E stained section. With additional step sectioning and IHC in the remaining six patients, the percentage of SLN positive patients increased from 79%, 83%, 83%, 90%, to 93% in the H&E sections through levels 1–5. With IHC these figures were 83%, 86%, 90%, 97%, and 100%, respectively. At the first level, IHC revealed one additional SLN positive patient and the additional levels 2–5 revealed metastases in one, one, two, and one more patient, respectively (table 1). Of the six patients with SLN metastases only detected at levels 2–5, one had further metastases in the subsequent additional lymph node dissection.

Looking at all harvested nodes in these 29 SLN positive patients, 31 tumour positive SLNs were found. When taking

the cumulative total of detected metastases at level 5 as 100%, the percentage of positive SLNs increased from 81%, 84%, 84%, 90%, to 94% in the H&E sections through levels 1–5. With IHC, the increase was from 84%, 87%, 90%, 97%, to 100%, respectively. The first level failed to detect metastases in five SLNs. Additional levels 2–5 yielded metastases in one, one, two, and one more positive SLN, respectively (table 2).

### DISCUSSION

Because validation studies have shown high accuracy rates for the SLN procedure,<sup>11</sup> it can now be used as a staging procedure without the need for completion lymph node dissection in patients with negative SLNs.<sup>4 5 11 12</sup>

As long as the surgeon has performed a reliable SLN biopsy procedure, it is up to the pathologist to determine whether lymph node metastases are present or not.

In the days of routine elective lymph node dissection, it was shown that a large proportion of patients are converted from node negative to node positive with a more detailed histological examination of lymph nodes.<sup>13 14</sup> This has important consequences for the clinical management of these patients because, in general, lymph node positive patients will have a worse prognosis.<sup>2</sup> For these reasons, the histopathological examination of SLNs tends to be even more elaborate, with the additional argument that this can be done with an acceptable workload for the pathologist because only a few nodes need to be examined this way.

Our study clearly shows that more patients are converted to node positive with each additional step of sectioning (at 250  $\mu\text{m}$  intervals), and that with each additional step IHC has a higher sensitivity than H&E.

However, even with this intensive protocol not all nodal metastases will be detected. Such a 100% sensitivity can theoretically only be reached with complete serial sectioning of the SLN at 12  $\mu\text{m}$  intervals.<sup>10</sup> In the study of Cook *et al*, reverse transcription polymerase chain reaction analysis slightly increased sensitivity for the detection of metastases, but at the cost of false positivity.<sup>14</sup> Therefore, the place of this technique in routine diagnosis remains to be established. However, the question arises whether all metastatic cells need to be found. From a clinical point of view, only SLN metastases that are accompanied by second echelon or distant metastases need to be identified. Therefore, a method that has a sufficiently high clinical sensitivity with an acceptable workload for the pathologist needs to be defined.

Complete step sectioning of the SLN in patients with breast cancer has been done in only a few studies. For instance, Cserni serially sectioned the SLN up to extinction with 3–5  $\mu\text{m}$  thick slices and examined every 10–20th level.<sup>15</sup> In the final analysis, 15 of the 21 patients with metastases limited to the SLN were positive on the initial central cross section. Dowlatsahi *et al* performed complete serial sectioning at 250  $\mu\text{m}$  intervals and found that only six of 30 SLN positive patients were positive on the initial section examined by H&E and IHC.<sup>16</sup> This difference is remarkable, also in view of the

**Table 1** Cumulative number of patients with melanoma sentinel node metastases found with haematoxylin & eosin (H&E) and immunohistochemistry (IHC) in each additional level (250  $\mu\text{m}$  intervals) in 29 patients

	H&E	%	IHC	%
Level 1	23	79	24	83
Level 2	24	83	25	86
Level 3	24	83	26	90
Level 4	26	90	28	97
Level 5	27	93	29	100

**Table 2** Cumulative number of metastases found with haematoxylin & eosin (H&E) and immunohistochemistry (IHC) in each additional level (250  $\mu\text{m}$  intervals) in 31 cutaneous melanoma sentinel nodes

	H&E	%	IHC	%
Level 1	25	81	26	84
Level 2	26	84	27	87
Level 3	26	84	28	90
Level 4	28	90	30	97
Level 5	29	94	31	100

fact that Cserni's intervals were smaller and therefore it would be expected that a greater number of additional metastases would be found. The greater yield on the initial section in Cserni's study could be ascribed to the fact that they tried to cut the central cross section guided by the blue lymphatic vessel, which we did not, or the different biological properties of breast cancer and melanoma cells. In addition, the average size of the metastases may have been greater in patients in Cserni's study (68% T2 tumours compared with 25% in the study by Dowlatshahi *et al*). No studies of complete sectioning of the SLN in patients with melanoma have been published, but the same results would be expected in melanoma.

"Immunohistochemistry facilitates the detection of single metastatic cells, and speeds up the screening of the sections dramatically"

Nevertheless, the fact remains that the use of IHC and additional step sectioning improves the detection rate of metastatic deposits. The question for practical purposes then remains: how many additional levels (and at what interval) need to be examined?

Turner *et al* examined 10 levels at 40 µm intervals in 42 patients with SLN positive breast cancer in whom the initial H&E section was negative.<sup>17</sup> With the use of IHC, the first two levels of SLN examination found additional metastases in all but one of these patients. Therefore, they concluded that the additional eight levels of examination did not significantly contribute to the detection of additional metastases. However, with 10 levels at 40 µm intervals, only 400 µm of the entire SLN slice (up to 5000 µm in thickness) is examined, which is unlikely to be sufficient for the detection of all metastases. Rather than taking many sections at small intervals, it may be more efficient to take fewer sections at larger step intervals.

Cook *et al* showed that in different patient groups, the percentage of SLN positive patients with IHC protocols involving only bivalving through the hilar plane, two additional levels with 50 µm interval, and four additional levels at 50 µm were 17.8%, 25.2%, and 33.8%, respectively. However, no direct evaluation of the contribution of each additional level was provided.

Our present study shows in detail the yield of step sectioning and IHC at five levels with an interval of 250 µm. In practice, this has proved to be an acceptable workload. Clearly, the yield increased with additional levels. The first level failed to detect metastases in five SLNs (16% of the total number of metastases found). Additional levels 2–5 yielded metastases in one, one, two, and one more SLN, respectively. Thus, additional levels clearly reveal more metastases and even the fourth and fifth level together reveal 10% additional metastases. Not surprisingly, the yield with IHC was higher than with H&E only. IHC facilitates the detection of single metastatic cells, and speeds up the screening of the sections dramatically. Therefore, some investigators omit H&E staining when IHC is performed. However, we prefer to make H&E control sections because they are helpful in the detection of artefacts and benign inclusions.

These results are comparable to the number of additional metastases found by Torrens *et al* in the SLNs of patients with breast cancer.<sup>9</sup> They studied SLNs of patients with breast cancer processed in our institute according to the same protocol.

In conclusion, step sectioning of SLNs with IHC is very useful for finding the smallest metastases in SLNs of breast cancer that may be clinically relevant. Therefore, we propose step sectioning and IHC at four additional levels, separated by 250 µm intervals, when the original H&E section is tumour negative. Despite the fact that our protocol might still miss

## Take home messages

- To optimise the detection of metastases in patients with melanoma, we propose step sectioning of sentinel lymph nodes and immunohistochemistry at four additional levels, separated by 250 µm intervals, when the original haematoxylin and eosin section is tumour negative
- Although more levels of sectioning might increase the yield even further, this protocol ensures a reasonable workload for the pathologist with an acceptable sensitivity

some metastases we believe that this protocol ensures a reasonable workload for the pathologist with an acceptable sensitivity.

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