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The impact of immunohistochemistry on sentinel node biopsy for primary cutaneous malignant melanoma

G.L. Ross, T. Shoaib, J. Scott, I.G. Camilleri, H.W. Gray*, R. MacKie and D.S. Soutar†

*Plastic Surgery Unit, Canniesburn Hospital; *Department of Nuclear Medicine, Glasgow Royal Infirmary; and †Department of Dermatology, Robertson Building, University of Glasgow, Glasgow, UK*

SUMMARY. Sentinel node biopsy (SNB) has emerged as an accurate means of identifying nodal disease in patients with malignant melanoma. Superselection of pathological nodes has allowed improved pathological staging of disease. The aim of this study was to look at the impact of immunohistochemistry on pathological staging of sentinel nodes. The first 100 patients undergoing SNB for primary cutaneous malignant melanoma were included in this study. Sentinel node harvesting was performed with the aid of preoperative lymphoscintigraphy and the intraoperative use of both a gamma probe and blue dye. If the sentinel nodes contained tumour on either routine pathology or immunohistochemistry, patients were offered a therapeutic lymph node dissection (TLND). Patients underwent no other treatment to the primary lymph node basin if the sentinel node was free of metastases. In all, 95 patients had at least one node identified, and 25 were staged SNB positive and offered subsequent TLND. We found that 76% (19/25) of SNB positive patients were staged positive on routine pathology, and 24% (6/25) were staged with immunohistochemistry. Immunohistochemistry upstaged disease in 8% of patients (6/76). In all, 21 of the patients staged positive with SNB underwent TLND; 50% (8/16) of the patients staged sentinel node positive with routine pathology showed no further disease in the TLND, compared with 100% (5/5) of the patients staged sentinel node positive with immunohistochemistry only ($P < 0.05$). Three patients have developed recurrence within the nodal basin following a negative SNB. The sensitivity of the procedure is currently 89% (25/28), with a mean follow-up of 24 months. Immunohistochemistry is an essential part of identifying micrometastasis in sentinel nodes, upstaging 8% of patients in our series. Patients with micrometastatic disease may well have a different prognosis from those with occult disease, and careful delineation of these patients is required to determine the prognostic influence of micrometastasis. © 2003 The British Association of Plastic Surgeons. Published by Elsevier Science Ltd. All rights reserved.

Keywords: sentinel node biopsy, melanoma, micrometastasis, prognosis.

Morton et al first described the concept of sentinel node biopsy (SNB) in the treatment of malignant melanoma.[1] They initially used a tracer injection of blue dye in each patient to identify the sentinel node. Their method was subsequently adapted to improve the detection of potentially elusive nodes using both preoperative lymphoscintigraphy and an intraoperative gamma probe. Thus, the triple diagnostic approach of preoperative lymphoscintigraphy and intraoperative blue dye and gamma probe detection is becoming the standard method of sentinel node identification.

Experience has shown that, for clinically N0 melanoma patients, the technique is undoubtedly more accurate than all prior non-surgical staging methods.[2–4] Patients who require therapeutic lymph node dissection (TLND) are accurately identified, and the clinician can confidently recommend a programme of clinical observation for patients whose sentinel nodes are negative. Therefore, only those patients whose sentinel nodes are positive for metastatic disease are exposed to the morbidity of lymphadenectomy and adjuvant therapies.

By using SNB to target lymph nodes for pathological evaluation, additional pathological techniques have been used to analyse the sentinel nodes, including serial sectioning of the sentinel node and immunohistochemistry staining. Immunohistochemistry is essential as H&E staining misses up to 12% of positive nodes.[5] The importance of these micrometastases was highlighted by Gershenwald et al, who reviewed patients who were staged sentinel node negative on routine pathology and who developed subsequent regional disease, and found that 80% of these patients had evidence of micrometastatic disease within the original sentinel node.[6] The aim of this study was to look at the impact that immunohistochemistry has had on the pathological staging of sentinel nodes within our practice.

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Patients and methods

We studied 100 patients who underwent SNB for primary cutaneous malignant melanoma using preoperative lymphoscintigraphy and intraoperative gamma probe and blue dye. All patients had a diagnosis of malignant melanoma proven by excision biopsy. Wider excision of the melanoma and SNB were performed at the same time. The day before surgery, patients attended the Nuclear Medicine Department, where up to 40 MBq of 99m Tc–99m labelled colloidal human serum albumin (Nanocoll) with a mean particle size of 80 nm (Nycomed Amersham, High Wycombe, UK) in approximately 0.5–1 ml of saline was injected intradermally at as many points as necessary to surround the excision-biopsy scar completely. Static lymphoscintigraphy was performed at 15 min, 30 and 60 min following injection, or until the first appearance of sentinel nodes.

During surgery, approximately 0.5–2 ml of Patent Blue V dye (Laboratoire Guerbet, Aulnay-Sous-Bois, France) was injected into the same site as the radiocolloid, completely surrounding the previous excision-biopsy scar. Through a small incision over the identified regional basin, blue-stained lymphatic vessels were sought and followed to the site at which they drained into a lymph node. The Neoprobe-1500 hand-held gamma probe (Ethicon Endosurgery UK), fitted with a straight collimated probe 14.0 mm in diameter, was used to identify radioactive sentinel nodes, including those marked preoperatively during lymphoscintigraphy. Sentinel nodes were identified in their lymph node basin and were labelled according to colour and presence of radioactivity. Radioactivity was confirmed within the sentinel node ex-vivo. If no blue-stained lymphatic vessels were found, the probe was used to guide the dissection. Once the sentinel lymph node had been excised, the Neoprobe was used to search the resection bed to ensure that there were no residual areas of high radioactivity. Once the operator was satisfied that all first echelon nodes had been completely removed, the wound was closed.

The sentinel nodes were sent to histology, where they were fixed in 10% neutral buffered formalin and, after fixation, bisected through their longest axes. If the thickness of the halves was more than 2 mm, the slices were further trimmed to provide additional 2 mm thick blocks. One half of each node was processed for histological examination. One H&E stained section was prepared from each block and examined. If the sentinel node was positive, the patient was offered a TLND on the basis of occult disease having been demonstrated in the sentinel node. If the sentinel node was negative, additional pathology was performed. Ten sections were taken from the second half of the node; sections one, three and five were examined by H&E, and sections two and four were examined with immunohistochemistry using S-100 and HMB-45. If the sentinel node was staged positive by immunohistochemistry (Fig. 1), the patients were offered a TLND on the basis of micrometastatic disease having been demonstrated in the sentinel node.

The pathological evaluation of the TLND in all cases was with routine H&E. Patients staged positive with SNB were offered entry into adjuvant randomised control trials. Patients who were staged sentinel node negative were followed up in clinic.

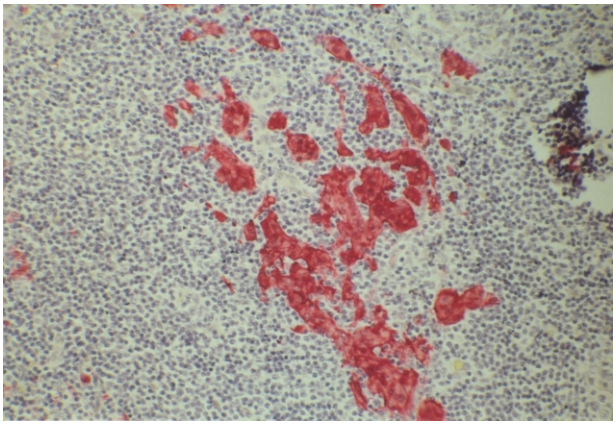

Figure 1—Melanoma detected by immunohistochemistry using S-100 staining.

Results

In all, 95 patients had at least one node identified (identification rate: 95%), and 25 were staged SNB positive and offered subsequent TLND. We found that 76% (19/25) of SNB positive patients were staged positive on routine pathology, and 24% (6/25) were staged with immunohistochemistry. Immunohistochemistry upstaged disease in 8% of patients (6/76).

In all, 21 of the patients staged positive with SNB underwent TLND; 50% (8/16) of the patients staged sentinel node positive with routine pathology showed no further disease in the TLND, compared with 100% (5/5) of the patients staged sentinel node positive with immunohistochemistry only ($P < 0.05$) (Fig. 2).

Three patients have developed recurrence within the nodal basin following a negative SNB. The sensitivity of the procedure is currently 89% (25/28) after a mean follow-up of 24 months (Fig. 3).

Discussion

The prognostic accuracy of SNB is reflected in the new proposed American Joint Committee on Cancer (AJCC)

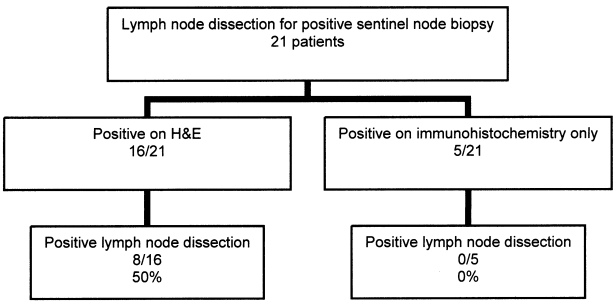

Figure 2—Results of lymph node dissections following upstaging by H&E versus immunohistochemistry only.

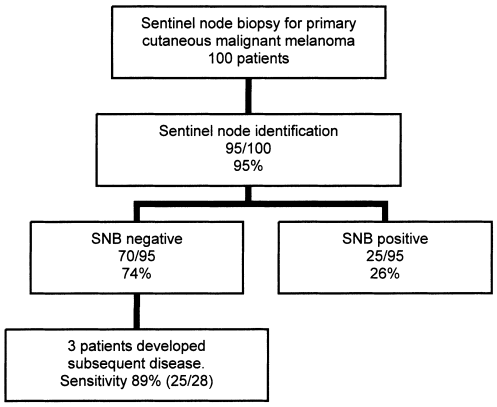

Figure 3—Sensitivity of sentinel node biopsy.

staging classification for melanoma, which will be used from 2002.[7]

Immunohistochemistry is an essential part of identifying micrometastasis in sentinel nodes, upstaging 8% of our patients. It would seem that conventional histological techniques may considerably underestimate the prevalence of disease.

In melanoma, it has been proposed that the presence of occult disease or non-palpable disease in the absence of any other disease be classified as N1a.[7] In our series 17 patients would be classified as N1a. Micrometastasis is defined as a lesion measuring less than 2 mm within a lymph node.[8] In our series all patients undergoing a TLND following the demonstration of micrometastatic disease in the sentinel node had no further disease. This compared with 50% of patients undergoing a TLND following the demonstration of occult disease in the sentinel node. Patients with micrometastatic disease may have a different prognosis from patients with occult disease.

The new AJCC classification does not distinguish between occult or non-palpable disease and micrometastasis because, as yet, the prognostic influence of micrometastatic disease is unknown. However, in order to determine the role of micrometastases, careful recording and sub-classification is required. In our series, 11 patients had occult disease and six patients had micrometastatic disease only.

It has previously been suggested that micrometastasis within a node be given the nomenclature 'mi'.[8] Therefore, in our series, the six patients with only micrometastasis in the sentinel node should be classified as N1a(mi), separating them from the 11 patients with occult disease in the sentinel node, who should be classified as N1a.

The role of SNB in clinical practice is still being evaluated in large multicentre trials.[5] It is a cost-effective[9] means of identifying micrometastasis, and careful classification of such disease as N1a(mi) may allow the determination of the prognostic influence of micrometastases in melanoma.

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The Authors

Gary Ross MRCSEd, Head and Neck Research Fellow
Taimur Shoaib FRCSEd, Registrar in Plastic Surgery
John Scott, Consultant Plastic Surgeon
Ivan Camilleri FRCS(Plast), Consultant Plastic Surgeon
David Soutar ChM, Consultant Plastic Surgeon

Plastic Surgery Unit, Canniesburn Hospital, Switchback Road, Bearsden, Glasgow G61 1QL, UK.

Henry Gray FRCP, Consultant in Nuclear Medicine

Department of Nuclear Medicine, Glasgow Royal Infirmary, Glasgow, UK.

Rona Mackie MRCP, Consultant Dermatologist

Department of Dermatology, Robertson Building, University of Glasgow, Glasgow, UK.

Correspondence to Mr G. L. Ross, Plastic Surgery Unit, Canniesburn Hospital, Switchback Road, Bearsden, Glasgow, Scotland G61 1QL, UK

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