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Original Paper

Role of Lymphatic Mapping and Sentinel Node Biopsy in Melanoma Detection

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The identification of micrometastases to the regional lymph node basin presents a challenging problem in the clinical management of patients with early-stage malignant melanoma (stage I and II). These patients generally have a good prognosis following surgical excision of the primary tumour; however, the presence of clinically undetectable nodal disease is the most important prognostic factor. Traditionally, these patients are either monitored closely for development of palpable metastases at which time therapeutic lymphadenectomy would be performed or, if they have poor prognostic factors, lymphadenectomy is immediately performed. The high rate of morbidity and lack of proven survival impact for immediate lymphadenectomy limit its use. Lymphatic mapping and sentinel node biopsy allow a more selective approach to regional lymph node dissections. Only node-positive patients undergo selective lymphadenectomy, thereby reducing the morbidity associated with the procedure. Several studies have confirmed that lymphatic mapping identifies the lymph node most likely to contain disease and confirms the orderly progression of lymphatic metastases. Use of radioactive colloid and a hand-held gamma probe improves the localisation of the sentinel lymph node. Because adjuvant systemic therapy has been proven to prolong survival in patients with nodal involvement, lymphatic mapping and sentinel node biopsy, by identifying patients with clinically negative, but pathologically positive disease, may improve their outcome. © **1998 Elsevier Science Ltd. All rights reserved.**

Key words: melanoma, prognostic factors, lymphatic mapping, sentinel node biopsy
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INTRODUCTION

An increasing number of melanoma patients are diagnosed with clinically localised melanoma, classified as American Joint Commission on Cancer (AJCC) stage I (< 1.5 mm thick) and stage II (> 1.5 mm thick) disease. However, many of these patients have high-risk tumours (i.e. thick primary lesions) that increase the likelihood of micrometastases in regional lymph nodes at the time of diagnosis.

Regional lymph node status is one of the most powerful predictors of disease progression and patient outcome [1]. Ten-year survival rates for patients without lymph node involvement are 70–80% in contrast to those for patients with tumour-positive lymph nodes of 20–30% [2]. In addition, a direct correlation exists between the number of positive lymph nodes and survival (Figure 1) [1]. However, the role of routine lymphadenectomy in the clinical management of patients with AJCC stage I and II melanoma has been a subject of considerable controversy. Currently, there is no conclusive data that lymphadenectomy in patients with clinically uninvolved lymph nodes improves survival [3, 4].

Following wide surgical excision of the primary tumour, there are three approaches to the treatment of clinically uninvolved regional lymph node basins: observation of the nodal basin with therapeutic lymph node dissection (LND) performed when palpable metastases are found, elective lymph node dissection (ELND), or sentinel node biopsy and selective LND.

Elective lymph node dissection (ELND)

The goal of ELND at the time of wide local cutaneous tumour resection is to remove occult microscopic lymph node tumour foci, a potential source of tumour dissemination,

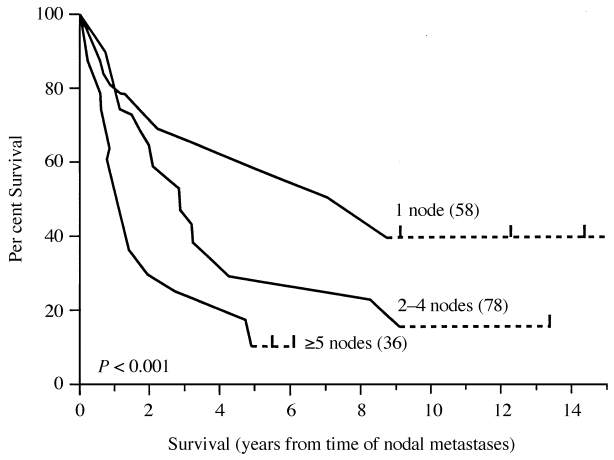

Figure 1. Correlation between survival and number of positive lymph nodes. Reprinted with permission from *Ann Surg* 1981 [1].

when the systemic tumour burden is minimal [5]. Unfortunately, many patients with clinically localised melanoma do not have regional lymph node involvement. Furthermore, ELND is associated with significant morbidity.

The efficacy of ELND has been evaluated primarily in retrospective, nonrandomised trials of heterogeneous patient populations with tumours of varying thickness and anatomical location, both of which are known prognostic factors [6]. More recently, several prospective, randomised clinical trials were conducted to assess the efficacy of ELND. In one such study conducted at the Mayo Clinic (Rochester, Minnesota, U.S.A.), no significant difference in survival or metastasis-free survival was observed among 171 patients with stage I melanoma ranging in thickness from ≤ 0.76 to ≥ 3 mm treated with delayed ELND, immediate ELND, or observation [4]. Multifactorial analysis indicated that the level of invasion and the thickness of the lesion were the most important prognostic factors, followed by age (60 years or older), site (legs) and tumour type (nodular). Similar results were obtained in a World Health Organization (WHO) study of patients with nonmidline trunk melanomas > 1.5 mm [7]. Neither of these trials delineated patients on the basis of prognostic factors. In contrast, the Intergroup Melanoma Committee of the National Cancer Institute (NCI) stratified 740 patients with stage I and II melanoma of intermediate thickness (1–4 mm) according to tumour thickness, anatomic site and ulceration [3]. The patients were randomised to receive either ELND or observation. The median follow up was 7.4 years. Although overall 5-year survival was not significantly different for patients who received ELND or observation, patients 60 years of age or younger had significantly better 5-year survival with ELND than with observation (88 versus 81%, respectively; $P = 0.04$). Additionally, among these patients, 5-year survival was better with ELND versus observation in patients with tumours 1 to 2 mm thick or without tumour ulceration or both. These data support an early surgical approach to nodal disease to not only improve survival rates but also to accurately stage patients, particularly in light of recent data indicating that interferon alfa-2b prolongs relapse-free and overall survival in node-positive patients [3, 8].

The risk of lymph node involvement has most often been assessed according to clinical features of melanoma, including tumour thickness [2]. Retrospective analyses of more than 1000 patients demonstrated that patients with primary tumours of intermediate thickness (i.e. 1–4 mm) have up to a 45% incidence of occult nodal metastases [9]. Primary tumours < 0.76 mm have < 5% incidence of occult nodal disease and tumours > 4 mm thick are likely to have clinically evident lymph node metastases [2]. Although occult nodal disease can be correlated with clinical features of cutaneous melanoma for the population, the clinical features of the primary lesion are neither a direct nor an accurate indicator of nodal status in an individual patient. Traditionally, the only method to determine the degree of nodal involvement has been complete LND with pathologic examination of the specimen.

LYMPHATIC MAPPING AND SENTINEL NODE BIOPSY

Lymphatic mapping procedures that allow evaluation of regional lymphatic basins with minimally invasive surgical procedures have been developed [10–12]. These procedures are based on the concept that melanoma nodal metastases, unlike most solid tumour metastases, occur in an orderly, sequential manner based on lymphatic flow [12]. According to this mechanism, the afferent lymphatic channel in each finite region of the skin drains to a specific initial node, defined as the 'sentinel node', within a nodal basin; different regions of the skin drain to different sentinel nodes. Several sentinel nodes, each accepting lymphatic drainage from several different regions of the skin, may be present within one nodal basin.

In theory, the histologic status of the sentinel node reflects the status of the total nodal basin. Therefore, the ability to identify and evaluate the sentinel lymph node allows more accurate evaluation of the regional lymphatic basin with a minimally invasive surgical procedure. Using this approach, only patients with tumour-positive sentinel nodes undergo therapeutic regional LND, while patients with tumour-negative sentinel nodes are not subjected to the morbidity of complete LND.

Lymphatic mapping in conjunction with sentinel node biopsy has demonstrated accuracy in detecting regional lymph node metastases in patients with melanoma [10–14] as well as axillary lymph node metastases in patients with breast cancer [15].

Intra-operative lymphatic mapping with vital blue dyes

Initial studies of lymphatic mapping used vital blue dye alone to visually identify the sentinel node [6, 11]. The dye, either isosulfan blue lymphazurin (1%) or patent blue-V (2.5%), is injected intradermally at a site adjacent to the intact primary tumour or biopsy scar. Following injection of the dye, 5–10 minutes is required to allow the dye to travel through the afferent lymphatic channels to the sentinel node. The dye mimics the passage of metastatic melanoma cells through the afferent lymphatics to the sentinel node in a regional nodal basin; thus, the sentinel node is the first node to be stained as dye enters the lymphatic basin. An incision is made over the sentinel node, which is stained blue and then removed for biopsy and histologic analysis.

A 4-year clinical trial in 223 consecutive patients with clinical stage I melanoma was performed to evaluate the sentinel node identification rate and accuracy of the technique [11]. All patients in this study underwent lymphadenectomy

after sentinel node biopsy in order to establish the incidence of metastases within nonsentinel nodes compared to the incidence within the sentinel nodes. The accuracy rate, or incidence of false-negatives, was defined as the identification of disease in nonsentinel nodes when the sentinel node was histologically negative.

Sentinel nodes of patients with early stage melanoma were successfully identified with a high degree of accuracy. Overall, at least one sentinel node was identified in 194 of 237 (82%) lymphadenectomy specimens [11]. In the majority of cases (72%), only one sentinel lymph node drained a particular primary melanoma. The success in identifying the sentinel node was directly correlated with the number of procedures performed by each surgeon, with at least 50 procedures necessary in order to achieve a high rate of success. The anatomical site of the drainage basin also affected success in identifying sentinel nodes; the highest success rate was obtained for lesions on the trunk or leg that drained to inguinal nodes. Of the 194 specimens, 259 nodes were stained blue and considered sentinel nodes; metastases were found in 47 of these nodes (18%). Only two of 3079 nonsentinel lymph nodes were the exclusive site of metastases, providing a false-negative rate of much less than 1%. Histologic examination of the sentinel node resulted in at least a 95% accuracy rate in staging the nodal basin for metastases [11]. This study demonstrated that the sentinel lymph node accurately reflects the histologic status of other nodes in regional lymphatic basins of melanoma patients.

Our experience at M.D. Anderson Cancer Center and University of South Florida in more than 50 patients undergoing sentinel node biopsy followed by lymphadenectomy supports the results of Morton and colleagues [6, 11]. The overall sentinel node identification rate was greater than 80%, with the axillary region the most difficult region in which to identify the sentinel node [6].

Lymphoscintigraphy

In order to improve the identification rate and localisation of the sentinel node, particularly in regions with complex lymphatic drainage patterns such as the head, neck and midline of trunk, pre-operative and intra-operative lymphoscintigraphy was added to the dye procedure [10, 14]. Pre-operative, lymphoscintigraphy identifies regional basins at risk and determines the number and location of sentinel lymph nodes. Intra-operative lymphoscintigraphy assists in the localisation of the sentinel node and decreases the size of the surgical incision. Sulphur colloid radiolabeled with 99-m technetium is injected intradermally at the site of the primary lesion either prior to or in conjunction with blue dye. A hand-held gamma probe is then used to localise the sentinel node transcutaneously prior to skin incision, while the blue dye allows visual identification of the sentinel node after the incision is made. In addition, if significant counts exist after the removal of the first sentinel node, other sentinel nodes that may not have been visualised can be located and removed.

Recently, a clinical trial of 106 consecutive patients presenting with clinically localised stage I and II cutaneous melanomas (mean tumour thickness = 2.24 mm) was performed to evaluate the combined use of pre-operative and intra-operative lymphoscintigraphy plus blue dye to detect sentinel lymph nodes [13]. Identification of one or more sentinel lymph nodes was possible for 124 out of 129 (96%) basins sampled using the combination of techniques. When just the vital blue dye technique was used, the success rate was 80%.

We also have observed in a consecutive series of patients that the use of vital blue dye plus radioactive colloid significantly increased the sentinel node identification rate from 89 to 99% (Table 1) and provided the following advantages:

- Colloid plus the hand-held gamma probe localises the node prior to skin incision.
- Vital dye allows visualisation of the 'hot' node.
- Hand-held gamma probe ensures that the sentinel node is identified.
- Gamma probe can identify in-transit sentinel nodes.

CURRENT CLINICAL TRIAL

A large prospective clinical trial is ongoing at University of Texas M.D. Anderson Cancer Center (Houston, Texas, U.S.A.) and H. Lee Moffitt Cancer Center and Research Institute, University of South Florida (Tampa, Florida, U.S.A.) to determine the prognostic value of sentinel lymph nodes and examine the patterns of failure following negative sentinel lymph node biopsy. Eligible patients include those with a primary cutaneous tumour > 1 mm and those with a primary tumour < 1 mm that is Clark's Level IV or ulcerated.

Pre-operative lymphoscintigraphy is used to identify the lymphatic basin in ambiguous drainage sites. Intradermal injection of technetium-labeled sulphur colloid 1 to 4 hours prior to surgery is used to determine the number and location of sentinel lymph nodes. Intra-operative lymphazurin injection allows visualisation of sentinel nodes once the incision is made. Sentinel node evaluation includes frozen section biopsy and serial sectioning with histochemical analysis. Only patients with positive sentinel lymph nodes undergo complete therapeutic lymphadenectomy.

Preliminary analyses of 612 patients have been conducted. Characteristics of enrolled patients are summarised in Table 2. The sentinel lymph node was identified in 580 (95%) patients. Of these patients, 85 (15%) had positive sentinel lymph nodes and 82 underwent complete therapeutic

Table 1. *Sentinel lymph node identification rate by mapping technique*

	Lymphatic mapping techniques		
	Dye alone	Dye + colloid*	*P*-value
Basins	291	225	
% Identified	89%	99%	< 0.01
Mean no. of nodes	1.25	1.88	< 0.001

*In patients with > 1 sentinel node, 30% of the time the second node had more counts than the first node.

Table 2. *Patient characteristics*

Age (% > 50 years)	55
Gender (% male)	58
Tumour thickness (mm)	
median	1.8
mean	2.4
Level of invasion (% > III)	63
Location (% axial)	52
Ulceration (%)	32

Table 3. Distribution of mapped basins

Basin	Basins mapped	
	Left *n* (%)	Right *n* (%)
Cervical	38 (6)	39 (6)
Axillary	209 (30)	192 (28)
Inguinal	104 (15)	101 (15)

lymphadenectomy. Of the 495 sentinel-node-negative patients, 72 underwent LND, while the other 423 were observed for development of metastases.

The observation that melanoma is a symmetrical disease was confirmed in these patients; the cervical, axillary and inguinal lymph node basins on both sides were mapped with approximately equal frequency (Table 3). Although the sentinel lymph node identification rate was > 90% for all three basins, the identification rate was highest for the inguinal basin (97%) and equal for the cervical and axillary basins (91% for each). As expected from prior prognostic studies [9], the incidence of positive sentinel lymph nodes was directly correlated with tumour thickness (Table 4).

Of the 82 patients with positive sentinel nodes who underwent lymphadenectomy, 65 (79%) had one positive sentinel lymph node only and the other 17 (21%) had one to four positive lymph nodes, of which the majority had only one additional positive node. Of patients with one or more positive sentinel lymph nodes, 80% of all sentinel nodes were positive and only 2% of nonsentinel lymph nodes were positive, supporting the concept that lymphatic melanoma metastases occur in an orderly manner based on lymphatic flow. A complete report of this trial, including disease progression, survival rates and analyses of risk factors, is expected.

The incidence of nodal disease was greater than anticipated by routine histologic examination of elective LND specimens. This indicates that routine histologic techniques may not identify a large portion of micrometastatic disease at the time of initial resection. More sensitive methods for detection of occult micrometastases have been developed, including serial sectioning, immunohistology, cell culture and the reverse transcriptase–polymerase chain reaction (RT–PCR) assay for tyrosinase messenger RNA (mRNA). Tyrosinase is involved in the production of melanin and is expressed in melanoma cells. Preliminary data indicate that the RT–PCR assay for tyrosinase mRNA is a sensitive method for detection of occult nodal micrometastases [16]. We performed RT–PCR analysis of specimens from 12 patients who developed positive lymph nodes after a negative sentinel lymph node biopsy. 10 of these 12 patients had disease that was missed by routine histology. As lymphatic mapping and sentinel node biopsy techniques are perfected, the use of more sensitive pathological evaluations are necessary to accurately identify the presence of nodal disease.

Table 4. Primary tumour thickness and sentinel lymph node status

Depth (mm)	Total *n*	Positive sentinel lymph nodes* *n* (%)
0–1.5	230	11 (4.8)
1.5–4.0	271	52 (19.2)
4.01 +	64	22 (34.4)
Total	565	85 (15.0)

*Overall $P < 0.000001$ by student's *t*-test.

CONCLUSIONS

For patients with stage I and II malignant melanoma, the presence of regional lymph node metastases is the most significant predictor of outcome. Because at this stage of disease lymph node metastases are not palpable, the most challenging problem is identifying those patients who may have undetected microscopic tumour foci. The role of elective LND in the clinical management of these patients remains an issue of debate. Few randomised studies have been conducted and a survival advantage for elective LND has only been identified in subgroups of patients, particularly those 60 years of age or younger, those with tumours of 1–2 mm and those without tumour ulceration [3].

Lymphatic mapping procedures that allow minimally invasive evaluation of the regional lymphatic basins have been developed. These procedures are based on the concept that melanoma nodal metastases occur in an orderly, sequential progression with initial drainage of the afferent lymphatic channel from each finite region of the skin to a specific sentinel node. The histologic status of the sentinel node has a high predictive value for detecting melanoma metastases in the lymph node basin. Therefore, therapeutic regional LND can be limited to those patients with tumour-positive sentinel nodes. Lymphatic mapping with sentinel node biopsy may not only improve patient outcomes by identifying those who are at high risk of relapse, but also these procedures are associated with significantly less morbidity than that observed with traditional LND.

The use of lymphoscintigraphy plus lymphatic mapping in our ongoing clinical trial successfully identified the sentinel lymph node in 95% of patients. This confirms the orderly progression of melanoma metastases and also supports the use of radiolabeled colloid in conjunction with the hand-held gamma probe to improve localisation of sentinel nodes. More sensitive pathologic techniques are being investigated to accurately stage patients once the sentinel node is identified and removed.

Lymphoscintigraphy, lymphatic mapping and sentinel node biopsy allow earlier therapeutic lymphadenectomy for patients with occult nodal micrometastases before development of palpable nodal disease and accurately identify the most powerful predictor of disease recurrence in stage I and II patients (i.e. nodal status). In addition, this technique has a low false-negative rate. However, the overall significance of lymphatic mapping and sentinel node biopsy will depend on the patterns of failure following lymph node dissection in patients with a positive sentinel lymph node. Because adjuvant systemic therapy has been proven to prolong survival in patients with nodal involvement, these techniques, by identifying patients with clinically negative, but pathologically positive disease, may improve their outcome.

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