Outcome of Patients With Melanoma and Histologically Negative Sentinel Lymph Nodes

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Hypothesis: Patients with melanoma and histologically negative sentinel lymph nodes identified by lymphatic mapping have a very good prognosis.

Design: Cohort study with follow-up information obtained from medical records and telephone interviews.

Setting and Patients: Of all patients with cutaneous melanoma who underwent intraoperative sentinel lymph node mapping between November 15, 1993, and April 18, 1997, at the Massachusetts General Hospital, Boston, 89 were found to have no evidence of melanoma in their sentinel nodes. Forty-six lesions (51%) were on an extremity and 44 (49%) were of axial location. The median tumor thickness was 1.8 mm (range, 0.36-12.0 mm) and 11 tumors (12%) were ulcerated.

Interventions: Patients underwent intraoperative sentinel lymph node mapping with lymphazurin and radiolabeled sulfur colloid. Sentinel lymph nodes were analyzed by standard hematoxylin-eosin staining. Only 2 patients received adjuvant therapy following wide excision of the primary lesion.

Main Outcome Measures: Site of initial recurrence and time to initial recurrence.

Results: The median follow-up for all patients was 23 months (range, 2-54 months). Eleven patients (12%) developed melanoma recurrences, and 78 (88%) patients remain disease free. Regional lymph nodes were the initial site of recurrence in 7 (8%) of 89 patients, and 7 (7%) of 106 mapped basins. Four patients had recurrence without involvement of regional lymph nodes: 2 with distant metastases and 2 with in transit metastases. The median time to recurrence was 12 months (range, 2-35 months). Sentinel lymph nodes were reanalyzed using serial sections and immunoperoxidase stains in 7 patients with recurrence and metastatic melanoma was identified in 3 (43%).

Conclusions: The risk for melanoma recurrence is relatively low in patients with histologically negative sentinel nodes identified by lymphatic mapping. Longer follow-up will improve our understanding of the prognostic value of this procedure.

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As with many other malignant neoplasms, melanoma metastasis may develop as a result of either lymphatic or hematogenous dissemination. The most powerful prognostic factor for patients with American Joint Committee on Cancer stage I and II melanoma is the presence or absence of metastatic melanoma in their regional lymph nodes. The hypothesis that some patients may harbor occult regional lymph node metastases prior to the establishment of distant metastases has served as the rationale for performing elective lymph node dissections (ELNDs). However, results of prospective randomized clinical trials designed to evaluate the efficacy of ELND have demonstrated either no survival benefit or survival benefit only to selected subgroups. In addition, the development of lymphatic mapping and sentinel lymph node biopsy has provided a less invasive method for determination of whether melanoma has spread to regional lymph nodes. Several refinements have improved the technical success rate of lymphatic mapping, including the use of radiolabeled sulfur colloid combined with a handheld gamma detector to guide operative dissection. The predictive value of histopathologic identification of metastatic melanoma within a sentinel lymph node is 100% for determination of whether melanoma has spread to regional lymph nodes. However, the predictive value of the absence of metastatic melanoma within the sentinel lymph node has not been clearly established. In all but 1 published report to date, the accuracy of lymphatic mapping and sentinel lymph node biopsy has...
PATIENTS AND METHODS

PATIENTS

We identified 89 consecutive patients who underwent intraoperative lymphatic mapping at the Massachusetts General Hospital (MGH), Boston, for cutaneous melanoma and were found to have no evidence of metastatic melanoma in their sentinel lymph nodes using routine histological techniques. All of the patients were operated on between November 15, 1993, and April 18, 1997. The pathological characteristics of the primary melanoma were reviewed at the MGH for nearly all of the patients in this study. In the few patients whose original primary melanoma tissue was not reviewed at the MGH, ulceration was scored as absent for statistical purposes unless indicated to be present in the referring institution’s pathology report. None of the patients had clinical evidence of metastatic melanoma at the time of their lymphatic mapping as assessed by clinical history, physical examination, chest radiograph, and liver function tests. None of these patients underwent a simultaneous ELND.

Nearly all patients were compliant with our follow-up recommendations, which consisted of quarterly history and physical examination, annual chest radiograph, and additional imaging studies for patients with signs or symptoms suggestive of metastatic melanoma. Routine surveillance was recommended every 3 months for the first 2 years, followed by every 6 months for the next 2 years, followed by yearly evaluation. The few patients who were not followed up according to these guidelines were contacted by telephone to determine their disease status.

SENTINEL LYMPH NODE MAPPING TECHNIQUE

One surgeon (K.K.T.) instructed 3 other surgeons (M.A.G., A.B.C., and W.W.S.) in the technique of sentinel lymph node mapping and directly participated in 3 to 5 operations for each. Simultaneous completion ELND was not performed on patients during this period. The patients described in this study represent approximately the first 110 patients who underwent lymphatic mapping for melanoma at the MGH. The number of lymphatic mapping operations performed by each of the surgeons during this period ranged from 15 to 50.

Approximately 3 hours prior to operation, 10.0- to 18.5-MBq technetium Tc 99m sulfur colloid (CIS-US Inc, Bedford, Mass) in a volume of 1 mL was injected into the dermis surrounding the site of the primary melanoma or biopsy scar. The total dose was typically divided into 4 equal parts of 0.25 mL each for injection into 4 sites. For patients with large biopsy scars the total dose was divided into more than 4 parts to permit more injections circumferentially around the scar. Planar gamma images were obtained 5 to 60 minutes following injection to define the location of the sentinel lymph nodes. In the operating room a majority of patients then received an injection of 0.5 to 1.5 mL of 1% lymphazurin dye (Zenith Parenterals, Rosemont, Ill) into the dermis circumferentially around the biopsy scar or melanoma. A handheld gamma detector (Care Wise Medical Products, Morgan Hill, Calif, or Neoprobe Corp, Dublin, Ohio) was used intraoperatively to precisely identify the location of the sentinel lymph nodes. Actual basin counts and lymph node counts varied depending on the gamma probe used and the dose of colloid administered. Basin counts were always at least 1 log order greater than background counts. Sentinel lymph nodes were defined as those that were stained with lymphazurin dye or concentrated 99mTc sulfur colloid. The lymph node resection bed was scanned carefully to ensure that all radioactive lymph nodes had been removed. Acceptable basin counts after sentinel lymph node excision were defined as less than 10% of the counts of the most radioactive lymph node. In a majority of patients, the entire sentinel lymph node was immediately placed into formalin. However, in a few patients, a portion of the sentinel lymph node was submitted fresh for an initial frozen section analysis. The primary melanoma site was widely excised during the same operation in most patients. The interval between radiolabeled colloid injection and operation typically ranged from 45 minutes to 3 hours.

HISTOLOGICAL ANALYSIS OF SENTINEL LYMPH NODES

Standard analysis of sagittally sectioned paraffin-embedded lymph nodes involved examination of 1 or 2 hematoxylin–eosin–stained sections of the central cut surface of each lymph node. A more detailed analysis of some lymph nodes was subsequently performed on archived tissue as described previously.6 Briefly, three 4-µm-thick serial tissue sections were obtained from the paraffin-embedded blocks at 3 levels 80 µm apart. One section from each of the 3 levels was stained with hematoxylin-eosin, and the remaining sections were stained with antibodies to S-100, HMB-45, NK1C3, and MART-1.

STATISTICAL ANALYSES

Variables were examined in a univariate analysis using the χ² test or Student t test where appropriate. Data from 1 patient in whom 2 separate primary melanomas were mapped during the same operation were analyzed as a single patient for recurrence status; however, the primary tumor characteristics and mapped basins for this patient were each counted individually.
lymph node mapping. In addition, long-term follow-up of patients with melanoma who undergo lymphatic mapping will identify those who will have a recurrence in sites other than regional lymph nodes (ie, distant metastases and in transit metastases). In the present study we assessed patterns of failure in a cohort of patients who underwent sentinel lymph node mapping without simultaneous ELND, and were found to have no evidence of melanoma in their sentinel lymph nodes using routine histopathologic analysis.

RESULTS

PATIENT CHARACTERISTICS

No evidence of metastatic melanoma was identified by standard histopathologic evaluation of the sentinel lymph nodes resected from 106 lymph node basins in 89 patients. One patient underwent simultaneous lymphatic mapping of 2 separate primary melanomas, and the sentinel nodes for these 2 lesions were located in separate basins (axillary and inguinal). The clinical and pathological characteristics of the patients are listed in Table 1. The median age was 51 years (range, 22-83 years) and 58% of patients were men. The primary melanoma site was on an extremity in 46 (51%) patients and axial location in 44 (49%). The most frequently mapped basin in this cohort of patients was the axilla (54%). Twenty-one patients (20%) underwent mapping of head and neck lymph nodes, and 4 other patients had much less common anatomic locations of their sentinel lymph nodes: epitrochlear, 2; chest wall, 1; and subscapular, 1. The median tumor thickness was 1.80 mm (range, 0.36-12.0 mm) and ulceration was present in 11 lesions (12%).

MELANOMA RECURRENCE

Eleven (12%) of 89 patients subsequently developed a recurrence despite having no evidence of metastatic melanoma with routine histological analysis of their sentinel lymph nodes (Table 2). In patients with recurrence, the median time to recurrence was 12 months (range, 2-35 months). The distribution of sex, age, primary melanoma site, and site of mapped lymph node basin was not statistically significantly different between those who developed a recurrence and those who did not (Table 1). Patients whose histologically negative sentinel lymph nodes were harvested from the inguinal lymph node basin accounted for the largest group of patients with subsequent recurrence. Of the 11 patients with subsequent recurrence, 7 (58%) initially underwent lymphatic mapping of the inguinal lymph node basin. The median tumor thickness was greater in patients with recurrence than in those without recurrence (2.85 mm vs 1.80 mm); however, this difference was not statistically significant. No difference was observed in the Clark microstaging level between these 2 groups. Although there was a trend toward a greater frequency of ulceration in the group of patients with recurrence compared with those without recurrence, the number of patients with recurrence was too small to allow a meaningful interpretation of the prognostic significance of this histopathologic feature.

The recurrence rate by site for the 11 patients with recurrence, given as number (percentage) of patients, is shown in the tabulation below.

Regional lymph node recurrence in a previously mapped basin was the first site of recurrence in 7 patients (8%) with recurrence. Two patients developed distant metastases and 2 other patients developed in transit metastases as their first sites of recurrence. Regional lymph node metastases in a previously mapped basin was the first site of recurrence in 7 (7%) of 106 lymph node basins.

Seven patients developed a single type of recurrence, whereas 4 patients developed more than 1 type of recurrence: 1 patient with in transit metastases subsequently developed regional lymph node metastases and additional in transit metastases, and 3 patients with regional lymph node recurrences subsequently developed distant sites of metastases (Table 2). Accordingly, 8 (9%) of the original 89 patients developed regional lymph node metastases as at least 1 component of their recurrence.

Table 1. Comparison of Clinical and Pathological Characteristics According to Recurrence Status*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients With No Recurrence</th>
<th>Patients With Recurrence</th>
<th>All Patients</th>
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<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>46 (59)</td>
<td>6 (55)</td>
<td>52 (58)</td>
</tr>
<tr>
<td>Female</td>
<td>32 (41)</td>
<td>5 (45)</td>
<td>37 (42)</td>
</tr>
<tr>
<td>Age, y (n = 89)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>51</td>
<td>47</td>
<td>51</td>
</tr>
<tr>
<td>Range</td>
<td>22-83</td>
<td>25-73</td>
<td>22-83</td>
</tr>
<tr>
<td>Primary tumor site (n = 90)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Axial</td>
<td>40 (51)</td>
<td>4 (36)</td>
<td>44 (49)</td>
</tr>
<tr>
<td>Extremity</td>
<td>39 (49)</td>
<td>7 (64)</td>
<td>46 (51)</td>
</tr>
<tr>
<td>Tumor thickness, mm (n = 90)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>2.01</td>
<td>3.82</td>
<td>2.23</td>
</tr>
<tr>
<td>Median</td>
<td>1.80</td>
<td>2.85</td>
<td>1.80</td>
</tr>
<tr>
<td>Clark level (n = 90)</td>
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<td></td>
</tr>
<tr>
<td>II/III</td>
<td>2 (3)</td>
<td>0</td>
<td>2 (2)</td>
</tr>
<tr>
<td>III</td>
<td>9 (11)</td>
<td>1 (9)</td>
<td>10 (11)</td>
</tr>
<tr>
<td>III/IV</td>
<td>6 (8)</td>
<td>1 (9)</td>
<td>7 (8)</td>
</tr>
<tr>
<td>IV</td>
<td>52 (66)</td>
<td>8 (73)</td>
<td>60 (67)</td>
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<tr>
<td>IV/V</td>
<td>1 (1)</td>
<td>1 (9)</td>
<td>2 (2)</td>
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<tr>
<td>Unknown</td>
<td>9 (11)</td>
<td>0</td>
<td>9 (10)</td>
</tr>
<tr>
<td>Ulceration (n = 90)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>9 (11)</td>
<td>2 (18)</td>
<td>11 (12)</td>
</tr>
<tr>
<td>Absent</td>
<td>70 (89)</td>
<td>9 (82)</td>
<td>79 (88)</td>
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<tr>
<td>Mapped lymph node basin (n = 106)</td>
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<td></td>
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<tr>
<td>Head and neck</td>
<td>21 (22)</td>
<td>0</td>
<td>21 (20)</td>
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<tr>
<td>Axillary</td>
<td>53 (56)</td>
<td>4 (33)</td>
<td>57 (54)</td>
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<tr>
<td>Inguinal</td>
<td>17 (18)</td>
<td>7 (58)</td>
<td>24 (23)</td>
</tr>
<tr>
<td>Other</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Epitrochlear</td>
<td>2 (2)</td>
<td>0</td>
<td>2 (2)</td>
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<tr>
<td>Chest wall</td>
<td>1 (1)</td>
<td>0</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Subscapular</td>
<td>0</td>
<td>1 (8)</td>
<td>1 (1)</td>
</tr>
</tbody>
</table>

*Data are given as number (percentage) unless otherwise indicated.
Of the 5 patients who developed distant metastases as at least 1 component of their recurrence, 3 (60%) had evidence of melanoma in their lymph nodes, and 2 (40%) did not based on routine pathological analysis. Univariate analyses of the prognostic factors with respect to recurrence revealed that male sex, Clark level greater than III, axial location, presence of ulceration, and mean tumor thickness were not statistically significant (P > .05) prognostic factors. The small number of patients with recurrences in this study reduces the statistical power of these analyses.

**SURVIVAL**

The median follow-up duration for the 89 patients in this study was 23 months (range, 2-54 months), and 78 (88%) of patients remain disease free. The median disease-free survival has not yet been reached. Of the 7 patients whose first recurrence was lymph node metastasis, all underwent regional lymphadenectomy. Four have remained disease free, 2 are alive with disease, and 1 died of disease. Of the 2 patients whose first recurrence was in transit metastasis, 1 underwent surgical resection of the in transit lesions and has remained disease free. The other patient was treated with hyperthermic isolated limb perfusion combined withinguinal lymphadenectomy and subsequently died of complications arising from retroperitoneal metastases.

**REEVALUATION OF SENTINEL LYMPH NODES WITH SERIAL SECTIONS AND IMMUNOHISTOCHEMISTRY**

The standard pathological assessment of the sentinel nodes in this study consisted of bisection of the lymph node along its long axis and histological examination of 1 or 2 hematoxylin-eosin–stained sections from each cut surface. Careful rereview of the original histological sections did not reveal evidence of metastatic melanoma in any patient in this study. We have also reported a more detailed examination of sentinel lymph nodes, including 78 patients in this study. This examination consisted of deeper sections into the lymph node and immunohistochemical stains with antibodies to S-100, HMB-45, NK1C3, and MART-1. An adequate amount of residual sentinel lymph node tissue was not available for further examination in some patients. Sentinel lymph node reanalysis revealed metastatic melanoma in 9 patients, 6 of whom have not had recurrences to date.

Of the 7 patients who developed regional lymph node metastases as their first site of recurrence, sentinel lymph nodes were reanalyzed in 4, and in 1 (25%) metastatic melanoma was identified in the sentinel node. Of the 2 patients who developed distant metastases as their first site of recurrence, a more detailed analysis of their sentinel lymph nodes revealed metastatic melanoma in 1 but not in the other. Of the 2 patients who developed in transit metastases as their first site of recurrence, metastatic melanoma was identified in 1 of the sentinel lymph nodes, and there was not sufficient additional tissue available for review in the other patient. Overall, of the 11 patients who developed a recurrence, sufficient additional tissue was available for review in 7 patients, and in 3 of them a more detailed pathological assessment of sentinel nodes revealed metastatic melanoma. Therefore, when taking into account the more detailed pathological review of sentinel lymph nodes, regional lymph node metastases in a previously mapped basin was the first site of recurrence in 6 (6%) of 97 lymph node basins harboring histologically negative sentinel lymph nodes. And when analyzed by patient, 86 patients had histologically negative sentinel lymph nodes of whom 6 (7%) subsequently developed regional lymph node metastases in the mapped lymph node basin as their first site of recurrence, and 8 (9%) had overall recurrence. Of the 5 patients who developed distant metastases as at least 1 component of their recurrence, 4 (80%) had evidence of metastatic melanoma in their sentinel lymph nodes using either standard techniques or serial sections with immunohistochemical staining. Only 1 patient presented with distant metastases without evidence of metastatic melanoma in their sentinel node.

**COMMENT**

In their original description of lymphatic mapping, Morton and colleagues used a vital blue dye alone to trace lymphatic channels to the sentinel lymph node. In this landmark study, the authors performed a simultaneous complete lymphadenectomy of the mapped basin to de-
termine the accuracy of the technique. In 38 (95%) of 40 regional node basins containing metastatic melanoma, the sentinel node was found to contain metastatic melanoma using hematoxylin-eosin staining as well as immunohistochemical staining with S-100 and NK1.C3. Of 156 basins with histologically negative sentinel lymph nodes, only 2 (1%) had metastatic melanoma in any of the remaining regional lymph nodes, thereby providing a predictive value of a negative result of 99%. In the years following this report, an analysis of published reports on sentinel lymph node mapping for melanoma has revealed interesting trends and concepts. First, the addition of radiolabeled sulfur colloid combined with the intraoperative use of a handheld gamma detector has improved the technical success rate of sentinel lymph node mapping. In addition, this improvement has presumably reduced the frequency of missed sentinel lymph nodes, especially in patients who have more than a single sentinel lymph node in a basin. Second, most patients are now undergoing sentinel lymph node mapping without simultaneous complete regional lymphadenectomy. Third, data have emerged that support the notion that more detailed analyses of sentinel lymph nodes will detect metastatic melanoma more often than routine analyses involving only hematoxylin-eosin staining of 1 or 2 sections.

The false-negative rate of sentinel lymph node mapping may be assessed by simultaneous ELND and comparison of the histological status of the sentinel lymph node with that of the remaining lymph nodes, as was originally described by Morton and colleagues. However, a more clinically relevant assessment of the accuracy of lymphatic mapping requires follow-up of patients who were found to have a histologically negative sentinel lymph node at the time of their lymphatic mapping and did not simultaneously undergo an ELND. The frequency of recurrence in this group of patients provides one measure of the prognostic accuracy of lymphatic mapping. Results from our study are in agreement with those previously published, indicating that the false-negative rate as assessed by clinical follow-up is higher than the false-negative rate as assessed by simultaneous ELND. In our study as well as in the largest follow-up study published to date, approximately 10% of patients with histologically negative sentinel lymph nodes detected by routine techniques have recurrences overall, with only 6% having recurrence in a previously mapped lymph node basin.

There are several possible explanations for false-negative results of lymphatic mapping, and these may be separated into 3 general mechanisms. The first general mechanism that may lead to false-negative results involves the biology of melanoma progression. For example, at the time of lymphatic mapping, metastatic melanoma cells may be within lymphatic channels but have not yet arrived in the regional lymph node basin. Subsequent to resection of a histologically negative sentinel lymph node, these cells may arrive in a regional lymph node and develop into clinically apparent metastases. Another example of melanoma biology that may explain false-negative results involves tumor cells spreading from the sentinel node to “secondary” (nonsentinel) nodes, followed by immune-induced regression of the original focus of metastatic melanoma in the sentinel lymph node. Alternatively, hematogenously circulating melanoma cells may spread to regional lymph nodes after the lymphatic mapping procedure has been performed.

The second general mechanism that may account for false-negative sentinel lymph nodes involves technical aspects of the lymphatic mapping procedure itself. There are several technical errors that may lead to misidentification of sentinel lymph nodes. For example, in patients who undergo lymphatic mapping without intraoperative use of a handheld gamma detector, the surgeon may believe that all of the sentinel lymph nodes have been resected, when in reality, an additional sentinel lymph node remains undetected. The presence of melanoma cells in this missed sentinel lymph node may give rise to a false-negative result. Similarly, the combination of lymphatic mapping using only vital blue dye without any preoperative lymphoscintigraphy may result in failure to identify sentinel lymph nodes residing in alternate lymph node basins or in unusual anatomic locations. Four patients in our series had sentinel lymph nodes located in uncommon anatomic sites. Disruption of the regional lymphatics prior to lymphatic mapping may also lead to misidentification of sentinel lymph nodes. The accuracy of the technique has been demonstrated only in patients in whom the procedure has been performed after a biopsy but prior to a wide excision of the primary melanoma. It is not clear that lymphatic mapping is accurate in patients who have undergone a definitive excision of their melanoma prior to lymphatic mapping. In other words, the lymphatic channels draining the residual scar resulting from a wide excision may not necessarily represent the same lymphatic channels that drained the primary melanoma. Although a radioactive, blue lymph node may be identified and resected in patients who have already undergone a wide excision of their melanoma, this lymph node may not be the primary node that received lymphatic drainage from the melanoma. Two of the patients with false-negative results in this study underwent wide excision of their melanomas prior to lymphatic mapping.

Virtually all investigators are in agreement that the addition of radiolabeled colloid to the technique of lymphatic mapping for melanoma has enhanced the robustness of the technique and shortened the “learning curve.” In general, primary melanomas located on the head and neck region are the most difficult to map because of the close proximity of the primary injection site and sentinel lymph nodes, as well as the technical expertise required to resect sentinel lymph nodes without injury to critical nerves. Conversely, the inguinal lymph node basin tends to serve as the least difficult basin from which to identify and resect sentinel lymph nodes. Interestingly, of the 11 patients in our series with recurrence, 7 had their sentinel lymph nodes resected from the inguinal basin. One possible explanation for this unexpected finding is that after resection of inguinal sentinel lymph nodes, we did not generally resect any additional iliac sentinel lymph nodes. Therefore, it is possible that some patients in our series had iliac sentinel lymph nodes with clinically occult metastases left unresected, thereby giving rise to false-negative results. However, none of the patients in our series developed iliac lymph node recurrences.
A third general mechanism that may give rise to false-negative results of lymphatic mapping involves the failure of routine histopathologic techniques to identify metastatic melanoma in the sentinel lymph node. In some patients, sentinel lymph nodes may contain metastatic melanoma below the limits of resolution for the analytic techniques applied. Although the use of serial sections and immunohistochemical stains clearly enhances the sensitivity of melanoma detection, these techniques are still limited by the possibility of sampling error, since not all of the lymph node is analyzed. The use of reverse transcription–polymerase chain reaction (RT-PCR) to detect messenger RNA for the tyrosinase gene has been reported to significantly enhance the sensitivity for detection of cells of melanocytic origin. This technique theoretically permits analysis of the entire sentinel lymph node without the limitations of sampling error. However, approximately 10% of patients have benign capsular nevi within their sentinel lymph nodes, thereby giving rise to false-positive results using RT-PCR analysis of the entire node. To help reduce the incidence of false-positive results using RT-PCR, it has been proposed that a portion of the lymph node should still be examined microscopically for capsular nevi. However, setting aside a portion of the lymph node again introduces the possibility of sampling error. Establishing primary cultures of lymph nodes is another sensitive method for detection of metastatic melanoma cells; however, this technique is labor intensive, expensive, and requires facilities that are not available in many hospitals.

The prognostic value of these more sensitive techniques for detection of microscopic melanoma in lymph nodes remains to be established. No long-term follow-up studies have been published of patients whose sentinel lymph nodes were examined by these microstaging techniques. It is possible that in some patients, the microscopic disease detected by these sensitive techniques may have been destroyed by the host immune response if left in situ. It is anticipated that such reports will be forthcoming, given that these microstaging techniques are being used more commonly. Because only a few lymph nodes are typically resected during lymphatic mapping, it has now become more practical to apply these microstaging techniques. One of the specific aims of the Sunbelt Melanoma Trial (Kelly McMasters, MD, PhD, University of Louisville, Louisville, Ky, oral and written communication, 1998) is to define the importance of these molecular techniques for analysis of sentinel lymph nodes.

At the MGH we consider lymphatic mapping for patients with cutaneous melanomas thicker than 1.0 mm and who have not already undergone a wide local excision of the tumor. We will also consider lymphatic mapping for patients with thinner lesions and other adverse prognostic factors including ulceration, axial location, and male sex. Based on the results of this study and others, we recently adopted a standard approach of sentinel lymph node evaluation using serial sections and immunohistochemical stains for S-100 and MART-1, which we believe will reduce the incidence of false-negative results. As indicated above, the false-negative rate may be a function of the rigor with which one examines the sentinel lymph node. However, the false-negative rate is also a function of the duration of clinical follow-up. The median follow-up for this study is only 23 months, and we anticipate that the false-negative rate will continue to rise with longer follow-up. Continued follow-up will also allow us to determine the value of serial sections and immunohistochemistry compared with routine hematoxylin-eosin staining. It is quite interesting that in the entire cohort of patients studied, only a single patient has developed distant metastases in the absence of regional lymph node metastases (excluding the patient in whom metastatic melanoma was identified in the sentinel node retrospectively using microstaging techniques). This supports the notion that melanoma follows an orderly progression of metastases, with disease first appearing in regional lymph nodes, followed by disease appearing in distant sites. However, this temporal relationship does not necessarily imply that regional metastases give rise to distant metastases. The presence of this temporal relationship merely implies that by the time that distant metastases are clinically detectable, melanoma has already spread to regional lymph nodes. These potential mechanisms of disease progression have obvious important ramifications on how complete resection of regional lymph nodes containing metastatic melanoma may or may not influence overall survival.


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REFERENCES


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DISCUSSION

Dougald MacGillivray, MD, South Portland, Me: Dr Souba and his coauthors have presented the outcome of 89 patients with cutaneous melanoma who had no evidence of metastases to the sentinel lymph nodes. The results of this report support the thesis that lymphatic mapping and sentinel lymph node examination is an accurate method and a logical alternative to elective node dissection for staging the regional lymph node basins in patients with melanoma. At a median follow-up of 23 months, 12% of these patients have had clinical evidence of recurrent disease. Eight patients, or 8.9%, eventually developed a regional nodal metastasis, and 5 patients, or 5.6%, had distant metastases. The rate of recurrent melanoma in this study is similar to that reported by Gerstenwald from the M. D. Anderson Cancer Center. In their experience with 243 patients with melanoma who had a negative sentinel lymph node, 11% had recurrent disease over a median follow-up of 35 months. However, their findings on reevaluation of the sentinel lymph node in those patients who recurred differed from those reported today. In the M. D. Anderson report, 80% of patients who had a regional nodal recurrence as a component of their initial recurrent disease had previously undetected metastases in the sentinel lymph node when it was reevaluated with special techniques, while none of the patients who had in transit or distant metastases as the first sight of recurrence had involvement of the sentinel lymph node on reexamination. In Dr Souba’s series, only 1 of 7 patients who had their site of recurrence in the regional nodal basin had occult disease detected on reexamination of the sentinel lymph node, while 4 of 5 patients, or 80%, who developed distant metastases had involvement of the sentinel lymph node. I suspect that some of this difference is due to the number of patients who had an adequate volume of residual tissue available for reexamination. However, of the 11 patients who developed recurrent disease, 8 of those patients, or 73%, had a regional nodal metastasis as a component of their recurrence. Do you think that this was due to a technical problem or failure to identify the sentinel lymph node at the initial procedure, or do you think this was possibly due to secondary involvement of the nodal basin from in transit or systemic disease? Has your experience with this group of patients resulted in a modification of your protocol for sentinel node identification and examination?

It is clear that a more extensive examination of the sentinel lymph node will detect more patients with occult microscopic disease. What is yet to be determined is a clinical significance of lymph node metastases detected by this more intensive scrutiny, particularly metastases detected by immunohistochemistry and molecular techniques.

Have you reexamined the sentinel lymph nodes of those patients who have not recurred to determine the frequency of occult metastatic disease that was not detected on initial examination?

Finally, your recurrence rate of 12% at 2 years of follow-up, while small, is not insignificant, particularly when there is adjuvant treatment that may improve survival in patients with occult metastatic disease. Are you currently investigating or utilizing other prognostic features or techniques such as ulceration, microscopic satellitosis, DNA ploidy, or molecular markers to select high-risk patients with sentinel lymph nodes that are negative who may benefit from adjuvant treatment?

Robert Quinlan, MD, Worcester, Mass: I wish you would give us some further information on which of the 2 techniques you think is most sensitive and reproducible in identifying the sentinel node—for instance, is it a lymphazurin dye technique or is it the radio technetium sulfur colloid technique? Have you had methodologic problems in technique as well as administration either of the dye or the radionuclide? Second question, What do you recommend in terms of the type of procedure? Would you do just a superficial node dissection once you identify, by immunohistochemistry, that you have positivity of the node, or should you extend that to a deep dissection and perhaps even if these patients are on protocol do you recommend any type of systemic chemotherapy?

Richard Swanson, MD, Worcester: Do you use rtPCR to analyze the sentinel nodes?

Giles Whalen, MD, Farmington, Conn: The question I have is just a point of technique. Were all of these melanomas in sight when they were injected, or were some of them previously excised and the biopsy site around it injected?

Dr Souba: I would like to thank the discussants for their thoughtful comments. Dr MacGillivray asked about the discrepancy among what M. D. Anderson found and what our group noted in terms of lymph node sections and the presence of melanoma cells. The answer depends, in part, on how hard you look. In the M. D. Anderson study, as many as 200 serial sections were made of the lymph nodes. This explains, at least in part, why they were able to identify melanoma cells more often in their “negative” nodes than we did. There are probably several explanations for false-negatives. One explanation relates to technical issues. There is a learning curve associated with performing lymphatic mapping. Secondly, it is conceivable that the patient has melanoma cells in the different lymphatics which have not reached the sentinel nodes. Under these circumstances, metastatic disease could appear after lymph node resection. The third explanation, as discussed above, relates to the extent to which the nodes are examined. In this light, Dr Swanson asked about reverse transcriptase PCR. This type of molecular biology technique is 1 example of some of the newer efforts that are being employed to examine lymph nodes.

Currently, the guidelines for lymphatic mapping in our group vary somewhat. In general, we map patient [tumors] that are greater than 1 mm in thickness. Those patients who do undergo lymphatic mapping have standard hematoxylin-eosin sections of the lymph node and immunohistochemistry.

Dr MacGillivray asked an interesting question about the significance of nodal micrometastases that are detected only by immunohistochemistry. It is quite possible that many of these patients are cured with surgery alone. However, the significance of micrometastases detected by newer techniques requires further study. As a general rule, patients who have a positive sentinel lymph node undergo a formal lymph node dissection under general anesthesia at a later date.