Nodal Melanocytic Nevi in Sentinel Lymph Nodes
Correlation With Melanoma-Associated Cutaneous Nevi

John B. Holt, MD,1 Omar P. Sangueza,2 Edward A. Levine, MD,3 Perry Shen, MD,3 Simon Bergman, MD,2 Kim R. Geisinger, MD,2 and Andrew J. Creager, MD1

Key Words: Nodal nevus; Sentinel lymph node; Melanocytic nevus; Breslow thickness; Congenital; Lymph node capsule

Abstract
Melanocytic nevi occurring in lymph nodes create diagnostic difficulty by mimicking metastases. Few studies describe nodal nevi in sentinel lymph nodes (SLNs) excised for melanoma. We evaluated 72 cases in which patients had undergone SLN biopsy for melanoma. Lymph nodes and cutaneous melanomas were evaluated according to a standard protocol. Nodal nevi were identified in 8 patients (11%). Of these, 6 (75%) had an associated cutaneous nevus (P = .006). Of 21 patients with an associated nevus, 4 (19%) with nodal nevi had a cutaneous nevus with congenital features (P = .01). The incidence of nodal nevus correlated with a Breslow thickness greater than 2.5 mm (P = .02). Nevi were not seen in non-SLNs. Nodal nevi appear more frequently in patients with melanoma-associated cutaneous nevi, particularly if congenital features are present. The increased frequency of nodal nevi in SLNs relative to non-SLNs suggests an etiology of mechanical transport of nevus cells.

Descriptions of intranodal melanocytic nevocellular inclusions (nodal nevi) have been reported periodically over the years.1-5 Nodal nevi predominantly form compact focal aggregates in the fibrous capsule and trabeculae of lymph nodes, although they recently have been described in the nodal parenchyma and sinusoids.6 The incidence in regional lymph nodes excised for malignancy has been reported to be between 1% and 22%.5,7,8 From a diagnostic standpoint, nodal nevi might mimic metastases when encountered in regional lymph nodes evaluated for staging patients with melanoma, and it has been reported that their occurrence in this group of patients is greater than in people undergoing regional lymph node excisions for other malignant neoplasms.7,9,10 The mechanism by which benign melanocytes are transported to lymph nodes is the subject of controversy. Prevailing theories include lymphatic transfer of melanocytes from a cutaneous nevus to a lymph node in the lymphatic drainage basin3,7 and an embryologic phenomenon whereby neural crest–derived melanocytes are transported to lymph nodes during in utero migration.

The increasing popularity of sentinel lymph node (SLN) mapping in patients with melanoma in conjunction with more stringent evaluation techniques has led to increased frequency of discovery of nodal nevi in regional lymph nodes excised for the treatment of melanoma.7 In addition, a correlation between the frequency of nodal nevi and the presence of melanoma-associated cutaneous nevi including those with congenital features has been reported.7,11 Distinguishing benign nodal nevi from melanoma metastases to an SLN may be difficult, and it is of critical importance for the determination of prognosis and systemic treatment.12 To our knowledge, only 2 systematic studies describing the characteristics of nodal nevi
in SLNs excised for melanoma have been published.\textsuperscript{7,11} SLN biopsy is an accepted staging procedure, and we expect that it will be used even more widely in the future. Accordingly, the diagnostic dilemma imposed by nodal nevi will continue to be encountered in this setting.\textsuperscript{13,14} For this reason, we conducted the present retrospective study in an attempt to define further the features of nodal nevi in patients with melanoma.

**Materials and Methods**

Between December 1998 and March 2002, material from all SLN mapping procedures performed for melanoma at the Wake Forest University Baptist Medical Center, Winston-Salem, NC, was retrieved. Only cases in which the corresponding primary melanoma had been reviewed were included in the study.

Melanoma was categorized by specific subtypes, Clark level, Breslow thickness, and the presence and type of preexisting melanoma-associated cutaneous nevi. The morphologic features of any associated precursor melanocytic lesion were characterized with particular attention given to the presence or absence of congenital features. For the purposes of this study, congenital features included small cutaneous nevi demonstrating the presence of nevus cells in the lower two thirds of the dermis; splaying of collagen bundles by singly or vertically oriented linear arrays of nevus cells; and extension of cells around nerves, vessels, and adnexa, as previously described.\textsuperscript{15} We acknowledge that there is overlap in the histologic features of congenital vs acquired nevi; however, for the purposes of this study, the cutaneous nevi with the aforementioned histologic features were considered to be congenital. In addition, the presence or absence of a geographically separate non–melanoma-associated melanocytic lesion in the wide excision specimen was noted.

A standard protocol was used to identify the SLN. The tumor bed was injected with technetium sulfur colloid (0.5-1.0 mCi [18.5-37.0 MBq]) preoperatively. A lymphoscintigram was obtained in the nuclear medicine department before the patient’s arrival in the operative suite, and a gamma probe (Neoprobe 2000, Neoprobe, Dublin, OH) was used intraoperatively to detect the SLNs. In addition, in all cases, perilesional intradermal injections of isosulfan blue were used intraoperatively to provide visual identification of the SLNs. SLNs then were harvested and sent fresh to the pathology department for intraoperative evaluation and subsequent permanent section evaluation. The SLN was obtained as the initial phase of the surgical procedure, with excision of the primary lesion generally being performed during intraoperative pathologic evaluation of the SLN. This minimized the impact of intraoperative evaluation on the total surgical time. Intraoperative evaluations were performed by imprint cytology as previously described\textsuperscript{16} and, if positive, resulted in immediate regional lymph node dissection. If the intraoperative evaluation was not performed, then a lymph node dissection was not performed during the same procedure.

SLNs were bisected along the long axis, fixed in 10% buffered formalin, processed in the usual manner, and paraffin embedded. A single H&E-stained section of the paraffin block was examined initially. If this section was negative for melanoma, 3 additional H&E-stained levels cut at 50-µm intervals in conjunction with immunohistochemical stains for S-100 and HMB-45 (DAKO, Carpinteria, CA) on the first of the 3 levels were evaluated.

Immunohistochemical studies were performed using the avidin-biotin-peroxidase complex method.\textsuperscript{17} Primary antibodies included S-100 and HMB-45. Immunohistochemical stains were considered positive for malignant melanoma if immunoreactivity was detected in cell clusters or individual cells that demonstrated architectural and cytologic features of metastatic tumor cells [Image 18] and [Image 21]. All metastases were compared with the primary melanoma. When discrepancies existed between the intraoperative and permanent section diagnoses, slides were reviewed in an attempt to determine the cause of the discrepancy.

**Nodal nevi** were defined as bland neovascular aggregates cytologically resembling those found in the intradermal component of the associated cutaneous nevus, when present. Generally, inclusions consisted of compact aggregates of cells with pale homogeneous eosinophilic cytoplasm and inconspicuous melanin pigment, indistinct cytoplasmic borders, round nuclei with delicate chromatin, inconspicuous basophilic nucleoli, and minimal, if any, mitotic activity [Image 30] and [Image 41]. Stereotypical locations in the lymph node included the fibrous capsule and trabeculae, although care was exercised to identify nodal nevi in the parenchyma and subcapsular sinus, as recently described.\textsuperscript{6} In all cases, nodal nevi were compared with the primary melanoma and any associated cutaneous nevus to distinguish cytologic differences.

Non-SLNs were examined using standard pathologic techniques. If the non-SLNs were more than 4 mm wide, they were sectioned; if they were less than 4 mm wide, the non-SLNs were submitted whole. Routinely, a single H&E-stained section of the non-SLNs was examined, but in select cases, multiple levels were obtained in an attempt to verify the presence of metastases.

Statistical comparisons were made using the Fisher exact test.

**Results**

A total of 72 cases with 198 SLNs (mean, 2.8 per patient) were suitable for inclusion in the study. The patients...
were 48 males and 24 females with an age range of 11 to 82 years (mean, 54.8 years). Primary melanomas were located on the head and neck in 19 patients, the trunk in 32 patients, the upper extremity in 10 patients, and the lower extremity in 11 patients.

The types were as follows: nodular, 31 (6 with associated cutaneous nevi); superficial spreading, 25 (15 with associated cutaneous nevi); lentigo maligna melanoma, 6 (0 with associated cutaneous nevi); desmoplastic, 6 (0 with associated cutaneous nevi); and not specified, 4 (0 with associated cutaneous nevi). The Clark levels of the primary melanomas were as follows: level II, 5; level III, 14; level IV, 49; and level V, 4 (mean, 3.7). The Breslow thickness ranged from 0.52 to 8.5 mm (mean, 2.3 mm).

Twenty-six patients additionally underwent completion regional lymph node dissection, yielding 287 non-SLNs.
Eighteen patients had SLNs containing metastatic melanoma, all of which were detected on the initial H&E-stained section evaluation. Nodal nevi were identified in 8 patients (11%) and 10 SLNs (5%). Of these, 6 patients (75%) with 8 SLNs (80%) had a melanoma-associated precursor melanocytic lesion ($P = .006$ and $P = .002$, respectively) [Table 1].

All nodal nevi were detected in the initial H&E-stained sections and were located in the lymph node capsule and/or trabeculae. All nodal nevi were positive for S-100 protein, while only 3 were positive for HMB-45. HMB-45 immunoreactivity in nodal nevi tended to be focal and weak, while metastatic melanoma cells demonstrated diffuse, strong immunoreactivity.

Of 21 patients with a melanoma-associated precursor melanocytic lesion, 4 patients (19%) with 6 SLNs (9%) had nodal nevi when the lesion demonstrated congenital features ($P = .01$ and $P = .004$, respectively) [Table 2]. The presence of nodal nevi was associated with a Breslow thickness greater than 2.5 mm [Table 3]. When a separate cutaneous nevus (not associated with the primary melanoma) was present in the excision, no correlation with nodal nevi could be identified [Table 4]. For patients who underwent SLN biopsy followed by completion regional lymph node dissection, no nodal nevi were identified in non-SLNs. Only 1 patient had a lymph node with both a nevus and a metastatic melanoma, a coincidence that did not reach statistical significance ($P = .67$ per patient; $P = 1.0$ per SLN).

### Table 1

<table>
<thead>
<tr>
<th>Nodal Nevi vs Melanoma-Associated Precursor Melanocytic Lesion (PML)</th>
<th>Nodal Nevus Present</th>
<th>Nodal Nevus Absent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients*</td>
<td></td>
<td></td>
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<tr>
<td>PML present</td>
<td>6</td>
<td>15</td>
</tr>
<tr>
<td>PML absent</td>
<td>2</td>
<td>49</td>
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<tr>
<td>Individual sentinel lymph nodes†</td>
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</tr>
<tr>
<td>PML present</td>
<td>8</td>
<td>57</td>
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<tr>
<td>PML absent</td>
<td>2</td>
<td>131</td>
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</table>

* $P = .006$.  
† $P = .002$.

### Table 2

<table>
<thead>
<tr>
<th>Nodal Nevi vs Precursor Melanocytic Lesion With Congenital Features (PML-CF)</th>
<th>Nodal Nevus Present</th>
<th>Nodal Nevus Absent</th>
</tr>
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<tbody>
<tr>
<td>Patients*</td>
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<tr>
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<td>PMLCF absent</td>
<td>2</td>
<td>14</td>
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<tr>
<td>Individual sentinel lymph nodes†</td>
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<td></td>
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<tr>
<td>PMLCF present</td>
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<td>12</td>
</tr>
<tr>
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* $P = .01$.  
† $P = .004$.

### Table 3

<table>
<thead>
<tr>
<th>Nodal Nevi vs Breslow Thickness</th>
<th>≤2.5 mm</th>
<th>&gt;2.5 mm</th>
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<td>3</td>
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<tr>
<td>Nodal nevus absent</td>
<td>15</td>
<td>0</td>
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<tr>
<td>Individual sentinel lymph nodes†</td>
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<td></td>
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<tr>
<td>Nodal nevus present</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Nodal nevus absent</td>
<td>53</td>
<td>4</td>
</tr>
</tbody>
</table>

* $P = .02$.  
† $P = .0007$.

### Table 4

<table>
<thead>
<tr>
<th>Nodal Nevi vs Separate Melanocytic Lesion (SML)</th>
<th>Nodal Nevus Present</th>
<th>Nodal Nevus Absent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SML present</td>
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<td>13</td>
</tr>
<tr>
<td>SML absent</td>
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<td>51</td>
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<tr>
<td>Individual sentinel lymph nodes†</td>
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<tr>
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<td>37</td>
</tr>
<tr>
<td>SML absent</td>
<td>7</td>
<td>143</td>
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</table>

* $P = .36$.  
† $P = .43$.

### Discussion

Since the initial description of benign nodal nevi by Stewart and Copeland in 1931, controversy has existed regarding the means whereby benign nevocellular aggregates come to reside in lymph nodes, and this issue is largely unresolved. Both embryologic and mechanical transport hypotheses have been postulated. The majority of investigators believe these inclusions to be benign, and similarities between nodal nevus cells and cutaneous nevocytes have been documented by electron microscopic and immunohistochemical studies. A recent study demonstrated some immunohistochemical differences between nodal nevi and primary melanomas in an attempt to aid in their distinction.

Substantial evidence favors the mechanical transport hypothesis. Early observations of cutaneous blue nevi that exhibited the apparent phenomenon of “benign metastasis” have been reported. Studies have demonstrated an increased incidence of nodal nevi in the lymph nodes excised from the drainage basin of a primary melanoma compared with lymph nodes excised for other malignant neoplasms. Correlations between the presence of nodal nevi and melanomas that have an associated precursor melanocytic lesion, with and without congenital features, have been reported. In the present study, nodal nevi were found only in SLNs, and their correlation with the presence of
precursor melanocytic lesions, particularly those with congenital features, was confirmed. The high incidence of nodal nevi in SLNs strongly supports the hypothesis of mechanical transport through lymphatics, although it must be pointed out that the low coincidence (1 case) of metastatic melanoma in conjunction with nodal nevi in SLNs might confound this finding. This is an interesting finding, as increased Breslow thickness correlated significantly and independently with the presence of metastatic melanoma and with the presence of nodal nevi.

In the context of a primary melanoma with an associated precursor melanocytic lesion, one potential explanation for the increased incidence of nodal nevi, particularly in SLNs, is that invading neoplastic melanocytes displace adjacent benign nevus cells that then are transported into lymphatics. Alternatively, the biopsy procedure itself might result in displacement of nevus cells into lymphatics, although this hypothesis is less attractive, as most nodal nevi in the capsule and trabeculae seem well encased in fibrous tissue, suggesting chronicity to their presence. In addition, a recent report of sinusoidal nevi\(^6\) suggests that mechanical disruption might be involved, whether it occurs by biopsy or invading tumor.

The relative paucity of descriptions of nodal nevi in deep intra-abdominal and intrathoracic lymph nodes, which presumably do not collect material from cutaneous drainage basins, also tends to support the mechanical transport theory, although, to our knowledge, this observation has not been well documented.

Some have argued that the statistically significant association of nodal nevi with congenital nevi supports an embryologic origin.\(^11\) In the present study, we compared melanomas with a melanoma-associated precursor melanocytic lesion or a geographically separate (non–melanoma-associated) melanocytic lesion present in the wide excision specimen. If the embryologic hypothesis of nodal nevus origin is correct, then one would expect nodal nevi to occur with relatively equal frequency in patients from both of these categories. However, we demonstrated a statistically significant increased frequency of nodal nevi only in patients whose melanoma had an associated precursor melanocytic lesion (Table 4).

If a mechanism exists that involves mechanical dislocation of nevocytes into lymphatics, it would intuitively make sense that nevi that show closer association with lymphatic vessels would be even more likely to exhibit benign metastasis behavior. Therefore, an increased frequency of nodal nevi when the associated precursor lesion has congenital features would be expected and was observed in this series.

In the present study, no non-SLNs contained nevi. Previous studies describing the incidence of nodal nevi in lymph node dissections performed for other malignant neoplasms, such as breast or prostate carcinoma, have demonstrated a very low incidence of nodal nevi.\(^5,7,26,27\) To our knowledge, only 1 other study exists that evaluated the incidence of nodal nevi in dissections for which SLNs and non-SLNs were distinguished.\(^7\) By using large numbers of cases, that study identified an incidence of nodal nevi in non-SLNs removed for breast carcinoma of 0.1%, while 3.9% of SLNs and 1.0% of non-SLNs removed for melanoma contained nevi. While none of the non-SLNs in our series contained a nevus, this is almost certainly the result of the relatively smaller number of cases evaluated in our study and would suggest that the numeric power was insufficient to detect the low incidence of nodal nevi in non-SLNs. In addition, it should be noted that non-SLNs were not examined at multiple levels of section or with immunohistochemical analysis, as is our protocol with SLNs. As all metastases and capsular nevi in the present study were identified with H&E staining, this correlation seemed appropriate; however, bias likely exists. Furthermore, the accuracy of the SLN technique is less than 100%, which limits the conclusions that may be drawn regarding “non-sentinel” nodal nevi. It also is important to note that none of the intraoperative touch preparations that were made from SLN material were interpreted as false-positive as a result of nodal nevi.

While definitive resolution of the question of nodal nevus origin awaits further study, we confirm the correlation between the incidence of nodal nevi and the presence of melanoma with an associated precursor melanocytic lesion. Moreover, the association of nodal nevi with Breslow thickness favors a mechanism of mechanical transport of benign nevocytes to lymph nodes. Although our study does not specifically address the issue, the appearance of nodal nevi in a large majority of SLNs for which there are no melanoma metastases might raise potential diagnostic and prognostic issues.

From the 1Department of Pathology, Duke University Medical Center, Durham, NC; and 2Department of Pathology and 3Surgical Oncology Service, Department of Surgery, Wake Forest University Baptist Medical Center, Winston-Salem, NC.

Address reprint requests to Dr Creager: Dept of Pathology, Box 3712, Duke University Medical Center, Durham, NC 27710.

References