Histopathologic Recognition of Involved Margins of Lentigo Maligna Excised by Staged Excision

An Interobserver Comparison Study

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Objectives: To assess interobserver and intraobserver concordance for identifying positive and negative margins in staged excisions of lentigo maligna and lentigo maligna melanoma and to determine if control biopsy specimens are useful to improve concordance.

Design: Retrospective, randomized interobserver and intraobserver comparison study of archived pathologic specimens. The study was conducted in 3 phases, and slides were evaluated blindly and independently by 5 pathologists: in phase 1, all slides were randomized and diagnosed as positive or negative. In phase 2, every third slide was evaluated again and diagnosed as positive or negative. In phase 3, slides were organized into cases, allowing evaluation of each margin in the context of the positive control (tumor from the center of the lesion) and negative control (control biopsy specimen), if available.

Setting: University referral center.

Study Material: A total of 301 glass microscopic slides from 27 patients who underwent staged excision for lentigo maligna or lentigo maligna melanoma from March 1997 to April 2001.

Main Outcome Measures: Interobserver and intraobserver concordance between original diagnoses and study diagnoses rendered on all slides by 5 pathologists.

Results: Phase 1 and 3 agreement was moderate (κ range, 0.4-0.5). Phase 2 (intraobserver) agreement was moderate to good for all pathologists (κ range, 0.6-0.9). Subset analysis revealed a statistically significant increase in agreement with the use of a control strip biopsy specimen for difficult slides.

Conclusions: Interobserver concordance for margin analysis in lentigo maligna and lentigo maligna melanoma is moderate, and intraobserver concordance is moderate to good. A control strip biopsy specimen may improve concordance in some cases.

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Lentigo Maligna (LM) is a malignant melanoma in situ and represents the precursor lesion of LM melanoma (LMM), an invasive form of malignant melanoma. These lesions occur predominantly on the sun-exposed skin of the head and neck in elderly patients and have an unpredictable clinical course—lesions may remain indolent for years before becoming invasive. Once invasion occurs, LMM has a prognosis similar to other subtypes of malignant melanoma.

The lifetime risk of developing invasive melanoma in LM is unknown because of a lack of longitudinal studies, but the most comprehensive study to date of more than 16,000 white subjects estimated the lifetime risk to be 2% to 5%. Others have suggested that the risk may be much higher, a third of patients or more. Once a lesion is brought to the attention of a physician and a biopsy is performed to confirm the diagnosis of LM, surgical removal is the standard of care. The currently recommended surgical margin for LM is 0.5 cm of clinically normal skin surrounding the lesion. For invasive tumors the surgical margin is 1 to 2 cm of clinically normal-appearing skin. However, complete extirpation of LM and LMM lesions presents difficulties because clinical examination, even with the use of a Wood lamp, often fails to identify the boundary of the lesion owing to subclinical extension of atypical junctional melanocytic hyperplasia. For this reason, some institutions such as the University of Utah Health Sciences Center, Salt Lake City, use a margin-controlled excision to ensure complete removal. This can be accomplished by Mohs micrographic surgery aided by rush permanent sections, first described in 1990 by Dhawan.
Sciences Center, a control biopsy specimen is some-

Assisted histopathologists. At the University of Utah Health

induced melanocytic hyperplasia can be a vexing problem

cytes indicates the presence of LM or simply solar-
mor atypical. At the University of Utah Health

mine whether a finding of increased atypical melano-
cytes disposed

to epidermis, and some of these melanocytes may be cyto-

can be useful (anecdotally) in the interpretation of LM and

LMM staged excision margins. However, we are un-

aware of any published reports that confirm or refute the

utility of a control biopsy specimen in this situation. Ob-

taining a control sample is not universally applied be-

cause patients may refuse to have additional specimens

taken from their face or neck when a lesion does not ex-

ist at that site.

Although there are published criteria for the diag-

nosis of LM and LMM, the criteria are not uniformly

applied among pathologists. (The reasons for this are

myriad, but it must be realized that ultimately, histopa-

thology is a subjective art.) Moreover, dermatopatholo-

ists interpreting melanocytic lesions are strongly influ-

enced by their mentors in how diagnoses are rendered.

In addition, the importance of experience in diagnosis

cannot be overemphasized. Consequently, the level of in-

terobserver concordance among pathologists in the di-

agnosis of melanoma and other pigmented lesions is not

very high.21,22 We have noticed from observation of sev-

eral dermatopathologists that each has a different thresh-

old for diagnosis of a positive margin, though all pur-

pose to use the same histopathologic criteria. These

differences could significantly affect patient care. Herein

we describe a study designed to establish interobserver

and intraobserver concordance for identifying positive

and negative margins among 5 pathologists and to test

the following hypothesis: A control specimen is useful

in interpreting difficult peripheral margins because by

using such a specimen, the physician can determine the

patient’s background level of junctional melanocytosis.

METHODS

The present study met all requirements of and was approved

by the University of Utah Health Sciences Center institutional

review board (IRB 8769-01; approved April 6, 2001). It is stan-

dard practice at the University of Utah Health Sciences Center

to identify the clinical margin of the lesion visually and also

with the aid of a Wood lamp. A surgical margin of 5 mm is out-

lined around the lesion in a straight-sided polygon. A strip of

skin up to 5 mm wide is then excised from each side (Figure 1),

oriented with different-colored inks applied to the surgical mar-

gin, then fixed in formalin and processed through paraffin

(Figure 2). Vertical perimeter sections of tissue are cut from

each of the peripheral margins, applied to glass microscopic

slides, and stained with hematoxylin-eosin (H&E) stain. The

center portion, containing the lesion, is serially sectioned or

“breadloafed” (to rule out invasion that could change the width

of the margin excision) and prepared similarly; the bread-

loafed center sections generally contain residual LM and/or LMM

and serve as a positive control (Figure 2). Sometimes a control

biopsy specimen in the form of an excisional strip or punch

(trephine) is taken, generally ipsilaterally but sometimes con-

tralaterally to the site of the LM and/or LMM, to represent the

negative control (Figure 1 and Figure 3). A pathologist then
reviews the slides in the context of the positive control and negative control (if available), and positive margin findings are communicated to the surgeon to perform additional excision, if necessary (Figure 4 and Figure 5).

CASE SELECTION

Cases of LM or LMM were identified from the dermatopathology archive at the University of Utah Health Sciences Center by computerized search (Figure 6). A total of 301 slides from 14 cases with a control slide and 13 cases without a control slide were retrieved; there were 159 slides from the group in which a control biopsy was performed (14 positive controls [breadloafed centers of lesions]; 14 negative controls from similarly sun-damaged skin; and 131 peripheral margins) and 142 slides from the group without a control available (12 positive controls and 130 peripheral margins). All of the H&E-stained slides were independently evaluated by 5 pathologists (S.R.F., F.A., J.C.M., G.M., and A.F.H.) without access to clinical or previous pathologic information. At the time the slides were evaluated for this study, 2 of the 5 pathologists were board certified in dermatopathology, and 2 were completing dermatopathology training. The fifth evaluator was a pathologist with an interest in skin specimens.

EXPERIMENTAL DESIGN

The study was conducted in 3 phases: In phase 1, all 301 slides were randomized, and the pathologists were asked to render a diagnosis of positive or negative, with each pathologist using his or her own criteria for diagnosis of LM and/or LMM. In phase 2, every third slide was evaluated again (100 slides) by the pathologists and diagnosed as positive or negative. In phase 3, the slides were organized into cases by accession number, but the peripheral margins were presented in random order to decrease sequence bias. Each peripheral margin was evaluated in the context of the positive control (tumor from the center of the lesion) and negative control (control biopsy specimen), if available, and diagnosed as positive or negative.

Just prior to the start of phase 3, participating pathologists met and discussed criteria for the diagnosis of a positive or negative margin according to criteria published by Weyers et al., the most useful of which, favoring a diagnosis of melanoma in situ, included presence of melanocytic nests, nonuniform pigmentation or distribution of melanocytes, presence of melanocytes far down adnexal structures, presence of melanocytes above the dermoepidermal junction, and melanocytic atypia. Examples of positive and negative margins were reviewed at a multiheaded teaching microscope.

After completion of all 3 phases by the 5 pathologists, their diagnoses were compared with the original diagnosis rendered at the time of excision. If the original diagnosis on the pathology report was atypical melanocytic hyperplasia and additional tissue was removed from that location, the margin was considered positive. Likewise, if the diagnosis was atypical melanocytic hyperplasia but no additional tissue was excised, the margin was considered negative. The 5 pathologists performed a total of 3310 independent evaluations of the 301 slides.

A random sampling of 86 peripheral margins were evaluated by 1 of the pathologists (S.R.F.) to determine melanocyte density. The length of each margin was measured visually to the nearest millimeter. Melanocytes were identified by mor-
Demographic information is summarized in Table 1. Sixteen (59%) of 27 patients were referred from community dermatologists for staged excision. Follow-up information for these patients is not available because the institutional review board did not give permission to contact physician’s offices or patients. Follow-up information for 9 of the patients showed no evidence of tumor recurrence after 1 to 42 months of observation (average, 16 months).

Twenty-seven patients underwent staged excision for LM, 14 of whom also underwent a control biopsy. The number of stages required to completely remove the tumor was not significantly different between the 2 groups (2.4 stages for those excisions with a control slide vs 2.8 stages for excisions without a control slide available; \( P = .34 \)). For the 14 patients who underwent a control biopsy, 6 of the control specimens were taken contralaterally to the site of staged excision, 4 were ipsilateral, and 3 were taken from the same anatomic area within 2 cm of the excision site (chest, scalp, or back). One LM tumor was excised from the nasal tip; the control specimen for this excision was done at the left preauricular area (Table 1).

Five pathologists each reviewed 261 margins and agreed with the original diagnosis on 942 (72%) of those 1305 evaluations. A total of 166 (64%) of the 261 margins were considered straightforward, and 95 (34%) were considered difficult. Overall agreement for positive control and negative control slides was 89% and 99%, respectively. Phase 1 agreement was moderate (\( \kappa \) range, 0.4-0.5; Figure 7). Phase 2 (intraobserver) agreement was moderate to good for all pathologists (\( \kappa \) range, 0.6-0.9; Figure 8). Phase 3 agreement was moderate (\( \kappa \) range, 0.4-0.5; Figure 9). Subset analysis revealed a statistically significant advantage for having a control strip biopsy specimen for difficult slides (\( P < .001 \); agreement was 46% without control, 51% with a punch biopsy specimen control, and 76% with strip biopsy specimen control [Figure 10]; \( P = .27 \) for punch biopsy vs no control). There was a statistical trend toward improved agreement between pathologists favoring ipsilateral control specimens (\( P = .07 \)), but there were no significant differences in agreement among head-and-neck vs trunk lesions or number of stages required to clear the tumor.

In a random sampling of peripheral margins evaluated for melanocyte density, 27 were difficult, and 59 were straightforward. A significant difference in the average number of melanocytes at the dermoepidermal junction across the breadth of the margin was not identified (mean±SD number of melanocytes per millimeter was 12±6 for difficult slides and 13±11 for straightforward slides). Similarly, no significant difference was identified in the average of the three 1-mm regions of highest melanocyte density or the number of enlarged hyperchromatic melanocytes (Table 2). Difficult margins were statistically more likely than straightforward margins to be diagnosed as positive (\( P < .001 \); Table 2). In addition, difficult margins often had an additional diagnostic abnormality, most often epidermal spongiosis (\( P < .001 \); Table 3).

Eight of the 14 control specimens were taken with a skin punch (trephine) having a mean±SD breadth of 2.4±0.8 mm, and 6 control specimens were in the form of an excisional strip having a mean±SD breadth of 8.1±4.0 mm (\( P = .003 \)). The average number of melanocytes per millimeter was similar in the punch specimen and strip control specimens. Other diagnostic abnormalities were present in 50% of the punch specimen controls and 67% of the strip specimen controls (Table 4).

Table 5 lists the total number of peripheral margins and type of peripheral margin by type of control specimen. The numbers of difficult peripheral margins with the same “other” diagnostic abnormality as the control specimen...
were 16 (44%) of 36 and 6 (60%) of 10 for punch controls and strip controls, respectively. Figure 11 shows examples of straightforward and difficult peripheral margins.

COMMENT

Identifying positive and negative peripheral margins in staged excisions for LM and/or LMM can be problematic. The pathologists in the present study demonstrated that they could reliably identify the positive controls (sections through the residual tumor) and negative controls (control specimens) with a high degree of concordance when confronted with them in random order. These, of course, represent the extreme ends of a continuum. It is the central region of this continuum that is the most troublesome for histopathologists and is fertile ground for uninformative diagnoses such as atypical melanocytosis or atypical melanocytic hyperplasia. Sometimes pathology reports with these latter diagnoses are appended with statements such as “recommend revision of this margin” or “follow-up observations should suffice”—comments that convey to the surgeon that the margin is either positive or negative, thus directing additional treatment, if necessary.

Overall, the lack of substantial interobserver concordance among several pathologists in our evaluation of LM and LMM is in keeping with other studies of melanocytic lesions reported in the literature. Farmer and associates22 distributed specimens from 37 melanocytic lesions to a panel of 8 expert diagnosticians. With 3 diagnostic categories (benign, indeterminate, and malignant), the panel reported a combined $\kappa$ value of 0.5, indicating moderate agreement. Similarly, Corona et al reported the findings of 4 experienced dermatopathologists reviewing 140 slides of cutaneous melanoma and...
benign pigmented melanocytic lesions. The κ value for malignant melanoma vs other melanocytic lesions was found to be 0.61, but when individual diagnostic categories were considered, κ values ranged from moderate agreement (0.59) for pigmented spindle cell nevus and Spitz nevus to poor for dysplastic nevus (0.17). In a study reported by Duncan et al,28 5 dermatopathologists reviewed 60 melanocytic lesions with an overall concordance for diagnosing dysplastic nevi of 77% and a κ value ranging from 0.55 to 0.84. Not surprisingly, a recent study of interrater reliability of the diagnosis of malignant melanoma in childhood was poor, even when the slides were evaluated by pairs of experts.29 Other groups have reported better diagnostic concordance with respect to melanocytic lesions.30,31

The interobserver concordance reported in the present study is maximized because only 2 diagnostic “bins” were used: positive or negative for LM and/or LMM. This type of reporting mimics clinical practice for extirpation of these lesions by a staged excision because the surgeon has essentially 2 treatment options: (1) resect more skin tissue from a problematic area or (2) conduct

follow-up observations of the site. It is interesting to note that the pathologists’ overall concordance herein did not improve substantially from the first phase of the study, in which slides were reviewed in random order, to the third phase of the study when the slides were organized into cases (similar to how they would be reviewed in a typical dermatopathology practice) even after histopathologic criteria were reviewed and examples were shown. These data underscore the need for developing improved methods for identifying in situ melanoma cells.

**CONCLUSIONS**

The present study revealed 2 types of peripheral margins: (1) those that were straightforward, as identified
by 4 or 5 of 5 pathologists agreeing on the original diagnosis, and (2) those that were difficult, about which none, or only 1, 2, or 3 of the 5 pathologists agreed with the original diagnosis. The fact that some margins were straightforward and some were difficult was not surprising because this has been our experience when interpreting these cases—straightforward margins show either no evidence of a melanocytic lesion or there is obvious margin involvement diagnostic of residual LM and/or LMM. The remainder of the peripheral margins occupy the difficult gray area between the 2 extremes.

The data reveal a marked difference in the original diagnosis between the straightforward and difficult groups. The difficult peripheral margins were diagnosed as positive 77% of the time, while only 28% of the straightforward margins were diagnosed as positive, indicating that when there is uncertainty about whether there is margin involvement, that margin is more likely to be diagnosed as positive or given a diagnosis that is less specific but with a recommendation for revision of that site. We were surprised to discover that in a random sampling of peripheral margins, a significant difference in basal melanocytes was not identified in difficult cases vs straightforward ones. It is possible that a difference exists between straightforward and difficult peripheral margins in the number of melanocytes positioned at the

### Table 2. Characteristics of Difficult and Straightforward Margins

<table>
<thead>
<tr>
<th>Type of Peripheral Margin (Phase 1)</th>
<th>No. of Peripheral Margins</th>
<th>Original Diagnosis Positive No. (%)</th>
<th>Original Diagnosis Negative, No. (%)</th>
<th>No. of Peripheral Margins Sampled</th>
<th>Mean ± SD of Three 1-mm Areas of Highest Melanocyte Concentration</th>
<th>Overall Mean ± SD No. of Melanocytes per Millimeter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Difficult (0, 1, 2, or 3 pathologists agree)</td>
<td>95</td>
<td>73 (77)</td>
<td>22 (23)</td>
<td>27</td>
<td>25 ± 13</td>
<td>12 ± 6</td>
</tr>
<tr>
<td>Straightforward (4 or 5 pathologists agree)</td>
<td>166</td>
<td>46 (28)</td>
<td>120 (72)</td>
<td>59</td>
<td>23 ± 17</td>
<td>13 ± 11</td>
</tr>
</tbody>
</table>

*Difficult slides were more likely to be diagnosed as positive than were straightforward peripheral margins (P < .001).
†The number of melanocytes at the dermoepidermal junction was not statistically different between the difficult and straightforward margins (P > .55 for both).

### Table 3. Additional Diagnostic Abnormalities in Specimens With Difficult and Straightforward Margins*

<table>
<thead>
<tr>
<th>Type of Peripheral Margin (Phase 1)</th>
<th>No. of Peripheral Margins</th>
<th>Total With Other Diagnosis‡</th>
<th>Multiple Diagnoses</th>
<th>Spongiosis</th>
<th>Actinic Keratosis</th>
<th>Lentigo</th>
<th>Tangential</th>
<th>Histology</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Difficult (0, 1, 2, or 3 pathologists agree)</td>
<td>95</td>
<td>79 (83)</td>
<td>54/79 (68)</td>
<td>44/79 (56)</td>
<td>30/79 (38)</td>
<td>10/79 (13)</td>
<td>10/79 (13)</td>
<td>3/79 (4)</td>
<td></td>
</tr>
</tbody>
</table>

*Data in parentheses are percentages.
†P < .001.

### Table 4. Additional Diagnostic Abnormalities in Control Specimens*

<table>
<thead>
<tr>
<th>Type of Control</th>
<th>No.</th>
<th>Mean ± SD Breadth of Control Specimen, mm‡</th>
<th>Mean ± SD No. of Melanocytes per Millimeter</th>
<th>Total With Other Diagnosis</th>
<th>Multiple Diagnoses</th>
<th>Spongiosis</th>
<th>Actinic Keratosis</th>
<th>Lentigo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Punch</td>
<td>8</td>
<td>2.4 ± 0.8</td>
<td>8 ± 6</td>
<td>4 (50)</td>
<td>2 (50)</td>
<td>2 (50)</td>
<td>4 (100)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Strip</td>
<td>6</td>
<td>8.1 ± 4.0</td>
<td>10 ± 3</td>
<td>4 (67)</td>
<td>1 (25)</td>
<td>2 (50)</td>
<td>1 (25)</td>
<td>1 (25)</td>
</tr>
</tbody>
</table>

*Data in parentheses are percentages.
†P = .003.

### Table 5. Margin Characteristics in Control Specimens*

<table>
<thead>
<tr>
<th>Type of Control</th>
<th>No. of Peripheral Margins</th>
<th>No. of Difficult Margins</th>
<th>No. (%) of Difficult Margins With Same Diagnosis as Control</th>
<th>No. (%) of Straightforward Margins With Same Diagnosis as Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Punch</td>
<td>87</td>
<td>36</td>
<td>51</td>
<td>16/36 (44)</td>
</tr>
<tr>
<td>Strip</td>
<td>44</td>
<td>10</td>
<td>34</td>
<td>6/10 (60)</td>
</tr>
</tbody>
</table>

*Other diagnoses from control biopsy specimens were compared with other diagnoses from peripheral margins of the same case.
Figure 11. Examples of straightforward (A and B) and difficult (C-H) peripheral margins (magnification ×200 unless otherwise noted). A, Straightforward example of residual lentigo maligna; B, a straightforward negative margin. Epidermal (C) and follicular (D) spongiosis with exocytosis of lymphocytes into the epidermis (C) and follicular epithelium (D); E, basilar keratinocyte atypia of the actinic keratosis type; F, mild increase in basilar melanocytes associated with solar lentigo-type change; G, tangential sectioning with the appearance of melanocytic hyperplasia (original magnification ×100); and H, banal aggregates of melanocytes at the tips of the rete that are usually interpreted as residual lentigo maligna.
dermoepidermal junction; this difference might be revealed with a larger sample size or immunohistochemical staining of sections with a marker of melanocytic differentiation.

Although we were unable to identify a significant difference between straightforward and difficult cases in basal melanocytosis, the difficult cases were statistically more likely to have an additional diagnostic abnormality, most frequently epidermal spongiosis. Epidermal spongiosis is often accompanied by exocytosis of lymphocytes, and these can sometimes be difficult to distinguish from melanocytes. The "typical" difficult slide has the following characteristics: (1) no definitive melanoma features (eg, no confluence of melanocytes at the dermoepidermal junction and no nests of melanocytes); (2) no increase in the number of basal melanocytes or only a small increase, insufficient to confidently make a diagnosis of melanoma; and (3) epidermal spongiosis or actinic keratosis change, often with an inflammatory component. Additionally, a technical abnormality may be present, such as tangential sectioning, incomplete epidermis, or poor staining quality. With this typical appearance, the slide is more likely to be diagnosed as positive or as atypical melanocytic hyperplasia (with recommended revision of the area) because the histopathologist is unable to confidently determine that the margin is negative.

The addition of a strip control specimen in difficult cases allows for increased agreement on a diagnosis for a particular margin, whether positive or negative. In the present study, availability of a punch biopsy control specimen failed to improve diagnostic concordance among pathologists and produced findings similar to having no control specimen available. This is an interesting finding and suggests that the additional information provided by the control strip may improve patient care because it provides a yardstick of normal skin that can be compared with and applied to the interpretation of a potentially abnormal area. The reason why a strip-type control specimen was helpful in interpreting difficult peripheral margins is unclear. We were unable to prove that control specimens were useful in determining the patient's baseline level of melanocytosis because the punch and strip control specimens showed similar basal melanocyte density. Likewise, not all of the strip biopsy specimens showed the same "other" diagnostic abnormality as seen in the peripheral margins from the same case. It is more likely that the reason a strip control specimen is helpful is because the diagnostican uses it to assimilate a myriad of subtle histologic features subconsciously rather than using a single objective histologic parameter. We are left with the finding that a strip-type control seems to improve diagnostic accuracy for difficult slides.

One problem with the present study is that a true gold standard diagnosis was not available. The diagnosis for each peripheral margin in this study was benchmarked against the original diagnosis because this dictated the treatment the patient received. The original diagnosis may not have been correct; likewise, the diagnosis agreed on by our multiple pathologists may not have been correct. For example, if all 5 pathologists agreed that the diagnosis for a particular margin was negative compared with a control specimen, and the patient developed a recurrence at that site, the pathologists most likely rendered an incorrect diagnosis (assuming that the diagnostic features were present on the sections examined). Another possibility is that the histologic features diagnostic of a positive margin might be insignificant clinically. Data addressing these issues are not currently available. Long-term follow-up of patients who have undergone a staged excision for LM and/or LMM is necessary to determine if this procedure, with or without having a control biopsy specimen available, definitively improves outcome.

In summary, we have investigated multiple aspects of observer concordance in evaluating peripheral margins in staged excisions for LM and/or LMM. Our results indicate the following: (1) Interobserver concordance was moderate, similar to that found in other studies of melanocytic lesions. (2) Intraobserver concordance was moderate to good. (3) Peripheral margins were either straightforward or difficult, mimicking clinical practice. (4) Difficult slides were much more likely to be diagnosed as positive. (5) Difficult and straightforward peripheral margins had similar numbers of melanocytes positioned at the dermoepidermal junction, but difficult margins were statistically more likely to have other diagnostic abnormalities, such as epidermal spongiosis or actinic keratosis change. Finally, (6) evaluation of a strip control biopsy specimen may be useful in evaluating difficult peripheral margins.

Based on the results of the present study, dermatologic surgeons at the University of Utah Health Sciences Center generally excise the first stage without obtaining a control biopsy specimen. The pathologist reviews the slides the following day, and if the peripheral margins are difficult to interpret, a recommendation is made to the surgeon that an ipsilateral control strip biopsy be performed within 2 cm of the primary excision site during the second stage.

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