Correlation of Inflammation in Frozen Sections With Site of Nonmelanoma Skin Cancer

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IMPORTANTCE During Mohs micrographic surgery of nonmelanoma skin cancer (NMSC), inflammation in histologic frozen sections has been found to occasionally presage the detection of tumors in frozen sections of adjacent excision specimens.

OBJECTIVE To quantify the correlation between the location of inflammation without visible tumor in histologic frozen sections and the location of subsequently detected NMSC.

DESIGN, SETTING, AND PARTICIPANTS A retrospective cohort study of 3148 cases pertaining to frozen sections associated with the staged excision of NMSC was performed from September 8, 2008, to September 18, 2009, at an urban academic medical center, with the collected data analyzed on May 9, 2013.

EXPOSURES Consecutive cases of Mohs micrographic surgery performed at an academic medical center.

MAIN OUTCOMES AND MEASURES For each wedge-shaped tissue segment corresponding with 1 hour of time on a clock face, the proportion of patients with inflammation at the source location of the segment who subsequently had a tumor at this same location; the proportion of patients who had neither inflammation nor subsequent tumor at the source location of the segment; the probability of subsequent tumor at this location given the prior finding of inflammation at the same location; and the probability that a location was without tumor in the absence of preexisting inflammation at that location.

RESULTS Of the medical records of 3148 cases of NMSC that were reviewed, 60 showed inflammation in histologic frozen sections from an excision specimen that was followed by tumor in the subsequent excision specimen. Of these 60, 39 (65%) were basal cell carcinoma and 21 (35%) were squamous cell carcinomas; 53 (88%) were Mohs stage 1. In 7 of 12 segments, a significant positive correlation was found between the presence of inflammation and the presence of nearby tumor with correlation coefficients ranging from 0.196 to 0.384 (P < .05). The probability that tumor was absent when inflammation was not seen at a particular location (ie, clock-face segment) in preceding sections from that location was 91%, with segment-specific probability values ranging from 82% to 96%.

CONCLUSIONS AND RELEVANCE During Mohs micrographic surgery of NMSC with the examination of frozen sections, histologic inflammation is modestly predictive of adjacent tumor whereas lack of inflammation strongly predicts that no additional tumor will be found.
The histologic interpretation of tumor in frozen sections during Mohs micrographic surgery may potentially be obscured by the presence of associated inflammation. Dense inflammation cannot only camouflage a tumor but can also be a harbringer of more tumor beyond the margin of a specimen. Prior work established this predictive utility of inflammation. Approximately one-fourth of a series of 22419 consecutive cases revealed sufficient inflammation and fibrosis to warrant harvesting of an additional Mohs stage, with 1.9% of the latter detecting skin cancer.

Although previous empirical work has shown that inflammation can presage the detection of more tumor, to our knowledge, the relationship between the microscopic location of the inflammation and the site of the additional tumor has not been elucidated. Adjacency between foci of inflammation and tumor, if confirmed, would be clinically significant in allowing a more circumscribed removal of peripheral tissue. Sparing tissue during a further Mohs stage would also translate into a smaller final wound defect and perhaps an improved preservation of structure and function. We sought to determine whether, when dense inflammation in frozen sections is followed by the detection of tumor in subsequent Mohs stages, the location of the inflammation provides information about the location of the tumor.

Methods

Study Design
A retrospective cohort study was conducted based on a review of glass slides and medical records from 3148 cases of Mohs surgery. All patient information was de-identified, and data were analyzed on May 9, 2013. The Northwestern University Institutional Review Board declared the study to be exempt from review.

Case Selection
Consecutive cases of Mohs micrographic surgery performed by 2 surgeons (M.A. and S.S.Y.) in the Department of Dermatology at Northwestern University from September 8, 2008, to September 18, 2009, were independently manually reviewed by 2 investigators, a board-certified dermatologist (M.K.) and a pathology assistant (A.F.). Mohs maps and glass slides were assessed to detect cases in which a stage revealed inflammation but no tumor was followed by a stage in which tumor was detected. Extraction of relevant cases required the agreement of both of the screening investigators.

For each case, the following information was recorded: the histologic type of skin cancer (eg, basal cell carcinoma, squamous cell carcinoma, or other), the anatomic location of the tumor, the diameter of the Mohs stage showing inflammation only, and the number of the relevant Mohs stage.

Grading of Inflammation
The general classification scheme for the findings in a frozen section was no inflammation, mild inflammation, moderate inflammation, and severe or dense inflammation, with the final classification category defined as the one requiring further consideration, even in the absence of adjacent visible tumor. As previously noted in work that included relevant illustrations, dense lymphocytic inflammation can obscure tumor in Mohs sections, and it is this inflammation that we sought to identify when it was present. The 2 surgeons whose Mohs slides were reviewed worked in the same office and had previously calibrated their customary practice so that they used a similar threshold for dense inflammation as the criterion for removing an additional Mohs stage.

Study Procedures
Glass slides of each stage in which only dense inflammation was seen were reviewed by 2 investigators (M.K. and A.F.), who recorded the location of such inflammation. Slides from the subsequent stages in which tumor was seen were similarly reviewed, and the location of the tumor was marked in the same manner as used for marking inflammation. For both sets of measurements, a clock-face configuration was used as a reference, with 1 o'clock specifying the wedge-shaped tissue segment from 12 to 1 on the clock face, 2 o'clock the segment from 1 to 2 on the clock face, and so forth. Forced agreement was used to resolve any interrater disagreements. A third investigator (M.A.) reviewed the resulting maps and compared them against the glass slides for verification.

Statistical Analysis
For each clock-face tissue segment, the following measures were computed: (1) the proportion of cases with inflammation at this location that subsequently had tumor at the same location; (2) the proportion of cases that had neither inflammation nor subsequent tumor at the same location; (3) the probability of subsequent tumor at this location given that inflammation was previously seen at the same location; and (4) the probability in which, in the absence of preexisting inflammation, subsequent tumor was likewise absent. Pearson correlation coefficients were also derived for the presence of inflammation and tumor at each hour of the clock face and for all of the hours of the clock face collectively.

Results
Of 3148 cases that were reviewed, 60 met the criteria for inclusion in the study, and their characteristics are displayed in Table 1. Most were basal cell carcinomas, present on the head or neck, in which inflammation was found only during the first Mohs stage, which had stage sizes typically less than 1 cm in diameter. There were no interrater disagreements in case selection or mapping.

Information about the colocalization of dense inflammation and cancer is presented in Table 2. Notably, although the presence of inflammation at a particular location (ie, clock-face segment) was not a strong predictor of adjacent tumor, the absence of inflammation at a particular location was a fairly strong predictor of the absence of tumor at this site. Specifically, the overall probability of tumor at a particular location given the absence of inflammation at this location was 91%, with segment-specific values ranging from 82% to 96%. By con-
tract, the probability of underlying tumor in a specific tissue segment given the presence of prior inflammation in that same segment was only 32%, ranging from 19% to 41% for each segment. (Because it was a criterion for inclusion, 100% of the assessed cases mentioned tumor after inflammation in at least 1 segment.)

A significant positive correlation between the presence of inflammation and the presence of tumor was found in 7 of 12 segments (Pearson correlations, *P* < .05), with correlation coefficients ranging from 0.196 to 0.384. When all segments of the clock face were considered collectively, there was a significant positive correlation between inflammation and the presence of tumor, with a correlation coefficient of 0.266 (Pearson correlation, *P* < .001).

Dividing the clock face into quadrants rather than hours led to similar results. Assuming 4 quadrants such that the first was from 1 o’clock to 3 o’clock and the last was from 10 o’clock to 12 o’clock, the likelihood of subsequent tumor in a given quadrant in which inflammation was previously seen ranged from 46.8% to 65.2% (mean, 57.3%). The likelihood of tumor being absent in a quadrant in which no inflammation was seen in the preceding stage ranged from 85.7% to 100% (mean, 94.7%).

**Discussion**

The data in this study indicate that when inflammation alone in a frozen section precedes the detection of nonmelanoma skin cancer on reexcision of tissue from the site from which the section was derived, the precise location of the inflammation is correlated with the location of the tumor. Although inflammation is only a weak predictor of adjacent tumor, the absence of inflammation at a particular microscopic location is a strong indicator of the absence of tumor at that location. If location is defined more broadly by specimen quadrant rather than clock-face hour, the associations between inflammation and tumor appear even stronger.

These findings have clinical relevance. When dense inflammation is detected in a Mohs section and additional tissue removal is required, avoiding the removal of tissue from margins of the section that are devoid of inflammation may...
sparing tissue without significantly increasing the probability of tumor being missed. Minimization of tissue resection in response to inflammation may result in a smaller scar and less sensory or functional impairment.

Katz and colleagues found that the issue of Mohs sections with dense lymphocytic inflammation in the absence of visible tumor could be resolved by sectioning more deeply into the excised tissue block rather than taking another layer of tissue. They found that in all cases in which occult tumor was present at the margins of a section, it was revealed by deeper sectioning. Accordingly, they recommended their technique as sufficient for disclosing tumor at a particular location and as more tissue-sparing than the excision of another layer. Although additional Mohs stages were excised in the context of dense inflammation in the current study, the method proposed by Katz and colleagues is an alternative (and potentially more tissue-sparing) initial approach to Mohs sectioning. If deeper sections did not reveal tumor and there remained a high suspicion that inflammation was masking tumor, additional stages could be taken as a precautionary second step.

Limitations of this study include its basis on data from a single center. On the other hand, the cases on which the study was based were treated by 2 Mohs surgeons (M.A. and S.S.Y.) who had been trained at different centers. In addition, the proportion of cases found to have dense inflammation preceding tumor was 2% (60 of 3148 cases), which was very similar to the 1.9% of cases in which Macdonald et al reported in the detection of additional tumor when inflammation prompted another Mohs stage excision. Notably, this 2% statistic represented only the 60 medical records among the 3148 reviewed in the present study in which dense inflammation without tumor in one stage was followed by visible tumor in the next stage. The study reviewed only cases of inflammation that were followed by tumor because the remaining cases of inflammation were less severe and hence not deemed comparable.

In the practice in which this study was conducted and in many Mohs surgical practices, the Mohs laboratory did not routinely conduct immunohistochemical staining with cytokeratins for cutaneous squamous cell carcinoma or cutaneous squamous cell carcinoma potentially masked by inflammation. However, the routine availability in the Mohs laboratory of immunostaining, as with cytokeratins, may improve the ability to more promptly exclude dense, masking inflammation and detect underlying tumor.

The results of this study appear to be most relevant to initial stages of Mohs surgery designed to remove small tumors in cosmetically and functionally sensitive locations such as the head and neck, genitalia, and toes. The deliberate effort to minimize (to the extent possible) the size of tissue sections excised in such initial stages may create a risk for failing to identify inflammation that requires an additional Mohs stage. However, the findings in our study indicate that, to reduce defect size and improve outcomes, excision of incremental stages can be guided by the localization of preexisting inflammation.

The measurement of specimens in this study was performed on glass slides after tissue fixation and processing. The diameter of lesions in vivo would be expected to be greater than that of the specimens examined on the slides. The shrinkage of skin samples ex vivo after their excision has been reported to range from 16% to 18%, suggesting that the tumors studied were correspondingly larger in vivo.

**Conclusions**

When dense inflammation alone precedes the detection of tumor in frozen sections, the location of the inflammation is modestly related to that of the subsequent tumor. Peripheral tumor is unlikely to be found where inflammation is absent.

**REFERENCES**