

# Reliability of Skin Biopsies in Determining Accurate Tumor Margins: A Retrospective Study After Mohs Micrographic Surgery

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**BACKGROUND** Skin biopsy reports of basal cell carcinoma and squamous cell carcinoma are often accompanied by comments on the margins. A physician's management can be influenced by such reports, particularly when the margins are reported as clear and no further interventions are pursued.

**OBJECTIVE** To retrospectively review pathology margins on Mohs micrographic surgery (MMS) cases performed at a University Center and to compare biopsy margins with the Mohs margins found on the first stage.

**MATERIALS AND METHODS** Data collection of 1,000 cases of Mohs surgery was obtained regarding margins on skin biopsy and compared with margins on the first stage of MMS.

**RESULTS** Overall, of the biopsies that showed only deep margin involvement, a lateral margin was seen on 32% of the first stages of MMS. Conversely, of the biopsies that showed only lateral margin involvement, a deep margin was seen on 14% of the first stages of MMS. Of the biopsies that showed clear margins, a margin was seen in 30% of the cases on the first stage of MMS.

**CONCLUSION** Skin biopsies processed through the "bread-loafing" technique are not reliable in detecting accurate margins, and therefore, a biopsy report should not include margin involvement within it.

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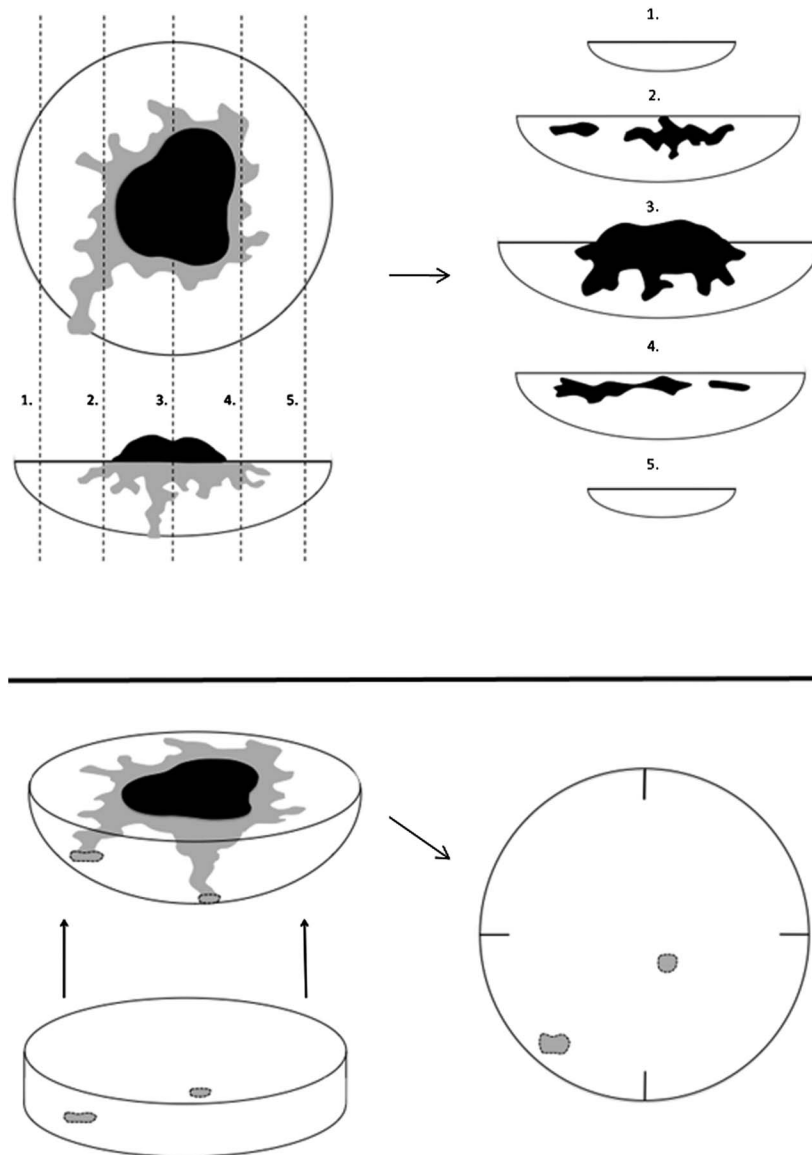
Treatment of basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) is usually preceded by a diagnostic biopsy. The reporting of positive or negative margins in these biopsies by dermatopathologists is something that might leave the treating physician in a predicament in terms of whether or not further treatment is required. A biopsy that is read out as having negative margins may give the false appearance of complete excision to both the patient and the physician. This, in turn, may result in confusion and ambiguity when it comes to a treatment decision.

Mohs micrographic surgery (MMS) is considered to be the gold standard for treatment of BCC and SCC

in view that it examines 100% of the specimen margins.<sup>1</sup> It is not uncommon for the Mohs surgeon to notice different margins on the first stage of surgery than those stated in the biopsy pathology report. An example of the processing of the biopsy specimen in comparison with the processing of the Mohs specimen is illustrated in Figure 1.

In fact, the bread-loafing technique used by pathology laboratories to report biopsy results is only 44% sensitive in accurately determining the involved margin.<sup>2</sup> We sought to determine the frequency in which biopsy-reported margins were contradictory to margins reported from Mohs and to make a preliminary

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**Figure 1.** An illustration on how the standard bread-loafing technique, used to process biopsy specimens, can result in a missed positive margin that will be detected by Mohs surgery (occurring between 7 and 8 o'clock). Numbers 1-5 correspond to 5 different sections taken while using this technique. The positive margin between sections 1 and 2 would be missed here while the margin would be considered positive when evaluated using the en face technique in the bottom of this figure.

conclusion on whether pathology reported biopsy margins should guide further treatment.

### Materials and Methods

The study was approved by the local institution review board committee. We reviewed the last 1,000 cases performed through MMS at the Dermatology Out-patient Clinic at Wayne State University. Pathology reports and Mohs maps were pulled, and data were collected. Inclusion criteria were cases of BCC and SCC tumors where data on age, sex, biopsy margin,

patient date of biopsy, gross measurement of the biopsy, tumor type, tumor margin on biopsy, pathology laboratory, the dermatopathologist's signed report, and Mohs maps for interpretation of margins were available. Exclusion criteria included cases where the above-mentioned criteria were unobtainable. Mohs surgery was performed based on common indications for the procedure such as tumor location, tumor size, histologic features, recurrent tumors, and tumor borders based on the pathology report. Mohs surgery was performed with a 1- to 2-mm margin around the biopsy site that was

confirmed by clinical photographs. Specimens were processed by the “en face” method, and slides and Mohs maps were available. Assessment was performed according to the last section of the specimen or the deepest one into the tissue either through the Mohs map or by examining the slide. The finding of tumor of the same kind as mentioned on the pathology report on the deep or lateral side of the tissue on the first stage of MMS was considered to be positive. The finding of a superficial BCC on the Mohs stage of an initial BCC tumor of any subtypes was considered to be the continuation of the tumor and was considered positive. Similarly, the finding of SCC in situ, but not actinic keratosis, on the Mohs stage of an initial SCC tumor of any subtype was considered to be the continuation of the tumor and was considered positive.

Information gathered from the excel sheet was further stratified based on tumor type and margin. Three main areas of focus were analyzed as follows: biopsies that indicated deep margin involvement and Mohs proving further lateral margin involvement and/or deep margin, biopsies that indicated lateral margin involvement and Mohs showing deep margin involvement and/or lateral margin, and biopsies that reported clear margins and then found to have a positive deep or lateral margin with Mohs. Based on the results of the analysis, the probability of finding tumor involvement in a margin cleared by pathological report was calculated and reported.

Data were tabulated initially using Microsoft Excel 2010 (Microsoft, Redmond, WA) and was then extracted and analyzed by SPSS, version 20.0 (IBM, Armonk, NY). Analysis involved mostly the margins on the biopsy compared with the margin on the first stage of MMS. Categorical variables were presented as number and percent, whereas continuous ones were summarized using the mean and the standard deviation. Associations were assessed using McNemar test. A *p*-value of <.05 was considered to indicate statistical significance.

## Results

Of the 1,000 cases, only 916 fit the inclusion criteria. Eighty-four cases were excluded as follows. In 64 cases, there were no comments on the margins in the

initial biopsy report. In 9 cases, a frozen biopsy was performed from the debulk tissue of the Mohs stage, and those were excluded. In the remaining 11 cases, the tumor involved was neither a BCC nor an SCC. In all, there was a total of 619 BCC and 297 SCC.

Comments on margins of the pathology report were found to fall into 1 of the 5 categories: (1) no comment on margins, (2) deep and lateral margins involved, (3) deep margin involved, (4) lateral margin involved, or (5) margins clear. Cases that fell into the first category were excluded as their results did not help with the final assessment on the first stage of MMS. Cases that fell into the second category were also excluded as both margins were found to be involved on the biopsy report. In other words, the finding of a deep margin, a lateral margin, or both margins on the Mohs stage would have been accounted for and would not have contradicted the findings of initial biopsy report. A total of 423 cases were present in these 2 categories and were excluded accordingly.

A total of 26 pathology laboratories and 66 dermatopathologists reported on the included biopsy results. Pathologists were either board certified in pathology (*n* = 14) or in dermatopathology (*n* = 52). There were a total of 4 Mohs surgeons who performed the surgeries. All Mohs surgeons were certified by the American College of Mohs Surgery.

Our population included 63% males and 37% females with an average age of  $72 \pm 14$  years (range, 17–100 years). There was a median lapse of time between biopsy and surgery date of 46 days. Results were tabulated in Tables 1, 2, and 3.

### **BCC Results**

The number of biopsies reported with clear margins was 3 (Table 1). Of these 3, 1 had lateral margin involvement (33%) on the first stage of MMS. In view of the small numbers, statistical calculations were not performed.

The number of biopsies reported with deep margins was 229. Of these, 35 had deep and lateral involvement, 42 had lateral margin, and 34 had deep margin involvement on the first stage of MMS. In 77 of 229

**TABLE 1. Summary of BCC Margins as Reported by the Biopsy Report Compared With the One Reported by the First Stage of MMS**

BCC Cases	Margin on Mohs				Total
	Clear, n (%)	Deep, n (%)	Lateral, n (%)	Deep and Lateral, n (%)	
Margins on biopsy					
Clear	2 (66.7)	0 (0)	1 (33.3)	0 (0)	3
Deep	118 (51.5)	34 (14.8)	42 (18.3)	35 (15.3)	229
Lateral	27 (45)	6 (10)	21 (35)	6 (10)	60
Total	247	40	64	41	292

cases (34%) there was lateral margin involvement on Mohs surgery that was not detected by biopsy.

The number of biopsies reported with lateral margins was 60. Of these 60, 6 had deep and lateral margin, 21 had lateral margin, and 6 had deep margin involvement on the first stage of MMS. In 12 of the 60 cases (20%), there was deep margin involvement on Mohs surgery that was not detected by biopsy.

Statistical calculations using a McNemar–Bowker test revealed a statistically significant *p*-value of <.001.

### SCC Results

The number of SCC biopsies reported with clear margins was 7 (Table 2). Of these, 1 had lateral margin involvement on the first stage of MMS and 1 had deep and lateral involvement. Two of the 7 cases (29%) had lateral or deep margin tumor involvement that was missed by traditional biopsy. Statistical calculations were not performed again for this small sample.

The number of SCC biopsies reported with deep margins was 145. Of these, 11 had deep and lateral margin involvement, 30 had lateral margin involvement, and 7 had deep margin involvement on the first stage of MMS. In 41 of the 145 cases (28%), there was lateral margin involvement on Mohs surgery that was not detected by biopsy.

The number of biopsies reported with lateral margins was 49. Of these 1 had deep and lateral margin involvement, 14 had lateral margin involvement, and 2 had deep margin involvement on the first stage of MMS. In 3 of the 49 (6%), there was deep margin involvement on Mohs surgery that was not detected by biopsy.

Statistical calculations using a McNemar–Bowker test revealed a statistically significant *p*-value of <.001.

### BCC and SCC Combined Results

The number of biopsies reported with clear margins was 10 (Table 3). Of these, 7 were SCCs and 3 were

**TABLE 2. Summary of SCC Margins as Reported by the Biopsy Report Compared With the One Reported by the First Stage of MMS**

SCC Cases	Margin on Mohs				Total
	Clear, n (%)	Deep, n (%)	Lateral, n (%)	Deep and Lateral, n (%)	
Margins on biopsy					
Clear	5 (71.4)	0 (0)	1 (14.3)	1 (14.3)	7
Deep	97 (66.9)	7 (4.8)	30 (20.7)	11 (7.6)	145
Lateral	32 (65.3)	2 (4.1)	14 (28.6)	1 (2)	49
Total	134	9	45	13	201

**TABLE 3. Summary of Combined BCC and SCC Margins as Reported by the Biopsy Report Compared With the One Reported by the First Stage of MMS**

Combined Cases BCC/SCC	Margin on Mohs				Total
	Clear, n (%)	Deep, n (%)	Lateral, n (%)	Deep and Lateral, n (%)	
Margins on biopsy					
Clear	7 (70)	0 (0)	2 (20)	1 (10)	10
Deep	215 (57.5)	41 (11)	72 (19.3)	46 (12.3)	374
Lateral	59 (54.1)	8 (7.3)	35 (32.1)	7 (6.4)	109
Total	281	49	109	53	493

BCCs. After clear margins were found through biopsy, there was 1 Mohs section that was found to have deep and lateral margin involvement, and 2 sections found to have only lateral margin involved by tumor in the first stage of MMS. There were 3 of the 10 cases (30%) reported to have clear margins that subsequently were found to have a margin involved on Mohs surgery. With the low number of patients, statistical calculations were not performed.

The number of biopsies reported with deep margin involvement was 374. Of these, 46 had deep and lateral margin involvement, 72 had lateral margin involvement, and 41 had deep margin involvement on the first stage of MMS. The remaining 215 were clear on the first stage of MMS. In this selection, there were 118 of the 374 cases (32%) where lateral margin involvement was seen with Mohs surgery but was not detected by biopsy (Figure 2).

The number of biopsies reported with lateral margins was 109. Of these, 7 cases had deep and lateral margin involvement, 35 cases had lateral margin involvement, and 8 had deep margin involvement on the first stage of MMS (Figure 3). In 15 of the 109 cases (14%), there was deep margin involvement seen with Mohs surgery that was not detected by biopsy.

## Discussion

Tissue may be examined in the horizontal, vertical, and/or oblique planes.<sup>3</sup> Traditional histopathologic processing involves vertical planes similar to the slices in a loaf of bread.<sup>4</sup> The commonly used variants in vertical sectioning, which include bread-loaf sectioning,

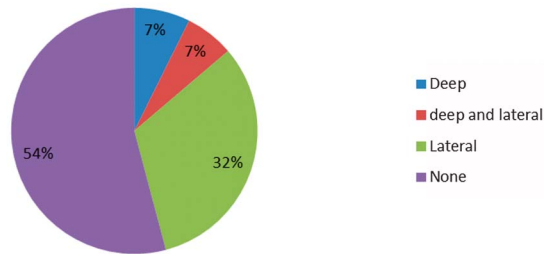
cross-sectioning, and peripheral sectioning, result in the visualization of only a small percentage of the tumor margin.<sup>3</sup> It is impossible to accurately determine the percentage of a surgical margin that is examined by these techniques as it would vary depending on the size of the biopsy, the thickness of the sections obtained, the number of tissue sections obtained from each cross-section, and the amount of tissue discarded between each of the sections.<sup>2</sup> Estimations of the actual percentage of the tumor margin that is typically viewed through bread-loafing technique is estimated to be <1%.<sup>3</sup> This is secondary to only a limited number of tissue sections being viewed. If every tumor were to be serially sectioned, it would yield approximately two thousand 5- $\mu$ m sections per centimeter of tumor, which is quite obviously impractical. Kimyai-Asadi and colleagues,<sup>5</sup> studying margins of melanoma in situ margins, confirm the impracticality of obtaining 100% margins through bread-loafing technique and add that the technique has to be performed at 0.1-mm intervals, instead of the commonly performed 4 mm, to detect 100% of the tumors involving the margin. They add that bread loafing at 1-, 2-, 4-, and 10-mm intervals

## Deep Margin Tumor Involvement in BCC and SCC on biopsy



**Figure 2.** An illustration of the proportion of margin involvement seen during MMS after biopsy-proven deep margin involvement in an SCC or a BCC. The key indicates the margins found during MMS.

### Lateral Margin Tumor Involvement in BCC and SCC on biopsy



**Figure 3.** An illustration of the proportion of margin involvement seen during MMS after biopsy-proven lateral margin involvement in an SCC or a BCC. The key indicates the margins found during MMS.

would have a 58%, 37%, 19%, and 7% chance of detecting positive margins, respectively. In addition to the limitations of vertical sectioning, BCCs have been shown to have highly irregular infiltration patterns and a predilection for small, finger-like outgrowths. This provides a setup for false-negative margins when only a small percentage of the biopsy margin is viewed. Previous studies have indeed indicated that this is the case. Jackson and colleagues<sup>6</sup> found residual BCC in the excision specimens of 77.8% of cases in which the biopsy margins were deemed negative, and Holmkvist and colleagues<sup>7</sup> found residual BCC in the Mohs sections of 66% of cases in which the clinical examination did not show evidence of tumor.

In our study, we retrospectively evaluated 493 patients with biopsy-confirmed BCCs and SCCs. Tumors from each patient were removed and examined by means of the MMS technique, which is considered the gold standard for margin examination, because it examines 100% of the margins. In this study, we have tried to elicit the number of times that the pathology report might miss on a margin that was later detected by the Mohs technique. Overall, there is a 32% chance of missing a lateral margin, when the pathology reports mention the involvement of only the deep margin, and there is a 14% chance of missing a deep margin, when the pathology report mentions only a lateral margin involvement (Table 4). One confounding factor that is worth to mention is that the distinction between deep versus lateral or deep and lateral margin involvement can be altered by tangential tissue cutting, or by retrospective review of the schematic Mohs map.

**TABLE 4. Percentage of Cases Where a Margin Was Missed on the Biopsy**

	Percentage Missed With Deep	Percentage Missed With Lateral	Percentage Missed With Clear
BCC	34	20	33
SCC	28	6	29
Combined BCC/SCC	32	14	30

Such interpretation could have potentially altered our data, although we do not believe that it has tremendously affected it, as the majority of cases were processed by the same histotechnician and interpreted by the same Mohs surgeon. Prospective studies that are present in the literature are in support of our results. Chuang and colleagues<sup>8</sup> looked at the incidence of biopsy-proven squamous cell carcinoma in-situ that were found to harbor invasive SCC on the Mohs stage. Of the 29 cases studied, 9 cases (31%) had a deeper component that was missed on skin biopsy. This is higher than our number (14%) and could be related to 2 main reasons: (1) possible regression of the tumor, as mentioned in the study by Swetter and colleagues,<sup>9</sup> who found that after shave biopsies, SCCs are twice more likely to regress compared with BCC, possibly from the wound healing inflammatory response; (2) curettage before removing the first section during Mohs surgery has also been shown to remove evidence of tumor.<sup>10</sup> Although this was not performed in all of the cases we retrospectively reviewed, it may have destroyed evidence of tumor and falsely decreased the percentage of tumor found during MMS.

The value of these studies comes into play when the dermatologist's evaluation and decision making might be altered by pathology reports that comment on margins. For example, some physicians might do a superficial shave removal when the pathology report mentions a lateral margin involvement. Alternatively, physicians might do a deeper punch or excision for cases where the deep margin is involved but the lateral margin is free. Finally, some patients might argue against re-excising a skin cancer when the biopsy margin is reported as clear. However, we have to realize that dermatopathologists are also pressured by dermatologists to give them margins on

their shave biopsy samples, probably in many cases for reimbursement purposes; however, as can be seen from this study, or from the previous studies by Kimyai-Asadi and colleagues,<sup>2</sup> Holmkvist and colleagues,<sup>7</sup> or Chuang and colleagues,<sup>8</sup> such margins are not always reliable and Mohs surgery, when appropriate, remains the gold standard treatment for nonmelanoma skin cancers.

In conclusion, this retrospective study revealed that there is a high probability that margins can be missed on skin biopsies, which are typically processed through the bread-loafing technique. We would encourage dermatologists not to ask their dermatopathologists for a margin on biopsied nonmelanoma skin cancers and not to rely on such margins for further management of such lesions.

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