Determination of Preexcision Surgical Margins of Melanomas from Fixed-Tissue Specimens

Frederick M. Golomb, M.D., Joyce P. Doyle, M.D., Caron M. Grin, M.D., Alfred W. Kopf, M.D., Mark K. Silverman, M.D., and Marcia J. Levenstein, D.Sci.

New York, N.Y.

The shrinkage of cutaneous surgical specimens of 199 malignant melanomas was analyzed. A formula was derived that makes it possible to calculate the in vivo (preexcision) specimen diameter from the in vitro (fixed-tissue) specimen diameter. The age of the patient was found to significantly influence specimen shrinkage and was incorporated into this shrinkage formula. The calculated in vivo specimen diameter was then used to determine the width of the in vivo surgical margins with reasonable accuracy. Thus this method permits calculation of the width of surgical margins from fixed-tissue specimens.

The standard management of primary cutaneous malignant melanoma has been surgical excision with wide margins.[1–6] In recent years, many authors have questioned the relationship between the width of the surgical margin and local recurrence, distant metastases, and overall prognosis. Many investigators feel that the traditional wide surgical margins are excessive and support the use of narrower surgical margins, especially in the treatment of thin melanomas.[7–15]

In order to assess the optimal surgical margin for the treatment of malignant melanoma, a number of investigators have compared the frequency of local recurrence and distant metastases in different groups of patients based on the width of their surgical margins. A recent randomized, prospective study compared the frequency of local recurrence, in-transit metastases, regional nodal metastases, and distant metastases in patients who had melanomas excised with either a 1-cm surgical margin or a 3-cm margin.[8] In that prospective study, which involved only patients with lesions that were less than or equal to 2 mm in Breslow thickness,[16] the margin measurements used for the analysis were those taken by the surgeon prior to excision of the lesion. Currently, there is an ongoing, randomized, prospective study of similar design that compares tumor recurrence in patients whose melanomas are treated with 2- versus 4-cm surgical margins (Intergroup Melanoma Committee of the National Cancer Institute).

In addition to the aforementioned prospective, randomized study, there have been several other analyses that evaluate the effect of the width of the surgical margin on recurrence of melanomas. Although some of these studies are based on in vivo margins measured by the surgeon,[9,10,15,17] others are based on measurements obtained from fixed pathology specimens.[7,13,14,18] When using fixed-tissue measurements, one must consider the degree of tissue shrinkage that has occurred in order to accurately obtain data regarding the in vivo surgical margins. While Day and Lew[18] computed an estimated width of the in vivo surgical margin using the pathology margin measurements and applying a 25 percent shrinkage factor,[19] the other authors who used the fixed-tissue measurements for surgical margins did not take into account any shrinkage factor. In their margin study, Breslow and Macht[7] acknowledged that specimen shrinkage occurs and stated that "these in vitro measurements were not corrected

From the Melanoma Cooperative Group at the New York University Medical Center, the Departments of Surgery and Dermatology at the New York University School of Medicine, and the Oncology Section, Skin and Cancer Unit, at the New York University Medical Center. Received for publication June 18, 1990; revised May 10, 1991.

for the retraction which occurs after the skin is excised." A number of other authors have made reference to shrinkage. In a study on the optimal resection margins for melanomas, Elder et al.[10] mentioned that specimen shrinkage occurred and did not exceed 29 percent. In an additional study, Bagley et al.[20] stated that the shrinkage of the excised specimens did not exceed 20 percent.

Although it is widely accepted that skin possesses retractile properties that result in shrinkage after its excision, there is a lack of published data indicating the degree and uniformity of this shrinkage. Before measurements obtained from fixed specimens can be used to draw conclusions that may have an impact on the course of treatment of malignant melanoma, the effects that specimen shrinkage may have on study results must be carefully considered.

The purpose of our study was to quantify the degree of shrinkage that occurs in cutaneous specimens from the time prior to surgical excision (in vivo) to the time after specimen fixation (in vitro). If this proved possible, this information could then be used to extrapolate backward from measurements obtained from pathology reports to determine the in vivo surgical margin measurements with reasonable accuracy.

This study involved the analysis of specimen shrinkage in 199 primary malignant melanomas treated by definitive surgical excision. The changes in the diameters of these specimens that occurred from the time of measurement in vivo to the time of measurement as a fixed specimen (in vitro) were analyzed to determine whether shrinkage is predictable with reasonable accuracy. We evaluated the following factors that might independently influence specimen shrinkage: (1) age and sex of the patient, (2) anatomic location, (3) diameter of the in vivo specimen, (4) whether or not a previous excisional biopsy had been done, and (5) direction of the axes of the surgical excision in relation to Langer's lines.[21–23]

Patients and Methods

One-hundred and ninety-nine patients with primary malignant melanomas treated by one of us (Golomb) in the years 1982 through 1989 were included in this study. Inclusion in the study required the following: (1) a definitive local surgical excision was performed, (2) the shape of the surgical excision was either an ellipse or a circle, and (3) records of the following measurements were available: minimum diameters of the specimen just prior to excision (in vivo), immediately after excision, and after fixation in formalin (in vitro).

In each case, prior to surgery, the planned excision was outlined with a marking pen. Precise cutaneous measurements of the diameters of the specimens and the width of the surgical margins were obtained at the time of operation by one of us (Golomb) using a millimeter ruler. The measurements obtained immediately prior to excision were defined as the in vivo specimen diameter and margin measurements. The specimen diameters, measured along the same axes, were then recorded after excision of the specimen but prior to its fixation. Final diameter measurements were then recorded from 1 to 5 days after tissue fixation, both by one of us (Golomb) and by either the pathologist or the pathology technician. These fixed-tissue measurements were defined as the in vitro specimen diameters. The fixative employed was 10% neutral buffered formalin.

All analyses were performed using SPSS-X Data Analysis System.[24] A linear regression analysis using the method of least squares[25] assessed the degree of linear correlation between the in vivo specimen diameters and the in vitro specimen diameters. The analysis was performed using the minimum diameters of the specimen, which reflects the axis used to determine the width of the minimum surgical margins.

A multiple regression analysis[25] was performed to determine which of the following factors independently influenced the degree of specimen shrinkage: (1) age, (2) sex, (3) in vivo diameter of the surgical specimen, (4) anatomic location, and (5) whether or not a previous excisional biopsy had been done.

A paired t test was used to compare in vitro diameter measurements obtained by one of us (Golomb) with the in vitro measurements obtained by the pathologist or pathology technician.

Langer's lines are thought to represent lines of maximal skin tension.[21–23] Thirty cases were identified in which the relationship between the specimen axes and Langer's lines was clearly defined. Only those specimens which had one axis that was parallel to Langer's lines (and therefore, the other axis was perpendicular to Langer's lines) were chosen for the analysis. In these 30 cases, the degree of shrinkage that occurred along the parallel axis was compared with the shrinkage along the perpendicular axis using analysis of variance.[26]

Finally, through the analysis of 199 melanoma

cases, a formula for the shrinkage of surgical specimens was derived. This formula allowed the determination of the width of in vivo surgical margins using a two-step process. Step one of this formula involved calculation of the in vivo specimen diameters from the in vitro specimen diameters. Step two of the formula resulted in the calculation of in vivo surgical margins from fixed-tissue specimens. This was done for 184 of the 199 cases, in which measurements of the in vivo diameters of the lesion or biopsy scar and of the in vivo surgical margins were available. The width of the surgical margin calculated using our formula was compared with the margin measurement that had been recorded in vivo by one of us (Golomb). The difference between the calculated margin measurements and the in vivo margin measurements was tested to see whether or not it was within the range of the value predicted by our formula (that is, ±3.5 mm).

Results

Of the 199 patients in this study, 93 were male and 106 were female. The mean age for the group was 51.9 years, with ages ranging from 22 to 89 years. Thirty-six patients had primary lesions located on the head and neck, 77 patients had lesions on the trunk, and 86 patients had lesions on the extremities. In 116 patients, the lesion was either intact or had undergone a previous shave or punch biopsy prior to the definitive surgical excision. Eighty-three patients presented with a linear scar from a previous excisional biopsy. Of the 199 lesions, 111 were removed using an elliptical surgical excision, and the remaining 88 were removed with a circular excision.

The mean in vivo diameter for all specimens was 32.3 mm (SD = 13.6 mm), with a range of 7 to 86 mm, and the mean in vitro diameter was 25.5 mm (SD = 11.0 mm), with a range of 6 to 74 mm. The mean fixed-tissue diameter measured by the pathologist or the pathology technician was 26.0 mm (SD = 11.0 mm), with a range of 6 to 73 mm.

The mean percent shrinkage that occurred from the time the specimen was measured in vivo until after tissue fixation was 20.7 percent. Of the total amount of shrinkage that occurred in these specimens, 94.2 percent occurred immediately after surgical excision but prior to tissue fixation. Thus formalin fixation contributed minimally to further specimen contraction.

The linear regression analysis used to assess the degree of linear correlation between the in vivo minimum specimen diameters and the in vitro minimum specimen diameters demonstrated a high degree of statistically significant correlation ($p < 0.0001$). These results indicated that 92.5 percent of the variation in the in vitro diameter measurement was explained by the in vivo diameter measurement. The remaining 7.5 percent was due to other factors that influence specimen contraction as well as to inherent measuring variability. Thus the analysis demonstrated that the factor that was most important in predicting the final diameter of the contracted specimen was the in vivo specimen diameter.

In the multiple regression analysis, the only other variable in addition to the in vivo diameter that proved to be an independent factor influencing shrinkage was the age of the patient. Two distinct cutoff points were identified, namely, age 50 and age 60. Thus this defined three separate groups for specimen shrinkage: (1) those patients less than 50 years of age, (2) those from 50 to 59 years of age, and (3) those 60 years of age or older. Patients who were less than 50 years old had the greatest degree of shrinkage, and patients who were 60 years of age or older had the least amount of shrinkage.

The remaining factors used in the multivariate analysis—sex, anatomic location, and whether or not the lesion had been treated with a prior excisional biopsy—were not statistically significant contributing factors influencing tissue shrinkage.

In the analysis of the effect of Langer's lines, the degree of shrinkage that occurred along the axes parallel to Langer's lines was not statistically different from that occurring along the axes perpendicular to these lines. However, this preliminary analysis used only 30 patients. The applicability of these results, therefore, is limited by this small sample size.

In order to assess the consistency between the in vitro measurements performed by one of us (Golomb) and the pathologist or the pathology technician, a paired t test was used. The t test showed a statistically significant degree of difference between these individual measurements ($p < 0.001$). However, the mean values of these two groups differed by only 0.55 mm. We feel, therefore, that while this difference was statistically significant, it is not clinically significant.

Finally, and most important, this data analysis resulted in the development of a mathematical formula for specimen shrinkage. This equation enabled us to calculate the in vivo specimen

diameter and the in vivo surgical margins using a two-step process. The first step used the measurement of the minimum diameter of the in vitro specimen (d) and the patient's age to determine the minimum diameter of the in vivo specimen (D). The second step involved subtracting the in vivo diameter of the lesion or biopsy scar (L) from this calculated in vivo diameter (D). This measurement is then divided by 2 to determine the width of the surgical margin (M). Thus the width of the in vivo surgical margins (M) used in the treatment of primary malignant melanoma can be determined using this two-step formula (Fig. 1).

Shrinkage Equation

Step 1: The determination of the in vivo specimen diameter (D):

$$D = 1.25 \times d + \text{age factor}$$

where D = in vivo specimen diameter (in mm)
d = in vitro specimen diameter (in mm)
age factor = 3 mm if age < 50
age factor = 1 mm if age = 50 to 59 years
age factor = −1 mm if age ≥ 60 years

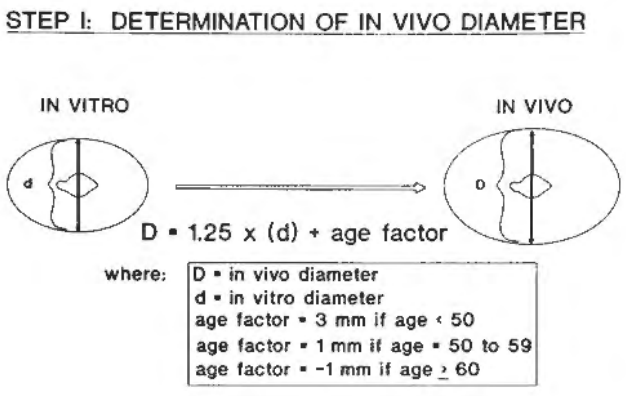

FIG. 1. A two-step formula for the determination of the width of the in vivo surgical margins.

The 95 percent confidence interval (2 standard deviations) for this equation is consistently less than ±7 mm. Therefore, in our data collection, 95 percent of the time the true value for the in vivo specimen diameter will fall within ±7 mm of the predicted value.

Step 2: The determination of the in vivo surgical margin (M):

$$M = \frac{D - L}{2}$$

where M = in vivo surgical margin
D = in vivo specimen diameter
L = in vivo lesion diameter

In order to verify the formula, a direct comparison of the calculated in vivo surgical margin and the actual in vivo margin measured by one of us (Golomb) was made for each specimen. This analysis, performed on 184 cases, showed 92.4 percent agreement within ±3.5 mm of these two measurements.

In addition, the mean of the absolute difference between the in vivo surgical margins calculated using our formula and the in vivo surgical margins measured by one of us (Golomb) was 0.08 mm.

DISCUSSION

To our knowledge, this represents the first study in which precise in vivo and in vitro specimen measurements, recorded during the definitive treatment for melanoma, were used to develop a mathematical formula to quantify specimen shrinkage. Using this formula, it is now possible to calculate the in vivo specimen diameter from the in vitro diameter, taking into account the patient's age. The fact that it is possible to develop such a formula with a 95 percent confidence interval of ±7 mm indicates that for our data, shrinkage is somewhat variable but can be predicted with a reasonable degree of accuracy. A 95 percent confidence interval of ±7 mm for the diameter of the whole specimen translates to approximately ±3.5 mm when dealing with margin measurements. Since the ultimate goal of this formula was to determine the width of the in vivo surgical margins, we tested our data to see if it were possible to calculate the in vivo surgical margins with this degree of precision (that is, ±3.5 mm).

The second step of our formula allows one to calculate the in vivo surgical margins using the in vivo specimen diameter determined in step 1. An analysis of the raw data demonstrated that in 92.4

percent of our patients, the difference between the predicted in vivo margin measurements (derived from step 2) and the in vivo margin measurements recorded by one of us (Golomb) prior to surgical excision was less than or equal to ±3.5 mm. Therefore, tissue shrinkage was predictable enough that our two-step formula could reliably estimate in vivo surgical margin measurements.

The optimal width of the surgical margins used to treat malignant melanomas has been a controversial issue. A number of authors have used fixed-specimen measurements to study the relationship between the width of the margins and the frequency of tumor recurrence without accounting for specimen shrinkage. Through our analysis we have derived a formula that can be used to correct for specimen shrinkage. Since pathology reports generally include measurements of the in vitro specimen diameters, our formula, which uses fixed-specimen diameter measurements to compute in vivo diameter measurements, should be widely applicable. This formula might then retrospectively be used on melanoma registries to determine surgical margins when only the age of the patient and the diameters of the lesion and fixed specimens are known.

In this study, the only factors that proved to contribute significantly to shrinkage were the patient's age and the diameter of the in vivo specimen. The other factors, i.e., sex, anatomic location, and whether or not a previous excisional biopsy had been done, were not shown on multiple regression analysis to influence shrinkage. The principal shrinkage occurred immediately after surgical excision. Only minimal additional shrinkage followed formalin fixation.

In our study, age was determined to be an independent variable influencing the degree of specimen shrinkage. Two clear cutoff points were identified: age 50 and age 60. Patients who were less than 50 years old were found to have the greatest amount of specimen shrinkage, whereas patients who were 60 years or greater had significantly less.

Dermal thickness varies from site to site on the body. Depending on anatomic location, the dermis is 15 to 40 times thicker than the epidermis and is thickest on the back.[27] We anticipated that the increased thickness in the skin of the trunk would impart a greater degree of cutaneous elasticity and thus a greater degree of specimen shrinkage. In addition, since the skin of the head and neck generally receives the greatest degree of sun exposure, one would expect the elastotic changes known to occur from chronic ultraviolet light exposure to be greatest in these regions, resulting in decreased skin elasticity compared with other areas of the body. However, in the multiple regression analysis of our data, anatomic location did not make a statistically significant contribution to the degree of specimen shrinkage.

In the analysis, one unexpected finding was that the presence or absence of a linear scar from a previous excisional biopsy did not significantly alter the extent of specimen shrinkage.

In sum, this report concludes that using measurements of in vivo tumor diameter and in vitro diameter of the postfixation shrunken surgical specimen, data that are generally available from medical records, it is possible with an acceptable degree of accuracy to determine what were the in vivo surgical margins used for the removal of the cutaneous malignant melanoma.

In conclusion,

1. Cutaneous specimen shrinkage occurs immediately following surgical excision. Formalin fixation contributes minimally to further shrinkage.
2. Specimen measurements (in vitro) recorded by the pathologist or the pathology technician showed a high degree of concordance with our own measurements and are therefore believed to be sufficiently reliable for use in the calculation of in vivo diameters.
3. Shrinkage varies inversely with age: Patients who were less than 50 had the greatest degree of shrinkage, whereas patients who were 60 years of age or older had the least amount of shrinkage.
4. Shrinkage was not influenced by sex, anatomic location, or whether a previous excisional biopsy had been done. In addition, in our small sample, shrinkage appears to be independent of the relationship of the excision to Langer's lines.
5. The formula for calculating the in vivo (preexcision) diameters (D) of melanoma specimens from fixed pathology specimens is

$$D = 1.25 \times d + \text{age factor}$$

where D = in vivo specimen diameter in mm
d = in vitro specimen diameter in mm
age factor = 3 mm if age < 50 years
age factor = 1 mm if age = 50 to 59 years
age factor = −1 if age ≥ 60 years

The formula for calculating the width of the in vivo surgical margins (M) excised in the treatment of melanoma is

$$M = \frac{D - L}{2}$$

where M = in vivo surgical margin
D = in vivo specimen diameter
L = in vivo lesion diameter

This two-step formula was tested on our data and resulted in predicted margin measurements that were within ±3.5 mm of the in vivo margins measured by one of us (Golomb) 92.4 percent of the time.

Frederick M. Golomb, M.D.
Department of Surgery
New York University Medical Center
560 First Avenue
New York, N.Y. 10016

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