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Comparison of Direct and Microslide Pathology Measurements of Uveal Melanomas

Many investigators have shown that large ciliochoroidal melanomas are more likely to be associated with an unfavorable outcome than small tumors by using data retrieved and measured from pathology files. In the past, the measurement of largest tumor dimension (LTD) may not have been recorded at the time of the gross examination, because the significance of this observation was not appreciated. If this information is not available, authors can eliminate cases from their studies, take all their measurements directly from glass microslides, or combine clinical estimates of tumor size for some cases with gross measurements for others. To date, there has been no formal study to compare the measurement of tumor dimensions from glass microslides with measurements made at the time of gross examination by the pathologist. This study of 112 ciliochoroidal melanomas reveals that measurements of the LTD made from the glass microslide correlate with direct measurements taken from the cut surface of the globe at the time of sectioning. Additionally, measurements of the LTD from the glass microslide are at least as effective in predicting patient outcome as direct measurements. These findings suggest that measurements of the LTD from the glass microslide provide as much prognostic information as direct measurements if it is known that the eye was cut to obtain representative sections of the tumor. Invest Ophthalmol Vis Sci 86:1788-1791, 1985

Many investigators who have studied the pathology of uveal melanomas have noted the importance of tumor size with relationship to prognosis.1-8 Yet, there is no standardized technique used by all authors for recording the measurement of tumor size: some investigators rely only on measurements taken from the tissue specimen at the time of gross examination,1,2 some investigators substitute measurement of tumor dimensions from glass microslides,3-5 while other investigators use both direct measurements and microslide measurements in the same study,6 sometimes combined with clinical data.7-8

Despite the mixture of measurements used in pathology studies, there is little evidence to prove that direct and microslide measurements are related. Davidorf and Lang7 concluded that “measurement of the specimen on the slide is an accurate method for the determination of tumor size,” but they did not present data to confirm their observation. On the other hand, Barr and coworkers,3 in attempting to study 49 small melanomas, found that in 11 cases (22%) initially selected for study, the maximum tumor dimension on the microslide did not match the gross description. Finally, when McLean and coworkers2 insisted that three-dimensional tumor measurements recorded at the time of gross examination be available for inclusion into their study, only 291 of 3852 cases (7.6%) were selected. While this low rate of case acceptance may be attributed to the desire to study three tumor dimensions instead of two, the authors may have been skeptical of measurements taken from microslides because of alterations in measurements induced by tissue handling and processing (personal communication, Ian McLean, M.D. to JWG).

A recent report suggests that measurement of the largest tumor dimension (LTD: maximum height or greatest length of scleral contact) is related to prognosis.3 We therefore compared 112 matched pairs of measurements, one obtained directly from the gross specimen and one taken from microslides, to explore Davidorf and Lang’s3 observation concerning the accuracy of substituting one type of measurement for another. We also compared the efficacy of each type


of measurement in predicting patient outcome using a variety of statistical tests.

Materials and Methods. As part of an ongoing retrospective study of the pathology of ciliochoroidal melanoma, all such cases accessioned by the Wills Eye Hospital pathology laboratory from 1963–1983 were identified. The starting date of 1963 was selected because it was at this time that the laboratory switched from celloidin embedding of globes to paraffin embedding. Cases for which measurements of tumor dimensions were based only on transillumination shadows were excluded from the study. Cases were included only if tumor dimensions appeared on the original pathology report as having been measured from the cut surface of the globe at the time of gross examination. In order to explore the relationship of the two types of LTD measurements (gross and microslide) to outcome, we included only patients known to have had metastatic melanoma or who had survived at least 5 yr free of disease. A total of 112 cases met these criteria.

Direct communication with all pathologists supervising the processing of material during the time that globes from this study were accessioned by the Wills Eye Hospital Laboratory (Charles Steinmetz, M.D., Merlyn Rodrigues, M.D. and Vitaliano Bernardo Jr, M.D., May, 1984) yielded the following information concerning how the globes were processed. An attempt was made to open each eye after fixation in formalin to generate pupil-optic nerve sections containing the largest dimension of the melanoma. Using transillumination of the globe, the area of shadowing was marked and the globe was opened in the corresponding plane. Measurements of the transillumination shadows were not included in this study. Such shadows may be larger than the actual tumor size because blood or viscous subretinal fluid in adjacent nonrhegmatogenous retinal detachments may also partially block the transmission of light through the globe. Tumor measurements were made without the aid of any magnification using calipers or a ruler applied to the cut surface of the tumor. Measurements were made by a variety of personnel including the aforementioned ocular pathologists as well as ophthalmology residents supervised by these pathologists. Only one level of the tumor was cut routinely, but, depending upon the interest in a particular case, 10 to 30 sections of the specimen were cut from the single level.

A glass microslide from each case representing the tissue section containing the largest amount of tumor per case was selected from the pathology files at Wills Eye Hospital and sent to one of the authors (JWG) for measurement of the LTD from the slide. Both the maximum length of scleral contact as well as tumor height were measured from the glass microslide by one technician using calipers without the aid of any magnification. The larger of these two measurements was designated as the largest tumor dimension and was used in the analysis of data. In the event that a mushroom-shaped tumor had a larger diameter in the subretinal head of the mass than in the choroid adjacent to the sclera, the technician was instructed to record the dimension in contact with the sclera in conformity with a previously published method.5 The LTD as measured on gross examination as well as the patient outcome was not provided to the technician. When all measurements of the LTD had been completed, the LTD at gross examination was compared to the microslide measurement using the following statistical methods.

To determine the relationship of LTD measurements from microslides to LTD measurements at the time of gross examination, a linear regression was applied to the matched pairs of measurements for each case and a correlation coefficient was determined, with the LTD-microslide measurement as the independent variable and the LTD-gross measurement as the dependent variable. Additionally, a paired t-test was performed to determine whether one method of measurement provided values significantly different from the other method.

Each type of measurement (gross and microslide) was tested separately for possible association with patient outcome by three different statistical methods: (a) the t-test for independent groups, comparing the measurements of LTD for survivors with measurements for tumor-related fatalities; (b) the Mantel-Haenszel test, comparing the survival of cases with LTD measurement above the mean to survival for those cases with LTD below the mean; and (c) the t-values derived from the Cox regression model.9 In addition, the mean of each pair of measurements was tested for possible association with patient outcome by each of these methods.

Results. The LTD measured from the glass microslides is strongly correlated with LTD measurements recorded at the time of gross examination with slope = .828, intercept = 2.8, R = .65, F = 82, and P < 0.001 (Table 1). Nevertheless, gross measurements were significantly larger than microslide measurements as determined by a paired t-test (Table 1, P < 0.005). By each of the three statistical methods used, the LTD as measured from the glass microslides proved at least as effective for predicting outcome as LTD measurements at the time of gross examination, as shown in Table 2.

Discussion. It is interesting that the LTD measured on microslides is significantly smaller than the LTD as measured directly from the cut surface of the fixed gross
specimen. There are several explanations to explain this discrepancy. (1) It is known that heating tissue in a paraffin bath will induce some tissue shrinkage, although the degree is shrinkage is not known. It is possible to study the degree of shrinkage prospectively by comparing gross measurements with microslide measurements of early sections from the block. However, the degree of shrinkage might vary from laboratory to laboratory unless tissue processing protocols are standardized. (2) The degree of expansion of the tissue section of a globe in the technician’s water bath may lead to variable LTD microslide measurements. (3) Because up to 30 slides were cut from the same level in the block, it is possible that the microslide we selected for study did not represent the surface measured at the time of gross examination. (4) It is possible to confuse dark red-brown blood in the choroid and subretinal space for melanoma at the time of gross examination. (5) It is possible to confuse the clinically important issue of how well either pathology measurement correlates with clinical measurement. (5) Many different people with different levels of experience performed the gross measurements.

This study does show that even though the microslide LTD measurements tend to be smaller than measurements from gross, formalin-fixed tissue, microslide measurements are at least as effective in predicting outcome as gross measurements (Table 2). It was not the purpose of this study to establish the relationship between LTD and the interval between enucleation and the discovery of first metastasis or the date of last contact if disease free; this study has been done by others. Instead, we included the LTD measurement by gross examination in the Cox model to predict the "force of mortality" and repeated the Cox model using the LTD derived from glass microslides. Each type of LTD measurement was highly effective; in addition, the mean of each measurement pair was tested for correlation with outcome and was also highly predictive (Table 2).

This study suggests that it is valid to substitute microslide LTD measurements for gross LTD measurements if it is known that an attempt was made to open the globe in a place that included the maximal area of scleral contact by tumor. However, by insisting that eyes be opened in a plane that passes through the tumor and which includes the pupil and optic nerve on the same section (the "standard" grossing technique), the zone of real maximal scleral contact by tumor may not lie in the tissue section generated unless the tumor is either perfectly round or, if not round, has a long axis directed along an optic nerve-pupil meridian.

Finally, while this study has addressed the problems of comparing glass microslide LTD measurements with gross pathology measurements, we have not addressed the clinically important issue of how well either pathology measurement correlates with clinical measurements. While a recent study shows correlation of echographic measurement of tumor height with measurements derived from glass microslides, the issue of correlation of clinically estimated maximal length of scleral contact with pathology measurements needs to be studied.

Key words: uveal melanoma, measurement, prognosis, largest tumor dimension

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**Table 1.** Comparison of largest tumor dimension measurements: measurements at gross examination versus measurements from microslides

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>S.D.*</th>
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</thead>
<tbody>
<tr>
<td>Measured at gross examination</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All 112 cases</td>
<td>11.5</td>
<td>4.3</td>
</tr>
<tr>
<td>47 cases dead of tumor</td>
<td>13.8</td>
<td>4.4</td>
</tr>
<tr>
<td>65 cases surviving</td>
<td>9.9</td>
<td>3.4</td>
</tr>
<tr>
<td>Measured from microslide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All 112 cases</td>
<td>10.6</td>
<td>3.4</td>
</tr>
<tr>
<td>47 cases dead of tumor</td>
<td>12.4</td>
<td>3.5</td>
</tr>
<tr>
<td>65 cases surviving</td>
<td>9.3</td>
<td>2.7</td>
</tr>
</tbody>
</table>

* Standard Deviation
† Regression analysis: independent variable = LTD microslide; dependent variable = LTD gross; R = .65 slope of regression line = .828; Intercept = 2.8; F = 82; P < 0.001. Paired t-test of LTD gross and LTD microslide measurements, all cases: t = 3, P < 0.005.

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**Table 2.** Comparison of the relationship of largest tumor dimension measurement techniques in predicting outcome in 112 uveal melanomas

<table>
<thead>
<tr>
<th>Test*</th>
<th>Gross</th>
<th>Microslide</th>
<th>Mean†</th>
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<tr>
<td>Student t-test</td>
<td>5.3</td>
<td>5.4</td>
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<tr>
<td>Mantel-Haenszel (Chi-sq., d.f. = 1)</td>
<td>19.2</td>
<td>25.0</td>
<td>20.3</td>
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<tr>
<td>Cox Model (t)</td>
<td>4.8</td>
<td>5.2</td>
<td>5.5</td>
</tr>
</tbody>
</table>

* See Materials and Methods; for all tests, P < 0.001.
† Mean of gross and microslide measurement for each case.
References


