Nucleolar Diameter and Microvascular Factors as Independent Predictors of Mortality from Malignant Melanoma of the Choroid and Ciliary Body

Rana’a T. Al-Jamal, Teemu Mäkitie, and Tero Kivela

PURPOSE. To determine whether nucleolar diameter and microvascular factors are independent predictors of mortality in malignant uveal melanoma of the choroid and ciliary body.

METHOD. A population-based retrospective cohort study was conducted of melanoma-specific and all-cause mortality in 167 consecutive patients who had an eye enucleated because of choroidal and ciliary body melanoma from 1972 through 1981. The largest nucleoli were measured from digital photographs of silver-stained tumors along a central 5-mm-wide linear field parallel to the base of the tumor. The mean of the 10 largest nucleoli (MLN) was calculated. Microvascular loops and networks and microvascular density (MVD) were assessed. Kaplan-Meier and Cox regression analyses were performed. Associations between MLN and other variables were determined.

RESULTS. The MLN could be determined in 126 (75%) melanomas. It ranged from 2.60 to 6.18 μm (median, 4.05). The association of large MLN with the presence of epithelioid cells (P = 0.017) and high MVD (P = 0.0053) was statistically significant. MLN was not significantly associated with tumor diameter and microvascular loops and networks. The 10-year melanoma-specific survival decreased with MLN (0.74, 0.60, and 0.42, arranged in tertiles; P = 0.0060), presence of loops and networks (P = 0.0001), and increasing MVD (P = 0.0001). By Cox regression, MLN was an independent predictor of survival, when adjusting in turn for presence of epithelioid cells, loops and networks, and MVD. In multivariate models with MVD, the independent prognostic information carried by MLN decreased, but the model as a whole was a better predictor of survival. The magnitude of this effect depended on whether MLN was modeled as a continuous or categorical variable.

CONCLUSIONS. In this population-based data set, MLN and microvascular loops and networks were unrelated, independent predictors of survival. MLN and MVD were found to be partially interrelated. Multivariate models that included MVD in addition to MLN fitted better with observed melanoma-specific survival than models that excluded MVD. (Invest Ophthalmol Vis Sci. 2003;44:2381–2389) DOI:10.1167/iovs.02-1215

During the past decade, two main lines of research have sought to derive an accurate prognosis for patients with uveal melanoma. One has emphasized characteristics of tumor cells, particularly their nucleoli and markers of proliferation, and the other characteristics of tumor blood vessels. Our goal was to clarify the relationship between MLN and microvascular factors in malignant uveal melanoma of the choroid and ciliary body and to determine how this relationship affects prognosis and survival, so as to improve understanding of this tumor. A special motivation was that the number of uveal melanomas that are available for histopathology is again likely to increase in the near future because of improved techniques for transscleral resection and the hope that such procedures would be more efficient than irradiation in preserving vision, especially when the tumor is medium sized.
PATIENTS AND METHODS

Study Design

The primary objective was to determine whether nucleolar size and microvascular factors are independently associated with survival of patients with uveal melanoma. The secondary purpose was to study the interrelationship between MLN, microvascular loops and networks, and MVD.

Study Population and Exclusion Criteria

We studied a cross-sectional, population-based cohort of 167 patients with uveal melanoma, previously used for analysis of microvascular loops and networks. The cause of death had been validated by reviewing all patient charts relating to malignant tumors and death, cross-checking with the Finnish Population and Cancer Registries, and by acquiring all histopathological material available from primary tumors, metastases, and second cancers.

The 167 consecutive patients, who had a choroidal or ciliary body melanoma enucleated between 1972 and 1981, were verified from the records of the Ophthalmic Pathology Laboratory, Helsinki University Central Hospital. During that time, enucleation was the only treatment available for uveal melanoma, and all removed eyes were submitted to this laboratory.

Complete follow-up data, with a median follow-up time of 25 years (range, 20–29) for those still alive, were available for 166 of the 167 patients. The diagnoses of all 9 secondary cancers and 49 of 53 specimens of metastatic uveal melanoma were confirmed by immuno-histochemistry. The study followed tenets of the Declaration of Helsinki and was approved by local review board.

Assessment of Nucleolar Size

Both hematoxylin-eosin staining and silver staining originally designed for labeling nucleolar organizing regions, have been used to identify nucleoli for measurement. The silver stain provides high contrast between nucleoli and other structures, allowing accurate discrimination of nucleoli. A comparative study found that measurements from silver-stained slides were easier to make and provided better prognosis than those from hematoxylin-eosin slides. The most frequent field selection for sampling has been a 5-mm-long linear strip from the center of the melanoma. Linear sampling was recently reported to be comparable to scanning nucleoli from the entire tumor section in predicting outcome. For these reasons, we chose silver staining and linear sampling for the present study.

Sections were cut at 5 μm on chromium-gelatin–treated glass slides and randomly coded. The code was broken only after all MLN measurements had been obtained. After deparaffinization, the sections were bleached with 0.25% potassium permanganate for 1 hour and 5.0% oxalic acid in distilled water for 5 minutes. One-step silver staining was performed using two solutions: first, 2.0% gelatin (Bacto Gelatin; Difco Laboratories, Detroit, MI) and 0.88% formic acid in distilled water; second, 50% silver nitrate in distilled water. The solutions were mixed 1:2 in the dark and poured into a dish to cover the specimens for 30 minutes. The sections were washed in distilled water and dehydrated, and coverslips were mounted (Mountex; Histolab Products AB, Gothenburg, Sweden).

Each slide was examined under a light microscope (BH-2; Olympus, Tokyo, Japan) at 20× magnification to orient the central longest axis of the tumor parallel to tumor base for digital photography (DP-10; Olympus). A photograph under low magnification was first taken for documentation of orientation. A series of color photographs under 400× optical magnification were obtained to image the nucleoli along this axis. A 5-mm strip was photographed, divided into 25 slightly overlapping images (resolution, 1280 × 1024 pixels, image area 218 × 175 μm). In case the tumor was less than 5 mm by largest basal diameter (LBD), the entire central axis of the tumor was photographed.

From each of the 25 photographs, the largest nucleoli were measured by image-analysis software (Olympus DP-10 Soft, ver. 3.0; Soft Imaging System GmbH, Münster, Germany). A strip one screen high at 300% digital magnification (final magnification on screen, ×4700; corresponding to 41 μm) was scanned from the top of each photograph. Measurements were taken along the longest axis of the nucleoli. The number of nucleoli measured was one to five per image (13 to 80 per
Assessment of Microvascular Factors

Closed microvascular loops and microvascular networks, consisting of at least three back-to-back loops, were identified according to Folberg et al. from sections bleached with potassium permanganate and oxalic acid and stained with periodic acid–Schiff without counterstain. They were viewed under a green filter (Wratten No. 58; Eastman Kodak, Rochester, NY). Loops of all sizes were taken into account.

Microvessels were identified with the monoclonal antibody QBEND/10 to the CD34 epitope of endothelial cells (lot 121202; Novocastra Laboratories, Newcastle-upon-Tyne, UK; diluted 1:25). They were counted at 400× magnification from the most highly vascularized area (hot spot), using an eyepiece with an etched graticule corresponding to 0.313 mm² (WK 10x/20L-H; Olympus). Any immunoabeled element, clearly separate from adjacent ones and totally inside the graticule or touching its top or left border, was counted as a microvessel.

Statistical Analysis

Analyses were performed on computer (Stata, ver. 7.0; StatCo., College Station, TX). Intraobserver agreement in measuring MLN was assessed by plotting the difference between the measurements against their mean and by calculating the mean difference with 95% confidence limits. Interobserver reproducibility was assessed similarly. To compare MLN in various types of uveal melanoma, the groups were compared with the nonparametric Kruskal-Wallis test. Nonparametric test for trend, which is an extension of Wilcoxon rank sum test, was used if the groups were ordered. The association between MLN and other continuous variables was analyzed by Spearman’s rank correlation. All tests used were two-tailed.

Univariate analysis of survival time data were based on the Kaplan-Meier product-limit method without taking competing risks into account. Patients judged to have died of causes other than uveal melanoma were censored at the time of death. Minimum sample size was calculated on the basis of a previous consecutive series, which reported the cumulative 10-year probability of survival to be 0.69 and 0.22 for patients who had a melanoma in which MLN was lower and higher than the median, respectively, corresponding to a survival difference of 0.47. Power analysis indicated that to detect a similar difference as significant with a power of 80%, the study should have a minimum of 58 patients (Power and Precision, ver. 2.0; Biostat, Englewood, NJ).

Cell type and tumor location were collapsed into two categories according to the presence of epithelioid cells (spindle, nonspindle) and ciliary body involvement (no, yes), respectively. Microvascular loops and networks were analyzed as a three-category variable that considered networks to be an advanced stage of loops (no loops, loops without networks, networks). LBD, MVD, and MLN were divided into tertiles.

Cox proportional hazards regression was used to adjust survival time data for the effect of other prognostic factors. LBD and MVD were modeled as continuous variables, and MLN alternatively as divided in tertiles to assess robustness of results. MVD was square-root transformed to obtain normal distribution. Independent variables were allowed in the model if $P < 0.10$, and different models were compared with the likelihood ratio test. The number of variables was restricted to four, based on a rule to have at least 15 to 20 events for each additional variable. The regression coefficients and hazard ratios (HR) with 95% confidence intervals (CI) were calculated. The assumption of proportional hazards was tested by the method of Therneau and Grambsch.

RESULTS

Mean Diameter of the 10 Largest Nucleoli

Nucleoli were reliably identified using the silver staining method in 126 (75%) of the 167 slides (Fig. 1A). The remaining 41 specimens were technically not satisfactory, because of loss

Table 1. MLN of 126 Choroidal and Ciliary Body Melanomas Studied Compared With Literature Data

<table>
<thead>
<tr>
<th>Source</th>
<th>MLN</th>
<th>Staining</th>
<th>Field</th>
<th>Measurement</th>
<th>Data Set</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present study/observer I</td>
<td>4.06±0.20±6.18</td>
<td>Silver†</td>
<td>5 mm parallel to tumor base through center</td>
<td>Largest diameter</td>
<td>Consecutive</td>
</tr>
<tr>
<td>Present study/observer II</td>
<td>3.71±2.18–6.61</td>
<td>Silver‡</td>
<td>5 mm parallel to tumor base through center</td>
<td>Largest diameter</td>
<td>Consecutive</td>
</tr>
<tr>
<td>Moshari/AgNOR17</td>
<td>3.13±1.10–5.50</td>
<td>Silver‡</td>
<td>Entire tumor area</td>
<td>Largest diameter</td>
<td>Selected[,]</td>
</tr>
<tr>
<td>Moshari and McLean/HE17</td>
<td>2.84±2.00–5.20</td>
<td>HE</td>
<td>Entire tumor area</td>
<td>Largest diameter</td>
<td>Selected[,]</td>
</tr>
<tr>
<td>Moshari and McLean/McCurdy17</td>
<td>2.69±1.03–4.84</td>
<td>HE</td>
<td>5 mm parallel to longest axis through center</td>
<td>Horizontal diameter</td>
<td>Selected[,]</td>
</tr>
<tr>
<td>Seregard et al.15</td>
<td>3.34‡ 2.07–5.53</td>
<td>HE</td>
<td>5 mm through tumor</td>
<td>Horizontal diameter</td>
<td>Selected[,]</td>
</tr>
<tr>
<td>Pe’er et al./Observer I*</td>
<td>3.76±2.31–6.62‡</td>
<td>HE</td>
<td>5 mm parallel to longest axis through center</td>
<td>Largest diameter</td>
<td>Consecutive</td>
</tr>
<tr>
<td>Pe’er et al./Observer II*</td>
<td>3.96±2.76–8.79</td>
<td>HE</td>
<td>5 mm parallel to longest axis through center</td>
<td>Largest diameter</td>
<td>Consecutive</td>
</tr>
<tr>
<td>Sorensen et al.7</td>
<td>2.77±1.61–4.85</td>
<td>HE‡</td>
<td>5 mm parallel to longest axis through center</td>
<td>Horizontal diameter</td>
<td>Selected[,]</td>
</tr>
<tr>
<td>Sorensen et al.5</td>
<td>3.06±2.20–4.23</td>
<td>HE</td>
<td>5 mm parallel to longest axis through center</td>
<td>Horizontal diameter</td>
<td>Consecutive</td>
</tr>
</tbody>
</table>

* Based on a randomly drawn subset of 63 tumors.
† Median.
‡ Excluding one outlier of 11.2 μm.
§ Mentions bleaching of melanin.
¶ Included 50% of patients who died of melanoma and 50% who survived without metastasis.
of tissue, because artifacts made it difficult to recognize the nucleoli in some heavily pigmented tumors or because the tumor was very small. Likewise, microvascular factors were reliably determined from 134 (80%) specimens. MLN and microvascular factors were both available for 113 (68%) tumors.

The median MLN based on the 10 largest nucleoli per tumor was 4.05 μm (range, 2.60–6.18), and the mean was 4.06 ± 0.54 μm (SD). The mean and range were of the same order of magnitude; Fig. 2B). Nevertheless, notable overlap was observed between categories (Figs. 2A, 2B; Table 2). No significant association was observed between MLN and gender, involvement of the ciliary body, LBD (P = 0.24, Spearman correlation; Fig. 2C), tumor pigmentation, presence of tumor-infiltrating macrophages, and microvascular loops and networks (P = 0.62, nonparametric test for trend; Fig. 2D; Table 2).

Kaplan-Meier Analysis of Survival

At the end of follow-up, 26 (21%) of 126 patients were alive, 60 (48%) had died of metastatic uveal melanoma, 8 (6%) of a histopathologically verified second cancer, 51 (25%) of unrelated nonmalignant disease, and 1 of an unknown cause.

Both melanoma-specific and all-cause survival rates were significantly associated with MLN (P = 0.0060 and P = 0.015, respectively, log-rank test for trend; Figs. 3A, 3B). The 10-year Kaplan-Meier estimate for melanoma-specific survival was 0.74 (95% CI, 0.56–0.85) for small, 0.60 (95% CI, 0.44–0.74) for medium, and 0.42 (95% CI, 0.25–0.58) for large MLN.

Melanoma-specific survival was also associated with LBD (P = 0.0007, log-rank test for trend; Fig. 3C), the presence of epithelioid cells (P < 0.0001, log-rank test; Fig. 3D) and microvascular loops and networks (P = 0.0001, log-rank test for trend; Fig. 3E), and MVD (P = 0.0001; Fig. 3F). The Kaplan-Meier estimate for 10-year melanoma-specific survival was 0.80 (95% CI, 0.64–0.90) if no loops, 0.48 (95% CI, 0.27–0.67) if loops were present without networks, and 0.40 (95% CI, 0.25–0.55) if loops forming networks were present. The corresponding estimates according to tertiles of MVD were 0.86 (95% CI, 0.70–0.94), 0.50 (95% CI, 0.33–0.65), and 0.41 (95% CI, 0.24–0.57), respectively.

Cox Regression Analysis of Survival

By univariate analysis, MLN was significantly associated with melanoma-specific survival, whether modeled as a continuous variable (HR 1.82 for each micrometer increase; P = 0.016) or a categorical one (HR 1.57 for each category increase, by tertile; P = 0.007; Table 3).

The second observer who graded a subset of 63 tumors drew conclusions qualitatively and quantitatively similar to those of the first observer, based on the same set (hazard ratio, 2.0 vs. 1.6; Wald χ² 5.29 vs. 4.80, P = 0.021 vs. 0.029, respectively). In addition, four of five of the bivariate associations reported for the entire data set of 126 patients (described later) were also identified by using data of either observer for the 63 patients.

The presence of ciliary body involvement; large LBD; presence of epithelioid cells, high grade of pigmentation; presence of microvascular loops and networks, as modeled by assuming networks to be an advanced stage of loops (HR 1.80 for each category increase; P < 0.001); and high MVD (HR 1.02 for each unit increase in square-root-transformed count; P < 0.001)
were also significantly associated with melanoma-specific survival (Table 3). MLN remained an independent predictor of prognosis, both as a continuous and categorical variable when adjusted in turn for the effect of ciliary body involvement, LBD, presence of epithelioid cells, and microvascular loops and networks (Table 3). When adjusted for MVD, it was of borderline significance as a continuous variable (P = 0.11; Table 3). Of the five bivariate models tested, those that combined MLN with cell type and MVD best predicted melanoma-specific survival, whether MLN was modeled as a continuous or categorical variable (e.g., model 3A vs. 4A, difference in −2 log likelihood 465.7 – 448.9 = 16.8, 1 df, P < 0.001; model 4A vs. 2A, 494.1 – 465.7 = 28.4, 1 df, P < 0.001 with Bonferroni correction). The model that combined MLN with microvascular loops and networks was significantly better than those that combined MLN with ciliary body involvement and LBD (e.g., model 4A vs. 2A, 494.1 – 465.7 = 28.4, 1 df, P < 0.001 with Bonferroni correction).

When the four best predictors were combined in trivariate models (see Table 3 for models that included MVD), MLN lost statistical significance when modeled as a continuous variable (P = 0.11–0.18) but retained significance as a categorical one (P = 0.023–0.067). No model was significantly better than the others. Combined in a single model, all four variables remained independent predictors of prognosis when MLN was modeled as a categorical variable (P = 0.084, model 8B), but as a continuous one, MLN was not statistically significant (P = 0.28, model 8A). Compared with the best trivariate models, the final models predicted survival significantly better (e.g., model 8B vs. 7B, 416.8 – 402.4 = 14.4, 1 df, P < 0.001; Bonferroni adjustment for three possible comparisons).

**DISCUSSION**

The mean MLN in this population-based, consecutive series of patients with primary choroidal and ciliary body melanoma was 3.71 to 4.06 μm, depending on the observer—on average, somewhat larger than in previous studies.6–8,15,17 The range of observations, however, has been rather consistent between all studies.

Several factors affect mean MLN. Nucleoli measured in silver rather than hematoxylin-eosin-stained sections appear significantly larger.17 The mean difference in one comparative series was 0.29 μm overall, and as high as 0.44 μm for patients who died of melanoma.17 If the horizontal rather than the longest diameter of nucleoli is measured, obviously, MLN will be smaller.15 When MLN is sampled from the entire tumor, the mean value is reported to be 0.15 μm larger than if a 5-mm linear field through tumor center is scanned.17 Because MLN is associated with death from uveal melanoma,5–7,10,15,17 enrollment criteria affect the mean value. Most previous studies were based on analysis of unconventional, selected data sets in which one half of patients died of melanoma and the other half survived for at least 10 years without metastasis,2,4,7,17 or a variation thereof.15 Because these analyses excluded patients who survived in the short term but were still at risk of dying of uveal melanoma, the mean MLN was probably biased toward

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**Table 2. MLN According to Clinicopathological Characteristics of 126 Choroidal and Ciliary Body Melanomas**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Median (Range)</th>
<th>Mean (SD)</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>3.98 (2.60–6.18)</td>
<td>4.01 (0.56)</td>
<td>0.21*</td>
</tr>
<tr>
<td>Male</td>
<td>4.18 (2.70–5.18)</td>
<td>4.12 (0.49)</td>
<td></td>
</tr>
<tr>
<td>Tumor location</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Choroid only</td>
<td>4.02 (2.60–6.18)</td>
<td>4.04 (0.55)</td>
<td>0.44*</td>
</tr>
<tr>
<td>Ciliary body involved</td>
<td>4.13 (2.91–4.95)</td>
<td>4.11 (0.51)</td>
<td></td>
</tr>
<tr>
<td>Largest basal diameter</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤10</td>
<td>3.85 (2.70–4.86)</td>
<td>3.90 (0.50)</td>
<td>0.11*/0.23†</td>
</tr>
<tr>
<td>&gt;10–15</td>
<td>4.17 (2.86–6.18)</td>
<td>4.15 (0.55)</td>
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<tr>
<td>&gt;15</td>
<td>4.05 (2.60–5.18)</td>
<td>4.05 (0.56)</td>
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</tr>
<tr>
<td>Cell type</td>
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<td></td>
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</tr>
<tr>
<td>Spindle</td>
<td>3.96 (2.70–6.18)</td>
<td>4.02 (0.56)</td>
<td>0.017*</td>
</tr>
<tr>
<td>Nonspindle</td>
<td>4.27 (3.32–5.18)</td>
<td>4.24 (0.45)</td>
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<tr>
<td>Pigmentation</td>
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<tr>
<td>Weak</td>
<td>4.06 (2.86–6.19)</td>
<td>4.07 (0.64)</td>
<td></td>
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<tr>
<td>Medium</td>
<td>4.06 (3.02–5.18)</td>
<td>4.10 (0.47)</td>
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<tr>
<td>Strong</td>
<td>4.01 (2.70–4.86)</td>
<td>4.02 (0.55)</td>
<td></td>
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<tr>
<td>Macrophages</td>
<td></td>
<td></td>
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<tr>
<td>Few</td>
<td>4.18 (2.86–5.18)</td>
<td>4.07 (0.51)</td>
<td>0.70*/0.84†</td>
</tr>
<tr>
<td>Moderate</td>
<td>4.08 (3.02–4.86)</td>
<td>4.12 (0.46)</td>
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</tr>
<tr>
<td>Many</td>
<td>3.79 (2.60–4.54)</td>
<td>3.71 (0.52)</td>
<td></td>
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<tr>
<td>Microvascular pattern</td>
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<td></td>
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<tr>
<td>No loops</td>
<td>4.06 (2.70–6.18)</td>
<td>4.07 (0.59)</td>
<td>0.83*/0.62†</td>
</tr>
<tr>
<td>Loops only</td>
<td>4.22 (2.86–4.95)</td>
<td>4.08 (0.60)</td>
<td></td>
</tr>
<tr>
<td>Networks</td>
<td>4.06 (3.38–4.86)</td>
<td>4.12 (0.41)</td>
<td></td>
</tr>
<tr>
<td>Microvascular density</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤23 vessels</td>
<td>3.87 (2.70–4.95)</td>
<td>3.88 (0.49)</td>
<td>0.0029*/0.017†</td>
</tr>
<tr>
<td>24–42 vessels</td>
<td>4.27 (2.86–4.86)</td>
<td>4.25 (0.39)</td>
<td></td>
</tr>
<tr>
<td>&gt;42 vessels</td>
<td>4.15 (3.02–6.18)</td>
<td>4.19 (0.38)</td>
<td></td>
</tr>
</tbody>
</table>

* Kruskal-Wallis test, two-tailed.
† Nonparametric test for trend, two-tailed.
lower values. Finally, the measurements of one observer may be higher than those of another. One study found a mean difference of 0.20 H9262 m,8 and in our study it was 0.38 H9262 m.

In addition to interobserver variation, use of a population-based data set and silver staining probably contributed to the larger than average mean MLN in our study. If differences in measuring remain consistent from specimen to specimen, they should not seriously affect the prognostic association of MLN. In our data set, MLN was significantly associated with death of uveal melanoma, and this result was not affected by interobserver variation in measuring MLN. The 10-year cumulative proportion of patients who died was 0.32 units larger if patients had a melanoma with a large rather than small MLN, and Cox regression estimated the risk of dying to be 3.1 times higher for the former group. In another consecutive series, the 10-year survival difference was estimated to be 0.47.6

With one exception, MLN has consistently been associated with melanoma-specific mortality by univariate analysis.2,4,7,10,17 For each 1- H9262 m change, the regression coefficient has ranged from 0.58 to 1.27.2,4,7,10,17 Our estimate falls within this range. Most previous estimates were based on the selected data sets mentioned earlier herein,2,4,7,15,17 which may have introduced bias in estimating effect size and in hypothesis testing.

MLN was significantly higher in melanomas that contained epithelioid cells,15,17 and we found no significant difference in MLN according to ciliary body involvement15 and presence of microvascular loops and networks,8,15 as previously reported. MLN was not significantly associated with LBD, in contrast to previous reports that found a weak to moderate correlation.8,15,17 A new finding was that MLN correlated positively with MVD.

Despite the association between MLN and presence of epithelioid cells, both independently predicted survival by bivariate Cox regression, confirming some15 but not all previous analyses.2 Moreover, this model fitted significantly better to the data than competing ones that included ciliary body involvement, LBD, and microvascular loops and networks. Our findings that MLN retained significance when adjusting for LBD,2,4,5 loops,10 and networks15 are in line with previous multivariate studies. It is consequently well established that MLN and microvascular loops and networks in uveal melanoma are unrelated, but other microvascular patterns have not been evaluated in this regard.
MVD is emerging as an important prognostic indicator that is independent of microvascular loops and networks in uveal melanoma. It is evaluated by antibodies to endothelial cell markers—in particular, factor VIII-related antigen and the CD34 epitope. It is not known for certain whether all elements labeled with these antibodies, particularly CD34, are truly endothelial cells. Evidence has been provided that a population of uveal melanoma cells may be immunopositive, suggesting a theory that the association of MVD with prognosis may, at least in part, reflect presence of more aggressive tumor cells that share features with endothelial ones.

When MLN and MVD were entered into a bivariate Cox model as continuous variables, the independent association of MLN with survival weakened, but the fit of the model significantly improved, compared with competing models. The fit further improved if multivariate models that additionally included microvascular loops and networks and epithelioid cells were constructed, with further erosion in the independent prognostic significance of MLN. However, if divided in tertiles and modeled as a categorical variable, MLN retained independent association with prognosis in multivariate models. The difference may in part depend on the relatively small sample size.

That microvascular factors and nucleolar size independently predicted prognosis in uveal melanoma is consistent with a hypothesis that they represent, to a significant extent, different processes or stages that contribute to metastatic efficiency, such as the ability to invade and seed metastases and the ability to proliferate. In addition to reflecting aggressiveness of uveal melanoma cells, loops, networks, and hot spots that contribute to high MVD harbor vascular channels that may directly be utile to high MVD harbor vascular channels that may directly be.
active transcription, translation, and gene activation. The ability to seed metastasis and to proliferate are likely to be interrelated, and we observed that MVD explains some of the prognostic association of MLN. Because uveal melanoma cells sometimes seem to express antigens on which measurement of MVD is based, one could assess nucleoli in tumors suspected of containing cells that share these antigens to look for a relationship at the cellular level.

In conclusion, using an independent population-based data set, we were not only able to confirm that MLN and microvascular loops and networks are unrelated, independent predictors of survival in uveal melanoma but also that multivariate models that include MVD in addition to MLN can step forward in managing patients with uveal melanoma. Progress in resection techniques is likely to not fully capture the process of clinical metastasis from the primary tumor. Progress in resection techniques is likely to provide in the near future fresh material for the ophthalmic pathologist to correlate angiographic data with genetic and histopathologic characteristics such as MLN.

References


