Intravascular Presence of Tumor Cells as Prognostic Parameter in Uveal Melanoma: A 35-Year Survey

Long V. Ly, Omar F. F. Odish, Didi de Wolff-Rouendaal, Guy S. O. A. Missotten, Gregorius P. M. Luyten, and Martine J. Jager

PURPOSE. Invasion of tumor cells into blood vessels is essential for metastasis of uveal melanoma. The occurrence of ingrowth of tumor cells in blood vessels in uveal melanoma was analyzed, and this parameter was compared with the survival of the patients.

METHODS. Between 1972 and 2007, 643 eyes primarily enucleated for uveal melanoma were evaluated histopathologically. Survival data were obtained from charts and from the Integral Cancer Center patient registry.

RESULTS. No vascular ingrowth of tumor cells occurred in 59% of the eyes, whereas 18% had tumor cell ingrowth in vessels inside the tumor, 10% in vessels outside the tumor, and 8% in vessels inside as well as outside the tumor. The presence of any intravascular ingrowth of tumor cells correlated significantly with the diameter (P < 0.01) and prominence of the tumor (P < 0.01), as well as with non-spindle-cell type (P = 0.03) and intrascleral ingrowth (P < 0.01), and was associated with a worse survival. When extravascular matrix patterns were not included in the multivariate analysis, intravascular ingrowth came out as an independent prognostic factor, but this was not the case when extravascular matrix patterns were included in the multivariate model.

CONCLUSIONS. Intravascular ingrowth of tumor cells in uveal melanoma occurs frequently in combination with well-known histopathologic factors such as large tumor size, epithelioid cell type, and intrascleral ingrowth. (Invest Ophthalmol Vis Sci. 2010;51:658 – 665) DOI:10.1167/iovs.09-3824

Uveal melanoma is the most common primary intraocular malignancy in adults, with an overall incidence of approximately six cases per million in the Western world.1,2 Many different treatment modalities exist for the intraocular tumor, but these therapies seem not to be effective in preventing metastases. This study was conducted to elucidate the importance of tumor cell ingrowth into blood vessels in uveal melanoma and to determine the prognostic importance of this phenomenon in a long-term study.

MATERIALS AND METHODS

Patients

Between August 1972 and August 2007, 715 eyes with uveal melanoma were enucleated in the Netherlands. The patients were observed, and when death occurred, the date was recorded. In addition to the patients’ charts, the database of the Integral Cancer Center West was used, which registers data on metastases and checks the survival status of each patient with uveal melanoma on a yearly basis. In the Netherlands, cause of death is reported according to a standard protocol to
Intravascular Presence of Tumor Cells in Uveal Melanoma

Pathology Specimens

Enucleation specimens were fixed in 4% buffered neutralized formalin. Between August 1972 and March 1995, enucleated eyes were embedded in celloidin after fixation for 48 hours and cut into 12-μm-thick mounted sections. The remaining sections were stored in 70% alcohol. From March 1995 onward, enucleated eyes were embedded in paraffin and serial sections of 4 to 5 μm were made of every globe and mounted on glass slides. From the embedded part of the eye, serial sections were made and every 10th section was mounted on a slide and examined. Some slides were stored for later diagnostic staining. Routine staining was performed with hematoxylin and eosin, and since January 1991, one periodic acid-Schiff (PAS) stain has been made of a representative section of each eye for examining extravascular matrix patterns.

Histopathologic Examination

During 35 years, hematoxylin and eosin 12-μm celloidin- and 4-μm paraffin-embedded sections have been reviewed by one ocular pathologist (DdWR) in a standard fashion for confirmation of the diagnosis and evaluated for histologic parameters, including tumor location, largest basal diameter (in millimeters), prominence (apical height; in millimeters), cell type, intravascular ingrowth of tumor cells, intrascleral ingrowth, and the PAS-stained slides for examining extravascular matrix patterns, such as loops and networks. Largest basal diameter was determined by measuring the curve-shaped base of the tumor on the pathologic slide. Extravascular matrix patterns have been scored since January 1991.15

Intravascular tumor cell growth was classified into four categories: no ingrowth, ingrowth in vessels inside the tumor, ingrowth in vessels outside the tumor (i.e., in an intrascleral thin-walled blood vessel, sometimes reaching the ocular surface, like the vorticose vein; Fig. 1), and ingrowth in vessels both inside and outside of the tumor. Intrascleral tumor growth curling around emissary nerves and transscleral blood vessels, thereby reaching the ocular surface, was not considered intravascular tumor growth as these tumor cells did not invade the blood stream.

The trabecular meshwork with its connection to Schlemm’s canal and aqueous veins was considered vascular tissue; therefore tumor growth into the trabecular meshwork was scored as ingrowth of tumor cells in vessels outside the melanoma.

Intrascleral ingrowth of tumor cells was classified into four different categories: no invasion of tumor cells, superficial (< one half of the sclera), deep (one half to three fourths of the sclera), and total scleral invasion. Episcleral and extraocular growth were included in the category total scleral invasion.

COMS criteria were applied to reclassify the diameter and prominence of the tumors, as recorded in the original pathology report, into the three groups small, medium, and large.19,20 The primary tumors were assessed on their pT categories (T1–T4) according the AJCC/UICC TNM classification (sixth edition).21,22

Data Analysis

ANOVA testing was used for comparing multiple groups (SPSS for Windows, ver. 12.0.1; SPSS, Chicago, IL). Tumor characteristics among categorized groups were compared with the χ² test. Survival was assessed with the Kaplan-Meier survival analysis accompanied by the log rank test. Patients were censored when they died of a cause other than metastasis of uveal melanoma. Univariate Cox proportional hazards modeling was used to evaluate the prognostic value of the different histopathologic parameters. Besides visual inspection of the log-minus-log curves, we also performed Cox regression analyses including interaction terms of relevant covariates with time in the model to assess the proportionality of the hazards. In case when the proportional hazards seemed to be violated, we kept the interaction term in the multivariate model. Multivariate analysis identified the independent significant prognostic variables for survival. P < 0.05 was considered to be statistically significant.
**RESULTS**

Between 1972 and 2007, 715 eyes with a uveal melanoma were enucleated: 643 (90%) eyes were primarily enucleated, whereas 72 (10%) eyes had received prior treatment: TTT (transpupillary thermotherapy) in 20 (3%), sandwich therapy (ruthenium plaque irradiation combined with TTT) in 11 (2%), ruthenium plaque enucleation: 643 (90%) eyes were primarily enucleated, whereas 72 (10%) eyes had received prior treatment: TTT (transpupillary thermotherapy) in 20 (3%), sandwich therapy (ruthenium plaque irradiation combined with TTT) in 11 (2%), ruthenium plaque irra-

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**Table 1. Baseline Characteristics of Patients and Histologic Data of Primarily Enucleated Eyes**

<table>
<thead>
<tr>
<th>Categorical Variables</th>
<th>Baseline Data</th>
<th>Intravascular Ingrowth of Tumor Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>% of n = 643</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>333</td>
<td>52%</td>
</tr>
<tr>
<td>Female</td>
<td>310</td>
<td>48%</td>
</tr>
<tr>
<td>Eye</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>511</td>
<td>80%</td>
</tr>
<tr>
<td>Left</td>
<td>322</td>
<td>20%</td>
</tr>
<tr>
<td>pTNM classification (6th edition)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>84</td>
<td>13%</td>
</tr>
<tr>
<td>T2</td>
<td>364</td>
<td>57%</td>
</tr>
<tr>
<td>T3</td>
<td>150</td>
<td>23%</td>
</tr>
<tr>
<td>T4</td>
<td>39</td>
<td>6%</td>
</tr>
<tr>
<td>Ciliary body involvement</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not present</td>
<td>514</td>
<td>80%</td>
</tr>
<tr>
<td>Present</td>
<td>122</td>
<td>19%</td>
</tr>
<tr>
<td>Cell type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spindle</td>
<td>263</td>
<td>41%</td>
</tr>
<tr>
<td>Mixed+epithelioid</td>
<td>366</td>
<td>57%</td>
</tr>
<tr>
<td>Intrascleral ingrowth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>52</td>
<td>8%</td>
</tr>
<tr>
<td>Superficial</td>
<td>331</td>
<td>52%</td>
</tr>
<tr>
<td>Deep</td>
<td>127</td>
<td>20%</td>
</tr>
<tr>
<td>Total sclera/episcleral</td>
<td>117</td>
<td>18%</td>
</tr>
<tr>
<td>Loops and/or networks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not present</td>
<td>76</td>
<td>12%</td>
</tr>
<tr>
<td>Present</td>
<td>140</td>
<td>22%</td>
</tr>
</tbody>
</table>

**Intravascular Ingrowth of Tumor Cells**

<table>
<thead>
<tr>
<th>Numerical Variables</th>
<th>Baseline Data</th>
<th>None†</th>
<th>Inside†</th>
<th>Outside†</th>
<th>Both†</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age, y</td>
<td>58.8 (±15.0)</td>
<td>57.2 (±15.0)</td>
<td>58.6 (±15.5)</td>
<td>63.0 (±13.8)</td>
<td>63.5 (±13.4)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Mean diameter, mm</td>
<td>11.4 (±3.6)</td>
<td>10.7 (±3.6)</td>
<td>11.7 (±2.9)</td>
<td>12.7 (±3.2)</td>
<td>14.1 (±4.4)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Mean prominence, mm</td>
<td>5.7 (±3.2)</td>
<td>5.3 (±3.3)</td>
<td>6.3 (±3.0)</td>
<td>6.1 (±2.7)</td>
<td>7.0 (±3.2)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Associations between intravascular ingrowth of tumor cells (none, no ingrowth of tumor cells in vessels; inside, ingrowth of tumor cells in vessels outside the tumor; outside, ingrowth of tumor cells in vessels outside the tumor; both, ingrowth of tumor cells inside and outside the tumor) and other histologic parameters. P-values for categorical parameters were obtained by χ² test and for the numerical data by ANOVA testing. Total, the number of patients in baseline data with this specific characteristic.

*p of n = 643 is the percentage of all patients with primarily enucleated eyes.
† Percentages in the columns of intravascular ingrowth represent the distribution of the total patients in each subpopulation of a categorical variable.

Most tumors (59%) showed no ingrowth of tumor cells in any blood vessels, 18% of the patients had tumor cell ingrowth in vessels inside the tumor, 10% in vessels outside the tumor, and 8% in vessels both inside and outside the tumor. With regard to scleral ingrowth of tumor cells, superficial ingrowth (52%) occurred most frequently.

The mean follow-up of patients was 8.8 years (SD ± 8.9 months), with a range of 0 to 31.6 years. Of all patients, 48% were alive at the last follow-up, 32% had died of metastases, and 21% had died of other causes. Eleven (2%) patients of the 643 were lost to follow-up. Other baseline characteristics can be found in Table 1.

**Association of Intravascular Ingrowth of Tumor Cells with Other Histopathologic Parameters**

Tumor cell ingrowth in vessels inside and outside the tumor was associated with a higher pT category and with scleral ingrowth (both P < 0.01; χ² test): A deeper scleral penetration was associated with more frequent tumor cell invasion in any blood vessels (Table 1, Fig. 2). Furthermore, ingrowth of tumor cells in vessels was positively correlated with the presence of...
epithelioid cells \( (P = 0.03; \chi^2 \text{ test}) \), higher age, a larger tumor diameter, and a greater tumor prominence \( (P < 0.001, P < 0.01, \text{ and } P < 0.01 \text{ respectively, ANOVA test}; \text{ Table 1}) \).

Survival Analysis

Kaplan-Meier analysis showed that patients with tumor cells in blood vessels had a significantly worse survival than did patients, who had no tumor cells in vessels \( (\chi^2 = 31.5 \text{ and } P < 0.01, \text{ Fig. 3 for the survival curve}) \).

Univariate Cox analysis demonstrated that intravascular ingrowth of tumor cells had a hazard ratio \( (HR) \) of 2.01 \( (P < 0.01) \) for vessels inside the tumor, of 2.29 \( (P < 0.01) \) with ingrowth in vessels outside the tumor, and of 2.49 \( (P < 0.01) \), if ingrowth occurred in both locations, compared with no vascular invasion of tumor cells. HRs for the other parameters can be found in Table 2.

We also plotted survival graphs, in which patients were categorized according to specific prognostic factors and analyzed whether ingrowth of tumor cells in blood vessels led to a different survival within those categories (Fig. 4). Except with regard to the absence of loops and/or networks, ingrowth of tumor cells into blood vessels constituted a significant additional risk factor for mortality.

Multivariate Analysis

Multivariate Cox regression analysis was performed to determine whether intravascular ingrowth is an independent significant prognostic factor for survival. Since extravascular matrix patterns have been scored since 1991, data are known concerning this parameter in 216 patients. We analyzed two multivariate models, one with and one without the presence of loops and networks as parameter. In the model without extravascular matrix patterns, ciliary body ingrowth \( (HR = 4.77, P < 0.01) \), largest basal diameter \( (HR = 1.02, P = 0.01) \), the presence of epithelioid cells \( (HR = 1.05, P = 0.04) \), age \( (HR = 1.03, P < 0.01) \), and intravascular ingrowth \( (HR = 1.60, P < 0.01) \) were independent prognostic factors for survival (Table 3A). If extravascular matrix patterns were added to this model, intravascular ingrowth turned out not to be a significant factor anymore, but ciliary body ingrowth \( (HR = 1.49, P = 0.02) \), largest basal diameter \( (HR = 1.11, P < 0.01) \), the presence of epithelioid cells \( (HR = 4.12, P < 0.01) \), and having extravascular matrix patterns “loops” and/or “networks” \( (HR = 4.43, P < 0.01) \) were independent predictive parameters for the survival of patients (Table 3B).

DISCUSSION

Several reports mention ingrowth of tumor cells into blood vessels in different types of malignancy, showing that the presence of tumor cells inside a blood vessel is associated with a worse survival.\(^2\) However, there is only a limited number of studies in uveal melanoma concerning ingrowth of tumor cells in blood vessels.\(^1\) In our center, 715 eyes were enu-
We observed that the presence of scleral invasion was associated with an increased frequency of tumor cell ingrowth in vessels inside and outside of the tumor. In general, melanomas use transscleral structures such as vessels and nerves to reach extracocular structures (e.g., the episclera); in those cases the tumor grows around the blood vessels and/or nerves. Regarding intravascular ingrowth: once tumor cells find their way inside a blood vessel, the bloodstream will carry the tumor cells into blood vessels was the patient’s age. In several articles, advanced age has been associated with a larger basal diameter, and multivariate analysis showed this to be one of the most reliable independent prognostic markers for metastasis development. However, RT-PCR positive results were observed in only 10% of all cases, whereas in 25% of the patients’ metastases occurred during the follow-up period. This result shows that an observation taken at one moment in time may not provide a complete picture.

We also observed that ciliary body involvement was associated with scleral invasion. This phenomenon can be explained by the fact that tumors located in the ciliary body have a simple route for invading the sclera via the trabecular meshwork and so can easily infiltrate the episcleral structures. This finding is also in line with the literature: Seddon et al.32 explained that contraction of the ciliary muscle facilitates the spread of tumor cells into the blood stream, leading to metastasis, and thus a bad prognosis.

An additional factor that was related to ingrowth of tumor cells into blood vessels was the patient’s age. In several articles, advanced age has been associated with a larger basal diameter, non–spindle-cell type and extracapsular extension, all of which are known prognostic factors for melanoma-related death.33–36 This was also the case in our study (data not shown). As tumor size and non–spindle-cell type were both clearly related to the presence of epithelioid cells is also associated with intravascular growth. In previous studies by Folberg et al.,29 and Vaupe and Gabbert30 it was described that different cell types determined the growth pattern of normal vessels and extracapsular matrix patterns in the tumor matrix. The different growth pattern of epithelioid cells compared to spindle cells can compress or even influence the constitution of the vascular lining, making it easier for tumor cells to invade the blood vessel.

Folberg et al.29 demonstrated previously with confocal microscopy that several patients, who had vascular loops in their tumors, lacked intravascular ingrowth of tumor cells and vice versa. Our larger study confirms this observation.

Although 94 patients had no apparent ingrowth of tumor cells into blood vessels, they still developed metastasis. There are several possible explanations for this observation: one could be that we did not analyze the section in which tumor cells invaded a blood vessel. Another explanation could be that the tumor cells metastasized via the extravascular matrix patterns, and ingrowth into blood vessels is not absolutely needed. Another physiological explanation for this observed phenomenon could be that the tumor cells at one time invaded the blood stream, but after this process, the defect in the vessel wall was repaired. It may be that the histopathologic situation after enucleation is not representative of what previously happened in the tumor, since tumor cells may have been shed already, and the normal vessel structure restored. Schuster et al.31 described that they could detect melanocyte-derived antigens with RT-PCR in peripheral blood samples of patients with uveal melanoma, and multivariate analysis showed this to be one of the most reliable independent prognostic markers for metastasis development. However, RT-PCR positive results were observed in only 10% of all cases, whereas in 25% of the patients’ metastases occurred during the follow-up period. This result shows that an observation taken at one moment in time may not provide a complete picture.

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The presence of intravascular tumor cell ingrowth, which may explain the observed correlation with old age. Another explanation may be related to the innate immune system: recent findings in experimental models show a relation between age and blood vessel growth. Apte et al. and Espinosa et al. demonstrated that blood vessel growth in old mice differed from young mice: When retinal neovascularization was induced by laser, young mice showed limited neangiogenesis, while old mice developed massive angiogenesis. Macrophages were thought to play a major role: proangiogenic M2-type macrophages are considered essential in helping tumor cells invade structures, such as blood vessels and the scleral matrix, by changing the integrity and construction of the tissue. Therefore, age may have important consequences for blood vessel growth and invasion of tumor cells into these structures, leading to more potential for metastatic spreading. Of interest, the presence of a high number of macrophages in uveal melanoma has been found to be associated with tumor size, the presence of epithelioid cells, and other unfavorable prognostic factors, demonstrating the relevance of macrophages.

Our data demonstrate that the presence of tumor cell invasion in blood vessels is associated with worse survival. Our survival results must be interpreted with care. Kujala et al. described that survival could be overestimated with Kaplan-Meier analysis for patients with a long follow-up, since death...
related to melanoma after the first event contributes more to the estimate. However, multivariate Cox regression analysis demonstrated that if all well-known prognostic factors, such as ciliary body involvement, largest basal diameter, epithelioid cell type, and the presence of loops and/or networks are put into a model, all were independent prognostic factors, similar to results of Seregard and Kock. Intravascular ingrowth is only an independent prognostic factor when extravascular matrix patterns are not added to the set of parameters analyzed.

We show in this study that it is not necessary to add extravascular ingrowth of tumor cells to the set of prognostic parameters that need to be analyzed, since some better prognostic markers, such as extravascular matrix patterns are known. Although histopathologic parameters are important predictors of survival in uveal melanoma, chromosomal analysis or microarray-based gene expression profiling may be even more precise predictors. However, that histopathologic analysis is still useful was shown by Damato et al. The best predictive index was not obtained from one test, but by using analysis of chromosome 3, basal tumor diameter, and cell type together.

References


