Inflammation and immunomodulation in uveal melanoma

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Chapter 6

Intravascular presence of tumor cells as prognostic parameter in uveal melanoma: a 35-year survey

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Abstract

Purpose: Invasion of tumor cells into blood vessels is probably essential for metastasis of uveal melanoma. We analysed the occurrence of ingrowth of tumor cells in blood vessels in uveal melanoma and compared this parameter with survival of the patients.
Material and methods: Between 1972 and 2007, 643 primarily enucleated eyes with uveal melanoma were evaluated histopathologically. For data analysis, we used the statistical program SPSS. 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, while 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 was significantly correlated with the diameter ($P < 0.01$) and prominence of the tumor ($P < 0.01$), as well as with non-spindle celltype ($P = 0.03$) 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.
Conclusion: Intravascular ingrowth of tumor cells in uveal melanoma occurs frequently in combination with well-known histopathological factors such as large tumor size, epithelioid cell type and intrascleral ingrowth.

Introduction

Uveal melanoma is the most common primary intraocular malignancy in adults with an overall incidence of around 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, which occur in about 50% of patients. The average survival after diagnosis of metastases is only 10 to 18 months[3]. Because of a lack of lymphatics within the uveal tract, uveal melanomas disseminate predominantly haematogeneously[4]. Due to this fact, vascular invasion of tumor cells is a prerequisite for the formation of metastases. Blood vessels have been an important area of investigation during the past decades, as vessel growth inhibition has been found to prevent tumor growth in many types of cancer[5-7].
The uvea is one of the most vascularised tissues of the human body, and blood vessels from the choroid are thought to extend into the uveal melanoma. In addition, it is assumed that the tumor itself creates blood vessels[8,9], which can be identified by fluorescein angiography[10,11]. Histologically, the density of blood vessels can be evaluated and specific areas with an increased microvascular density can be identified. Foss, Mäkitie and others showed that a high microvascular density (MVD) in primary uveal melanoma tissue is associated with a worse survival[12-14] .

In certain tumors, including uveal and cutaneous melanoma, specific fluid-conducting channels have been observed, which are known as extravascular matrix patterns, or as vascular mimicry[15]. The presence of specific patterns such as loops and networks is associated with metastatic disease. These fluid-conducting meshworks lack endothelium and do not stain with vessel markers CD 31 and CD 34, indicating they are not regular blood vessels. Erythrocytes may travel through these channels, and one can thus imagine that tumor cells could also migrate via these extravascular matrix patterns towards larger blood vessels[16].

The Leiden University Medical Center (LUMC) has been the main referral center for uveal melanoma in the Netherlands for almost 40 years. Since 1972, all enucleated eyes have been evaluated histologically by one pathologist according to a standard protocol. This analysis includes tumor cell infiltration into blood vessels inside the tumor, in transscleral vessels, and in the vortex veins; however, the prognostic value of this specific parameter has hardly been evaluated. In the medical literature, very little information is available on this parameter. One previous study by our group mentioned this parameter, but this study was limited in numbers and time[17], and Coupland recently studied extraocular extension in relation to vortex veins[18].
As mentioned above, many studies show the importance of blood vessels in tumor growth[6,7,13,15], and in the development of metastases. This study aims 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. Patients were followed 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 uveal melanoma patient on a yearly basis. In the Netherlands, cause of death is reported according to a standard protocol to the Central Bureau for Statistics (the CBS, the Hague, the Netherlands). Additionally, a specialized nurse registers information on clinical metastases or treatment for metastases. Follow-up time was measured in months. The data were updated in August 2007.
The research protocol followed the current revision of the tenets of the Declaration of Helsinki (world medical association declaration of Helsinki 1964; ethical principles for medical research involving human subjects).

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 onwards, enucleated eyes were embedded in paraffin and serial sections of 4-5µm were made of every globe and mounted on glass slides. From the embedded part of the eye, serial sections were made and each tenth section was mounted on a slide and examined. Some slides were stored for later diagnostic staining. Routine staining was performed with haematoxylin and eosin, and since January 1991, one Periodic acid-Schiff (PAS) stain is made of a representative section for examining extravascular matrix patterns.

Histopathological examination

During 35 years, hematoxylin- and eosin 12 mm celloidin and 4mm paraffin sections were reviewed by one ocular pathologist [DdWR] in a standard fashion for confirmation of the diagnosis, and evaluated for histological parameters, including tumor location, largest basal diameter (in mm), prominence (apical height) (in mm), 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 pathological 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 a intrascleral thin-walled blood vessel, sometimes reaching the ocular surface, like the vorticose vein (see Figure 1)), and ingrowth in vessels both inside as well as 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 the Schlemm's canal and aqueous veins was considered as 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, namely no invasion of tumor cells, superficial (< ½ of the sclera), deep (½ to ¾ of the sclera) and total scleral invasion. Episcleral and extra-ocular 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].

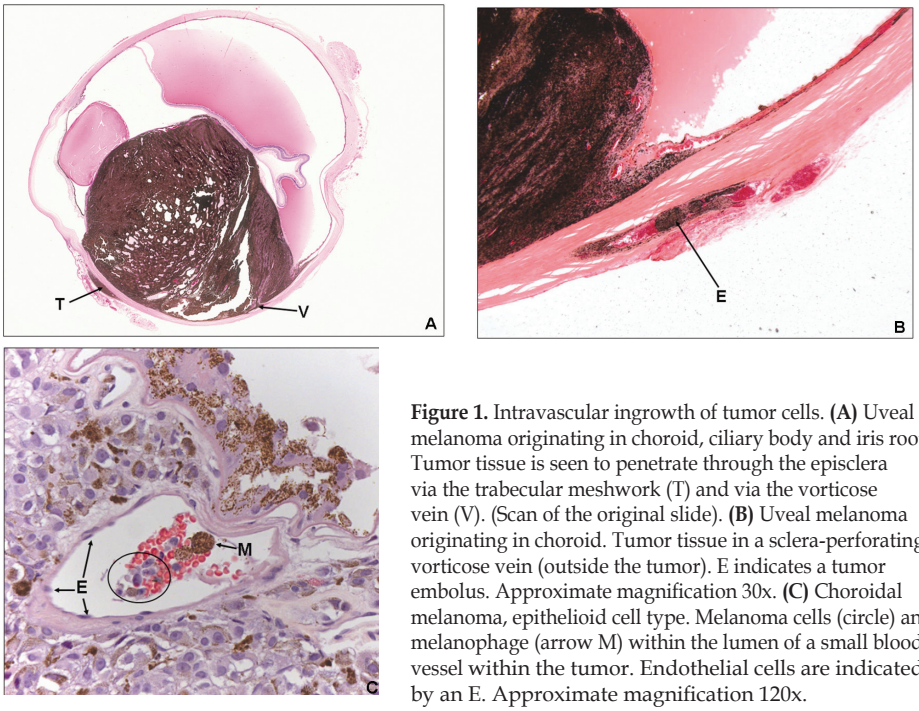

Figure 1. Intravascular ingrowth of tumor cells. **(A)** Uveal melanoma originating in choroid, ciliary body and iris root. Tumor tissue is seen to penetrate through the episclera via the trabecular meshwork (T) and via the vorticose vein (V). (Scan of the original slide). **(B)** Uveal melanoma originating in choroid. Tumor tissue in a sclera-perforating vorticose vein (outside the tumor). E indicates a tumor embolus. Approximate magnification 30x. **(C)** Choroidal melanoma, epithelioid cell type. Melanoma cells (circle) and melanophage (arrow M) within the lumen of a small blood vessel within the tumor. Endothelial cells are indicated by an E. Approximate magnification 120x.

Data analysis

All statistical analyses were performed with statistical software SPSS for Windows, release 12.0.1 (SPSS Inc., US). ANOVA testing was used for comparing multiple groups. Tumor characteristics among categorized groups were compared with the chi-square test.
Survival was assessed using the Kaplan-Meier survival analysis accompanied by the log rank test. Patients were censored, when they died of another cause than due to metastasis of uveal melanoma. Univariate Cox proportional hazards modeling was used to evaluate the prognostic value of the different histopathological 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. A p-value of less than 0.05 was considered to be statistically significant.

Results

Between 1972 and 2007, 715 eyes with a uveal melanoma were enucleated. 643 eyes (90%) were primarily enucleated, while 72 eyes (10%) had received prior treatment: TTT (transpupillary thermotherapy) in 11 (2%), Ruthenium plaque therapy in 20 (3%), sandwich therapy (Ruthenium plaque irradiation combined with TTT) in 28 (4%), and Proton Beam in 13 (2%) cases.
We only analysed the primarily enucleated eyes, since the group of eyes which had received prior treatment was relatively small and tumor characteristics may have been influenced by prior treatments.

Of the 643 enucleated patients, 333 (52%) were male and 310 (48%) were female. Mean age was 58.8 years (SD ± 15.0 years).
Tumors were classified as small in size in 14%, medium in 64%, and large in 21%, according to the COMS criteria and were mainly categorized as T2 (57%) or T3 (23%) according the TNM classification (6th edition) [21,22]. The mean prominence was 5.7 mm (SD ± 3.2 mm) and the mean diameter 11.4 mm (SD ± 3.6 mm).
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 as well as 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 % was still alive at the last follow-up, 32% had died due to metastases and 21 % had died from other causes. Eleven patients of the 643 (2%) were lost to follow-up. Other baseline characteristics can be found in Table 1.

Table 1. Baseline characteristics of patients and histological data of primarily enucleated eyes.

		Baseline data		Intravascular ingrowth of tumor cells					P value
Categorical variables		Total	% of N=643	Total N=612	None* N=376	Inside* N=118	Outside* N=67	Both* N=51	
Gender	Male	333	52 %	319	61 %	19 %	11 %	9 %	0.96
	Female	310	48 %	293	62 %	20 %	11 %	8 %	
Eye	Right	311	48 %	298	61 %	19 %	12 %	8 %	0.66
	Left	322	52 %	314	62 %	20 %	10 %	9 %	
pTNM classification (6th edition)	T1	84	13 %	82	88 %	5 %	4 %	4 %	*< 0.01*
	T2	364	57 %	348	53 %	26 %	13 %	8 %	
	T3	150	23 %	141	67 %	16 %	9 %	8 %	
	T4	39	6 %	36	56 %	6 %	11 %	28 %	
Cilary Body involvement	Not present	514	80 %	497	61 %	20 %	11 %	8 %	0.83
	Present	122	19 %	114	63 %	17 %	11 %	10 %	
Cell type	Spindle	263	41 %	252	67 %	19 %	8 %	6 %	*0.03*
	Mixed + Epithelioid	366	57 %	281	57 %	19 %	13 %	10 %	
Intrascleral ingrowth	None	52	8 %	51	90 %	8 %	0 %	2 %	*< 0.01*
	Superficial	331	52 %	318	66 %	21 %	9 %	4 %	
	Deep	127	20 %	124	55 %	21 %	12 %	12 %	
	Total/ episcleral	117	18 %	110	44 %	17 %	20 %	19 %	
Loops and/or networks	Not present	76	12 %	74	81 %	5 %	5 %	5 %	0.87
	Present	140	22 %	139	77 %	4 %	9 %	7 %	

	Baseline data	Intravascular ingrowth of tumor cells				P value
Numerical variables		None N = 376	Inside N = 118	Outside N = 67	Both N = 51	
Mean age	58.8 (±15.0)	57.2 (±15.0)	58.6 (±15.5)	63.0 (±13.8)	63.5 (±13.4)	*< 0.01*
Mean diameter (in mm)	11.4 (±3.6)	10.7 (±3.6)	11.7 (±2.9)	12.7 (±3.2)	14.1 (±4.4)	*< 0.01*
Mean prominence (in mm)	5.7 (±3.2)	5.3 (±3.3)	6.3 (±3.0)	6.1 (±2.7)	7.0 (±3.2)	*< 0.01*

P-values for categorical parameters were obtained by Chi-square test and for the numerical data by ANOVA testing. **Total:** the number of patients in baseline data with this specific characteristic. **% of N = 643:** percentage of all primarily enucleated patients. * Percentages in the columns of intravascular ingrowth represent the distribution of the total patients in each subpopulation of a categorical variable.

Association of intravascular ingrowth of tumor cells with other histopathological parameters

Tumor cell ingrowth in vessels inside as well as outside the tumor was associated with a higher pT categorie and with scleral ingrowth (both P<0.01; χ^2-test): a deeper scleral penetration was associated with more frequent tumor cell invasion in any blood vessels (see Table 1 and Figure 2). Furthermore, ingrowth of tumor cells in vessels was positively correlated with the presence of epithelioid cells (P = 0.03; χ^2-test), higher age, a larger tumor diameter, and a greater tumor prominence (P < 0.001, P < 0.01, and P < 0.01 respectively, ANOVA test; see Table 1).

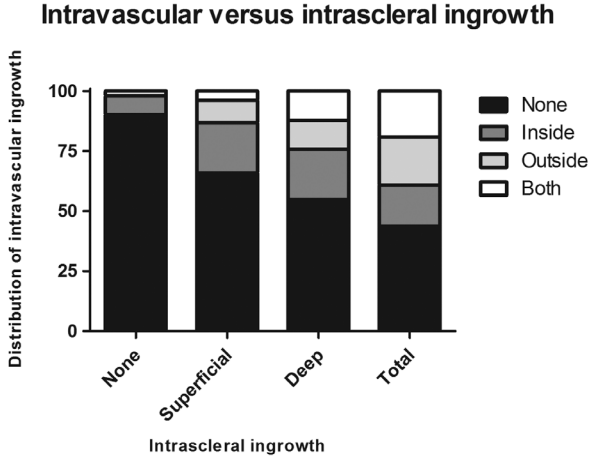

Figure 2. Distribution of intravascular ingrowth versus intrascleral ingrowth.

Survival analysis

Kaplan-Meier analysis showed that patients with tumor cells in blood vessels had a significantly worse survival compared to patients, who had no tumor cells in vessels (Log Rank test, $\chi^2 = 31.5$ and P < 0.01, see Figure 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. For the other parameters, hazard ratios can be found in Table 2. We also plotted survival graphs, in which patients were categorized according to specific prognostic factors and analysed whether ingrowth of tumor cells in blood vessels led to a different survival within those categories (Figure 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 survival.

Table 2. Cox proportional hazard survival analysis of different parameters with death due to metastasis as endpoint.

		Cox univariate			
		B	**P-value**	**HR**	**95% CI interval**
Gender	Male/ Female*	0.24	0.09	1.27	0.96-1.67
Eye	Right/ Left*	-0.08	0.57	0.92	0.70-1.22
pTNM classification (6th edition)	T1*	-	-	1	-
	T2	*1.31*	*<0.01*	*3.69*	*1.99-6.84*
	T3	*1.79*	*<0.01*	*5.97*	*3.07-11.57*
	T4	*2.27*	*<0.01*	*9.67*	*4.50-20.76*
Ciliary Body involvement	Present/ Not present*	*0.84*	*<0.01*	*2.31*	*1.69-3.17*
Cell type	Spindle*	-	-	1	-
	Mixed+ Epithelioid	*1.05*	*<0.01*	*2.86*	*2.09-3.92*
Intravascular ingrowth	None*	-	-	1	-
	In vessels inside tumor	*0.70*	*<0.01*	*2.01*	*1.44-2.81*
	In vessels outside tumor	*0.83*	*<0.01*	*2.29*	*1.52-3.46*
	In vessels both inside/outside	*0.91*	*<0.01*	*2.49*	*1.57-3.95*
Intrascleral ingrowth	None*	-	-	1	-
	Superficial	0.26	0.41	1.30	0.70-2.43
	Deep	0.60	0.07	1.83	0.95-3.53
	Total sclera	0.64	0.06	1.90	0.98-3.70
Loops and/or networks	Present/ Not present*	*1.65*	*<0.01*	*5.23*	*2.03-13.49*
Age	For each year	*0.03*	*<0.01*	*1.03*	*1.02-1.04*
Largest basal diameter	For each mm	*0.18*	*<0.01*	*1.20*	*1.16-1.24*
Prominence	For each mm	*0.13*	*<0.01*	*1.14*	*1.10-1.19*

* = indicates the reference group. HR = Hazard ratio.
For numerical variables, the HR is the hazard ratio for each unit (mm or years) for the parameter.
P-values ≤ 0.05 are shown in italics.

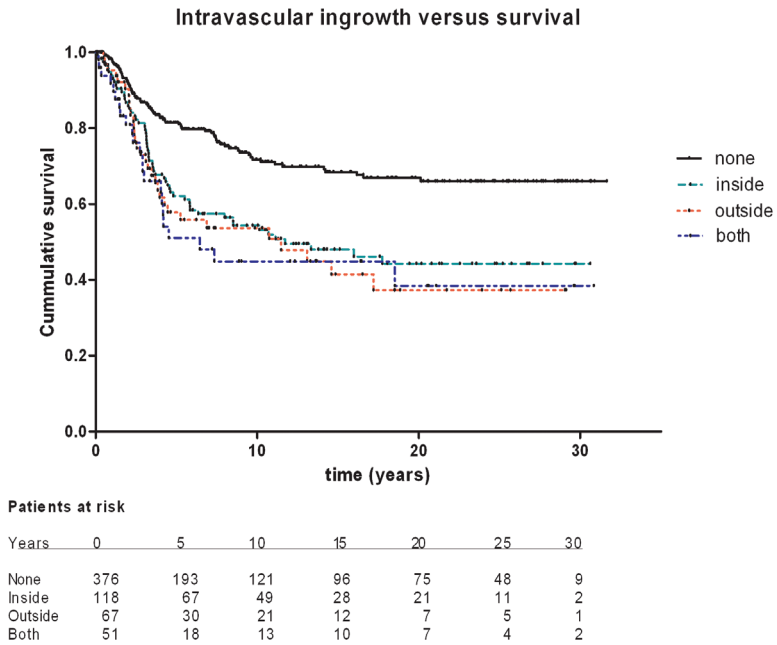

Figure 3. Kaplan-Meier survival curve of melanoma patients according to the different types of intravascular ingrowth. The numbers below represent the numbers of patients at risk.

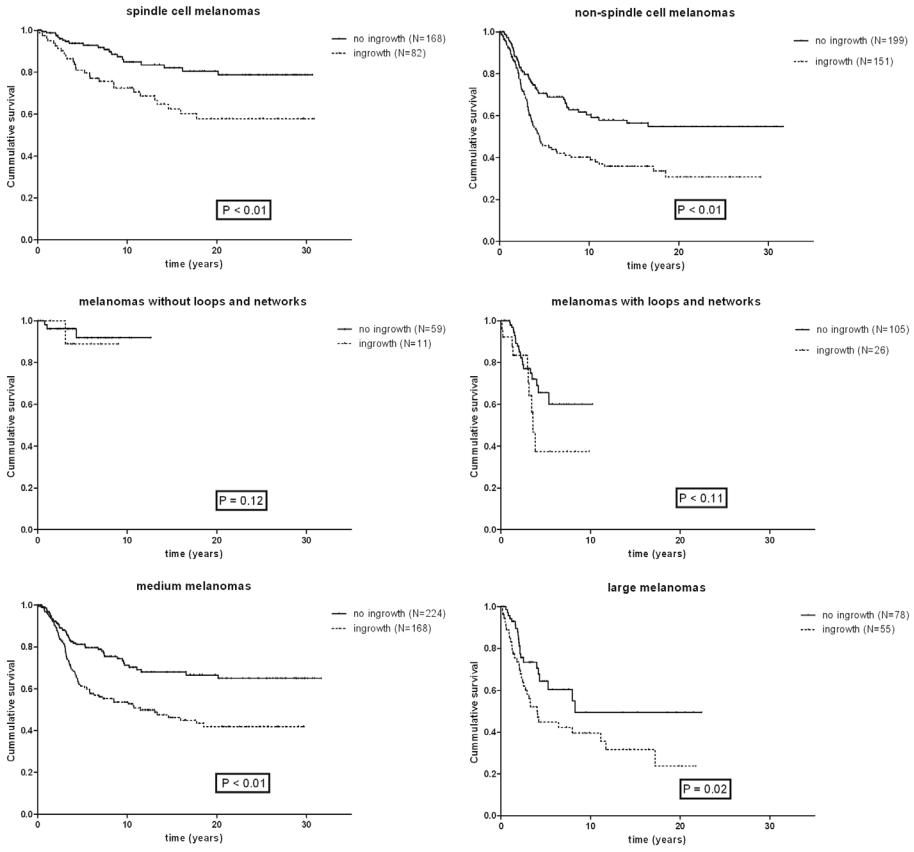

Figure 4. Kaplan-Meier survival graphs for intravascular ingrowth after categorization to some known independent prognostic factors. P-value is obtained by the Log rank test.

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 analysed 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 (see Table 3). 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 (see Table 3).

Table 3. Multivariate analysis performed for several known independent prognostic factors without and with extravascular matrix patterns

	Multivariate analysis without extravascular matrix patterns				
		B-value	**P-value**	**HR**	**95% CI interval**
Presence of Ciliary Body involvement		*1.56*	*< 0.01*	*4.77*	*1.85-12.28*
Presence of Epithelioid Cell type		*0.49*	*0.04*	*1.05*	*1.01-1.10*
Presence of Intravascular ingrowth		*0.47*	*< 0.01*	*1.60*	*1.17-2.17*
Presence of Intrascleral ingrowth		-	NS	-	-
Age	For each year	*0.03*	*< 0.01*	*1.03*	*1.01-1.04*
Largest basal diameter	For each mm	*0.02*	*0.01*	*1.02*	*1.01-1.03*
Prominence	For each mm	-	NS	-	-

	Multivariate analysis with extravascular matrix patterns				
		B-value	**P-value**	**HR**	**95% CI interval**
Presence of Ciliary Body involvement		*0.40*	*0.02*	*1.49*	*1.07- 2.09*
Presence of Epithelioid Cell type		*1.42*	*< 0.01*	*4.12*	*1.20-12.70*
Presence of Intravascular ingrowth		-	NS	-	-
Presence of Intrascleral ingrowth		-	NS	-	-
Presence of Loops and/or Networks		*1.49*	*< 0.01*	*4.43*	*1.46-13.48*
Age	For each year	-	NS	-	-
Largest basal diameter	For each mm	*0.11*	*< 0.01*	*1.11*	*1.03-1.20*
Prominence	For each mm	-	NS	-	-

HR = Hazard ratio. NS = not significant.
For numerical variables, the HR is the hazard ratio for each unit (mm or years) for the parameter.
P-values ≤ 0.05 are shown in italics.

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[23-25]. However, there is only a limited number of studies in uveal melanoma concerning ingrowth of tumor cells in blood vessels [17,18]. In our center, 715 eyes were enucleated for uveal melanoma over a 35-year time interval. Only the primarily enucleated eyes (N = 643) were analysed. We did not include eyes with prior treatment, such as Ruthenium-106 brachytherapy, in our study, due to the fact that the tumor characteristics could have been modified by the prior therapy [26,27].

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 extraocular 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 to other parts of the body. Figure 1 demonstrates this situation: it can be observed that ingrowth of tumor cells into the vorticose vein

occurred, which may then allow tumor cells to migrate outside the eye[28]. This can then be observed macroscopically as an extra large and dark vorticose vein on the sclera after enucleation.
The presence of epithelioid cells is also associated with intravascular growth. In previous studies by Vaupel and Folberg et al.[29,30], it was described that different cell types determined the growth pattern of normal vessels and extravascular 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 into 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. Several reasons can explain this observation: one of the reasons could be that we did not analyse 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 histopathological situation after enucleation is not representative for 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 uveal melanoma patients and with multivariate analysis this appeared to be one of the most reliable independent prognostic markers for metastasis development. However, RT-PCR positive results were only observed in 10% of all cases, while in 25% of the patients metastases occurred during the follow-up period. This 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 can be explained in tumors located in the ciliary body, as they have a simple route via the trabecular meshwork invading the sclera and so can easily invade the episcleral structures. This is also in line with the literature: Seddon et al. 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[32].

An additional factor that was related to ingrowth of tumor cells into blood vessels was the patients' age. In several articles, advanced age has been associated with a larger basal diameter, non-spindle cell type and extrascleral extension, which are all 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 celltype were both clearly related to the presence of intravascular tumor cell ingrowth, this 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[37-39]. Apte and Espinosa demonstrated that blood vessel growth in old mice differed from young mice: when retinal neovascularisation was induced by laser, young mice showed limited neo-angiogenesis, while old mice developed massive angiogenesis. Macrophages were thought to play a major role: pro-angiogenic 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 invasiveness by tumor cells in these structures, leading to more potential for metastatic spreading[14]. Interestingly, the presence of high numbers of macrophages in uveal melanoma were previously found to be associated with tumor size, the presence of epithelioid cells and other unfavourable prognostic factors, demonstrating the relevance of macrophages[40].

Our data demonstrate that the presence of tumor cell invasion in blood vessels is associated with a worse survival. Interpretation of our survival results has to be performed with care. Kujala et al.[41] 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 et al.[42]. Intravascular ingrowth is only an independent prognostic factor when extravascular matrix patterns are not added to the set of parameters analysed.

We show in this study that it is not necessary to add intravascular ingrowth of tumor cells to the set of prognostic parameters that need to be analysed, since some better prognostic markers, such as extravascular matrix patterns are known. While histopathological parameters are important predictors of survival in uveal melanoma, chromosomal analysis[43,44] or microarray based gene expression profiling[45] may be even more precise predictors. However, that histopathological analysis is still useful was shown by Damato et al.[46]: the best predictive index was not obtained from one test, but by using analysis of chromosome 3, basal tumor diameter, and cell type together.

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