Angiogenesis and screening in uveal melanoma

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Long-term follow-up in uveal melanoma after enucleation.

Comparison of histopathologic prognostic factors

SUBMITTED

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ABSTRACT

Objective

To evaluate the long-term survival after enucleation for uveal melanoma and to review the relative importance of the main prognostic histopathologic factors.

Methods

A total of 600 patients who underwent an enucleation for uveal melanoma between 1973 and 2003 were included in the study. Histopathological data on largest basal tumour diameter and height, the presence of retinal detachment, necrosis, pigmentation, mitotic rate, intravascular ingrowth, scleral ingrowth, PAS-positive patterns, ^{32}P uptake, integrity of Bruch's membrane and cell type were obtained, and related to survival using Cox regression analysis. Importance of different variables was compared before and after the introduction of Ruthenium brachytherapy (1982).

Results

The cumulative 5-, 10- and 15-year survival for melanoma-related death was 72.2 % (SE 2%), 63.1 % (SE 2%), and 58.1% (SE 2%), respectively. In univariate Cox regression analysis, the most significant prognostic factors were the presence of PAS-positive loop patterns (P <.0001; HR 5.448), largest basal tumour diameter (P<0.001; HR 2.139), and cell type (P<0.001; HR 1.804). The multivariate Cox-analysis showed the same parameters as most important prognostic factors. Largest basal tumour diameter and cell type both entered the multivariate models before and after the introduction of Ruthenium brachytherapy.

Conclusion

PAS-positive loop patterns were the most important prognostic factor in uveal melanoma. Largest basal tumour diameter and cell type were independent significant prognostic factors for uveal melanoma, both before and after the introduction of Ruthenium brachytherapy.

INTRODUCTION

Uveal melanoma is the most common primary malignant intraocular tumour, with an estimated incidence of 7-10 cases per million per year.[1] During the last decennia, the treatment of uveal melanoma has been improved by applying eye-sparing techniques such as brachytherapy (Ruthenium, Strontium, Iodine), brachytherapy in combination with transpupillary thermotherapy (sandwich therapy), and proton radiotherapy. Enucleation is still used in large uveal melanomas and when episcleral growth is diagnosed. Up to 50% of the patients who undergo enucleation die of metastatic disease within 10 to 15 years after diagnosis of uveal melanoma.[2,3]

To improve survival, it is essential to prevent metastases or to treat uveal melanoma metastases more effectively. Recently, the first positive results for metastases therapy were published; new therapies include isolated liver perfusion,[4] gemcitabine in combination with treosulfan[5] and vaccination with melanoma-specific antigens.[6]

Many prognostic histopathologic factors have been identified.[7,8] One of the most consistent prognostic indicators for metastases of uveal melanoma is tumour size.[9-12] Another important prognostic factor is cell type: presence of epithelioid cells in particular is a sign of poor outcome.[10,11,13,14] Heavy pigmentation has been shown to have some prognostic significance in univariate analysis, but not in multivariate models[11,12]. Mitotic rate has also shown some prognostic significance[15] as well as nuclear morphometry.[16] During the 1950s, the disodium phosphate ^{32}P isotope uptake test was developed to differentiate benign from malignant in choroidal and ciliary body tumours, because it was considered to be an indicator of nuclear activity.[17,18] Recently, a very long-term study showed that the ^{32}P isotope uptake test had no prognostic value for uveal melanoma.[19] Studies on tumour blood vessel morphology were introduced by Robert Folberg and colleagues in 1992 when they introduced a classification for nine morphological types of 'fibrovascular' architecture as demonstrated by PAS staining.[20] These patterns are to be regarded as fibrous sheets throughout the tumour, enclosing the larger venes and arteries of the tumour. The significance of the presence of loops, networks or both was demonstrated in multivariate models.[21-23] Also in the 1990s, microvascular density was shown to be of prognostic significance in some studies, depending on the antivascular antibody used (CD34, CD31, FVIII), and the selection of the area studied.[24-26] In recent years, chromosomal abnormalities, in particular chromosome three monosomy, have been shown to be of prognostic value.[27-30] The value of chromosome three monosomy still has to be evaluated in long-term follow-up studies and was not included in our study as we do not have long follow-up data on this test.

Frequently, studies include too small a number of patients to perform multivariate analysis. Another problem may be that changes occur in the histopathological evaluation. In our study group, the same pathologist performed all histopathological analyses in a standardized fashion.

This study compares the importance of the different histopathologic factors in a large group of 600 enucleations, performed at the Department of Ophthalmology of the Leiden University Medical Center. In 1982, Ruthenium brachytherapy was introduced. Before 1982, uveal melanoma was only treated by enucleation. To evaluate whether the importance of different variables changed after the introduction of Ruthenium brachytherapy, Cox regression was done in separed groups.

METHODS

Patients

All patients who underwent enucleation for melanoma between 1973 and 2003 at the Leiden University Medical Center were included in this study. The study protocol followed the tenets of the Declaration of Helsinki. The clinical, histopathological, and follow-up data were collected and registered at the Oncological Registration of Leiden University Medical Center. Histopathological analysis was performed by one ocular pathologist (D W-R). Every year, the Dutch Population Registry was consulted. The melanoma-related deaths were confirmed by general practitioners or specialists and a copy of the records was sent to the Oncological Registration service. Patients with an iris melanoma were excluded from this study.

Histopathological variables

The following 12 variables were analyzed (Table 1):

1 Largest tumour diameter in millimetres; largest basal tumour diameter was classified and divided into three groups according to the TNM standards: small (<10 mm), median (10-16 mm), and large (>16 mm) diameter.
2 Tumour height. The TNM classification was followed to separate three groups: thin (< 2.5 mm), median (2.5-10 mm), thick (>10 mm).
3 Integrity of Bruch's membrane: intact or broken;
4 Depth of scleral infiltration: none, partial, deep scleral infiltration, microscopic episcleral, macroscopic episcleral infiltration.
5 Cell type: spindle, mixed and epithelioid cell type.
6 Number of mitoses (mitotic rate per 15 high-power fields): < 10 mitoses per 15 fields, between 10 and 20 mitoses, and > 20 mitoses/ 15 microscopic fields.

7 Intravascular ingrowth of tumour cells: no ingrowth, ingrowth in vessels inside the tumour, ingrowth in vessels outside the tumour, or ingrowth in vessels both inside and outside of the tumour.
8 PAS-positive patterns. The effect of loops and networks was analyzed both separately and by a combined variable that considered networks to be an advanced stage of loops, leading to three categories: no loops, loops without networks, and networks.
9 Degree of pigmentation: none, light, intermediate, or heavy.
10 ^{32}P uptake. ^{32}P uptake was devided in two groups: above and below the median value (131%).
11 Tumour location was coded 'in front of' or 'behind' the equator; 'with' and 'without' papillary involvement.
12 Previous treatment (TTT, proton beam irradiation or Ruthenium plaque therapy) was registered.

Statistical analysis

All clinical, histopathological, and follow-up data of this study were registered in SPSS 11.0 (Chicago, Illinois) and Stata software (StataCorp, Texas). Univariate analysis was illustrated by Kaplan-Meier curves, compared with the log rank test, and analyzed by univariate Cox proportional hazards model. In this analysis, patients judged to have died of non-melanoma related causes were censored at their time of death.

Multivariate Cox analysis identified the independent significant prognostic variables. The same variable classifications were used for multivariate analysis as for univariate analysis. Forward stepwise regression was used to identify independent prognostic factors.

The regression coefficients and hazard ratios (HRs) with their 95% confidence intervals were calculated. Any P value less than 0.05 was regarded as statistically significant.

To investigate differences in the influence of variables before and after the introduction of Ruthenium plaque brachytherapy, we analyzed all variables in both groups (n=250 and n=350, respectively).

Characteristic	N	%	N before 1982	%	N after 1982	%
Cell type						
Spindle	249	41.5	126	50.4	123	35.1
Mixed	267	44.5	84	33.6	183	52.3
Epithelioid	70	11.7	36	14.4	34	9.7
Not clear (necrosis)	14	2.3	4	1.6	10	2.9
Localization						
before equator	259	43.2	107	42.8	152	43.4
behind equator	264	44	113	45.2	151	43.1
disk involvement	77	12.8	30	12.0	47	13.4
Largest basal diameter						
<10 mm	204	34	88	35.2	116	33.1
10-16 mm	338	56.3	145	58	193	55.1
>16 mm	58	9.7	17	6.8	41	11.7
Height						
<2.5 mm	146	24.4	75	30	71	20.3
2.5-10 mm	389	64.8	148	54.2	241	68.9
>10 mm	65	10.8	27	10.8	38	10.9
Instrascleral growth						
none	57	9.5	14	5.6	43	12.3
partial	310	51.7	141	56.4	169	48.3
deep	116	19.3	50	20.0	66	18.9
total	35	5.8	12	4.8	23	6.6
microscopic episcleral	21	3.5	10	4.0	11	3.1
macroscopic episcleral	45	7.5	18	7.2	27	7.7
Loops						
no loops	68	11.3	0	0	81	23.1
loops	81	13.5	0	0	68	19.4
not available	451	75.2	250	100	201	57.4
Networks						
no networks	93	15.5	0	0	56	16
networks	56	9.3	0	0	93	26.6
not available	451	75.2	250	100	201	57.6

P32						
above median	119	19.8	115	46	4	1.1
beneath median	118	19.7	113	45.2	5	1.4
missing	363	60.5	22	8.8	341	97.4
Retinal detachment						
no retinal detachment	169	28.2	139	55.6	30	8.6
retinal detachment	136	22.7	108	43.2	28	8
missing	295	49.1	3	1.2	292	83.4
Mitosis rate						
<10 mitosis/15 fields	396	66	149	59.6	247	70.6
10-20 mitosis/15 fields	107	17.8	59	23.6	48	13.7
>20 mitosis/15 fields	57	9.5	30	12	27	7.7
not available	40	6.7	12	4.8	28	8.0
Necrosis						
Yes	409	68.2	57	22.8	122	34.9
None	179	29.8	189	75.6	221	63.2
Not clear	12	2	4	1.6	7	2.0
Bruch's membrane						
intact	190	31.7	96	38.4	94	26.9
broken	337	56.1	136	54.4	201	57.4
not clear	73	12.2	18	7.2	55	15.7
Pigmentation						
none	43	7.2	25	10	18	5.1
slight	274	45.6	115	46	159	45.4
moderate	181	30.1	74	29.6	107	30.6
severe	85	14.1	33	13.2	52	14.9
not clear	17	3	3	1.2	14	4.0
Prior treatment						
no prior treatment	557	92.8	250	100	307	87.7
prior treatment	43	7.2	0	0	43	12.3
Intravascular ingrowth						
none	334	55.7	147	58.8	187	53.4
within tumor	115	19.2	48	19.2	67	19.1
outside tumor	62	10.3	26	10.4	36	10.3
both	35	5.8	13	5.2	22	6.3
not clear	54	9	16	6.4	38	10.9

TNM classification						
class 1	95	15.8	47	18.8	48	13.7
class 2	397	66.2	164	65.6	233	66.6
class 3	89	14.8	28	11.2	61	17.4
class 4	19	3.2	11	4.4	8	2.3
Median height (mm)	5		4.5		5.5	
Median diameter (mm)	11		10.5		11	
Median mitotic rate	5		7		4	
Median age at enucleation (years)	59.7		56.6		61.1	
Sex (M/F)	314/286	52.3/47.7	128/122	51.2/48.8	186/164	53.1/46.9
Eye (OD/OS)	290/310	48.3/51.7	123/127	49.2/50.8	167/183	47.7/52.3

Table1. Clinicopathological characteristics of 600 enucleated uveal melanomas

RESULTS

All included tumours (n=600) were treated with enucleation. The median age at enuclation was 59.7 years (SD 15.1). The study included 314 (52.3%) male and 286 (47.7%) female patients. No patients were lost during follow-up. At the end of the follow-up time (August 2004), 210 patients (35%) had died of melanoma-related disease, 141 patients (23.5%) had died of non-melanoma-related diseases, and 249 (41.5%) were still alive. The median follow-up time after enucleation was 6.4 years (range 0.1-31 years; mean 9.5 years). Before the introduction of Ruthenium brachytherapy in 1982, 250 patients were included. The number of patients included in five-year groups is shown in Figure 1.

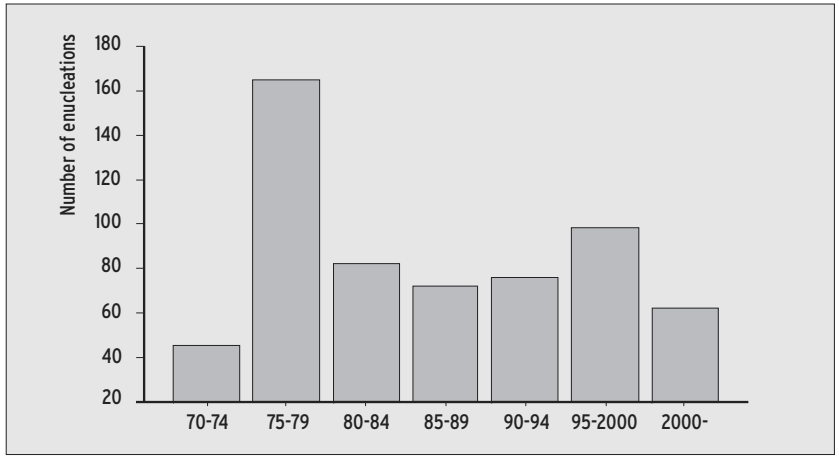


Figure 1. Overview of the number of patients enrolled in this study over time.

After 1982, 43 patients (7.2%) had a previous treatment before enucleation (TTT 3 (0.5%); Ruthenium application 12 (2%); Sandwich therapy (TTT + Ruthenium) 18 (3%); proton beam therapy 10 (2.7%). Reasons for secondary enucleation were a recurrent tumour (24/43; 56%), insufficient reaction to therapy (8/43; 19%), neovascular glaucoma (5/43; 12%), total retinal detachment (4/43; 9%), and cosmetical reasons (2/43; 5%).

All studied histological data were available for most of the 600 patients except for the ^{32}P uptake test that was only available for 237 patients between 1973 and 1984 (39.5%) and PAS patterns which had been registered since 1993, and were available for 149 patients (24.8%). If the number of available data is less than 600, n-value is reported for the individual tests (cfr Table 1).

Tumour characteristics

The median tumour diameter was 11 mm (mean 11.1 mm, SD 3.6): 204 tumours were small (34%), 338 tumours median (56.3%), and 58 large (9.7%). Regarding tumour height: 146 tumours were thin (24.4%) (2.5 mm), 389 median (64.8%) (2.5-10mm), 65 thick (10/8%) (>10mm). In 337 eyes (56.1%), Bruch's membrane was broken.
Intrascleral ingrowth of tumour cells occurred superficially in 310 cases (51.7%), deeply in 116 eyes (19.3%), and totally in 35 (5.8%). Microscopic episcleral invasion occurred in 21 eyes (3.5%), and macroscopical episcleral invasion in 45 eyes (7.5%);
Two hundred and forty nine tumours (41.5%) were of the spindle cell type, 267 of the mixed cell type (44.5%), and 70 of the epithelioid cell type (11.7%). In fourteen tumours,

the cell type could not be determined due to extensive necrosis (2.3%). Median mitotic rate was 5/ 15 fields. In 334 tumours (55.7%), there was no tumour cell ingrowth in vessels, 115 (19.2%) had tumour cell ingrowth in vessels within the tumour, 62 (10.3%) outside the tumour, and in 35 cases both inside and outside the tumour (5.8%). PAS-positive loop patterns were present in 81/149 patients (54.4%) and network patterns in 56/149 patients (37.6%). In 43 tumours (7.2%), there was no pigmentation, pigmentation was light in 274 tumours (45.6%), moderate in 181 tumours (30.1%), and heavy in 85 cases (14.1%). Localization was in front of the equator in 259 eyes (43.2%), behind the equator without papillary involvement in 264 eyes (44%) and with papillary involvement in 77 eyes (12.8%) (Table 1). The relation between different variables is shown in Table 2. The presence of loops is not related with largest basal diameter, indicating the independent prognostic value of these two parameters.

	Cell type	Localization	Largest basal diameter	Height	Scleral invasion	Retinal detachment	Mitotic rate	Necrosis	Pigmentation	Prior treatment	Bruch's membrane invasion	Loops	Networks	Combined patterns
Age	0.030	<0.001	<0.001	0.006	0.084	0.934	0.728	0.002	0.079	0.030	0.001	0.330	0.743	0.292
Cell type		0.092	<0.001	0.006	0.313	0.960	0.010	0.098	0.206	0.122	0.201	0.011	0.108	0.019
Localization			<0.001	<0.001	0.038	0.005	<0.001	<0.001	<0.001	0.098	<0.001	0.411	0.368	0.487
Largest basal diameter				<0.001	<0.001	<0.001	<0.001	<0.001	0.019	0.058	<0.001	0.145	0.003	0.982
Height					0.032	<0,001	0.001	<0.001	0.004	<0.001	<0,001	0.004	0.003	0.205
Scleral invasion						0.763	0.619	0.126	0.330	0.025	0.029	0.925	0.525	0.673
Retinal detachment							0.076	0.465	0.987	0.264	<0.001	n.a.	n.a.	n.a.
Mitotic rate								0.032	0.020	0.009	0.001	0.045	0.086	0.252
Necrosis									0.003	0.140	<0.001	0.141	0.587	0.702
Pigmentation										0.135	0.005	0.515	0.438	0.438
Prior treatment											0.288	0.251	0.943	0.418
Bruch's membrane invasion												0.155	0.126	0.063
Loops													<0.001	<0.001
Networks														<0.001

n.a. = not available
Age, Largest basal diameter and Height were analyzed as continuous variables.
All other variables as catagorical (see table 1 for classes) with Ttest, ANOVA or Chi-Square test.

Table 2. Relation between different histopathological and clinical variables in 600 enucleated patients.

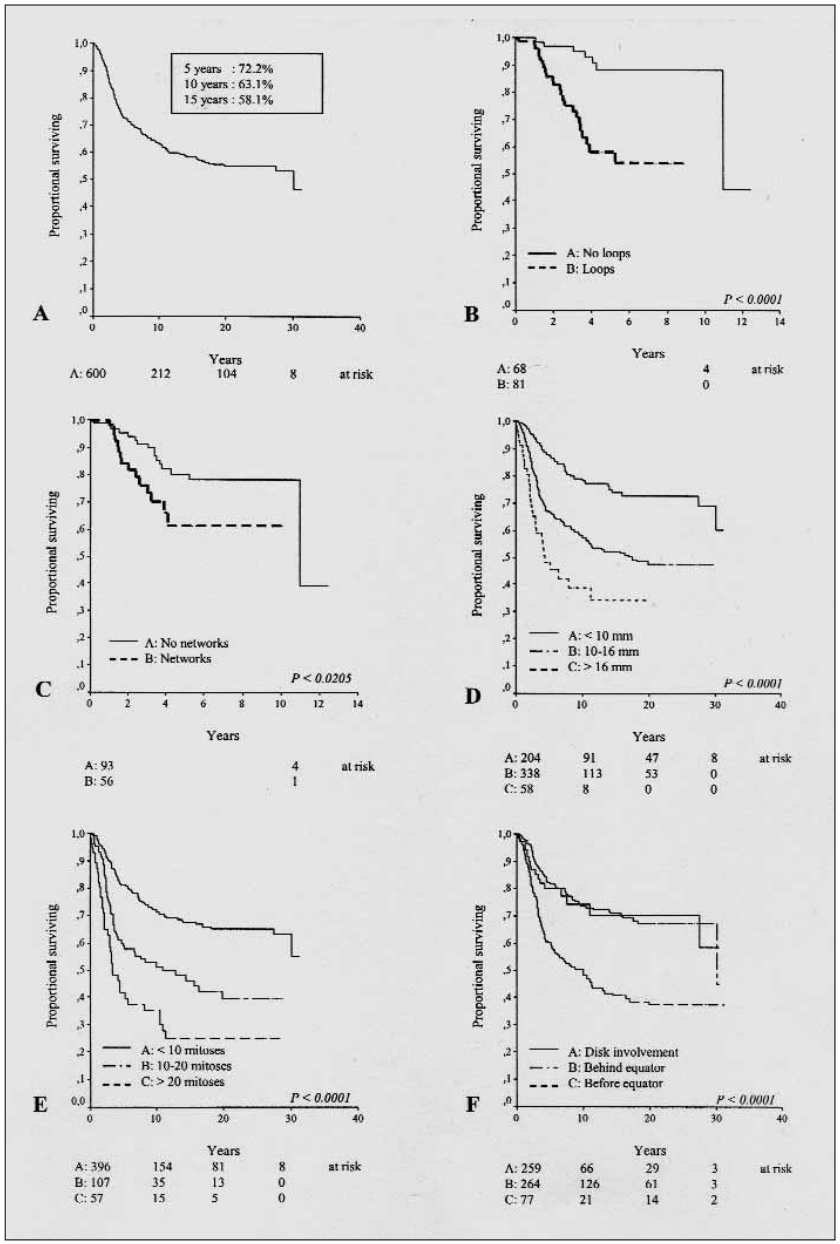
Univariate Cox regression Analysis

The cumulative 5-year survival for melanoma-related death was 72.2%, 10-year survival was 63.1%, and 15-year survival was 58.1% (illustrated with a Kaplan Meier curve, Figure 2A). Two hundred and ten patients had died of melanoma metastases by the end of follow-up. One hundred forty one died of non-melanoma-related causes.

The studied histopathological data are reported in order of their prognostic value: The most important unfavorable prognostic factors in this study were: the presence of PAS-positive loop patterns ($P < 0.001$; HR 5.448)(Figure 2B), PAS-positive network patterns ($P < 0.001$; HR 2.299) (Figure 2C), and the largest basal diameter ($P < 0.001$; HR 2.139) (Figure 2D). Considering networks to be an advanced stage of loops (as described by Mäkitie) and thereby combining them gave a similar result as compared to separate analysis of loops ('combined patterns' $P < 0.001$; HR 2.005) (Table 3).

Other significant, prognostic factors were mitotic rate ($P < 0.001$; HR 1.981) (Figure 2E), localisation of the tumour ($P < 0.001$; HR 1.938) with an unfavourable outcome for papillary tumours (Figure 2F), and higher TNM classification ($P < 0.001$; HR 1.872).

Thick tumours had a significantly worse outcome ($P < 0.001$; HR 1.860), as did epithelioid cell type tumours ($P < 0.001$; HR 1.804) (Figure 2G). The presence of necrosis ($P < 0.001$; HR 1.764) (Figure 1H) was a marker for poor outcome, as did the presence of Bruch's membrane invasion ($P < 0.001$; HR 1.741). Older age at the time of enucleation ($P < 0.001$; HR 1.515) was also significantly associated with worse outcome. Highly pigmented tumours did worse ($P < 0.001$; 1.379) than other pigment types. The invasion of vessels by tumour cells ($P < 0.001$; HR 1.286), or the presence of a retinal detachment ($P = 0.009$; HR 1.649) accompanying the tumour were significant as well. Other histopathologic factors were not found to be of significant prognostic value, namely invasiveness of the sclera ($P = 0.240$; HR 1.047), eye (OS/OD) ($P = 0.728$; HR 1.212), ^{32}P uptake ($P = 0.121$; HR 1.395), treatment before enucleation ($P = 0.731$; HR 1.089), and sex of the patient ($P = 0.164$; HR 1.212).



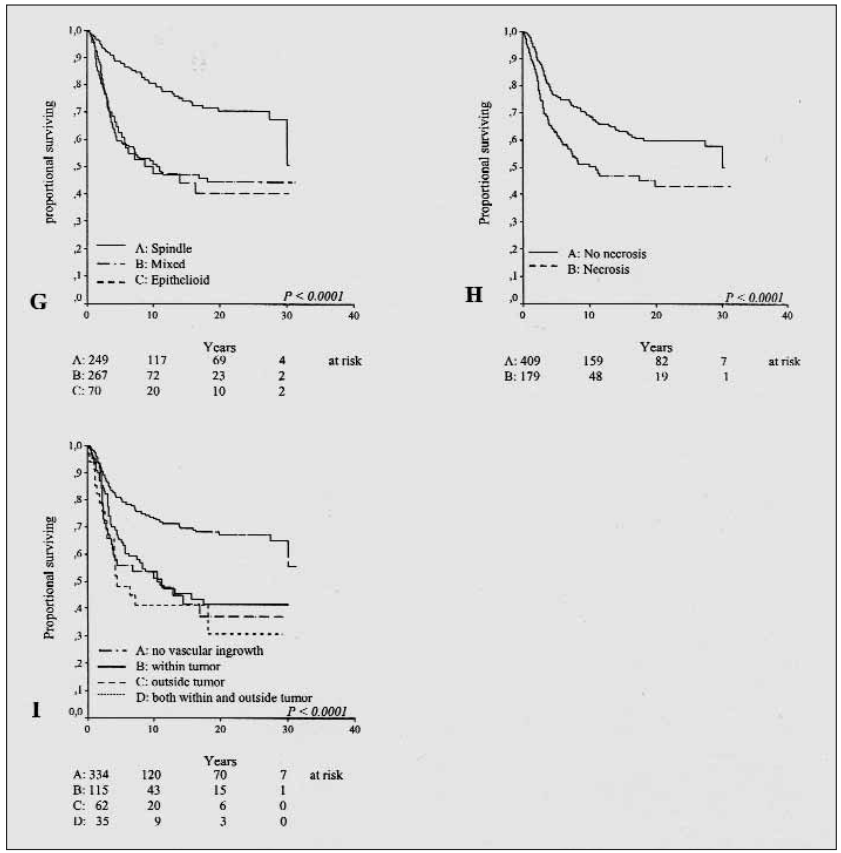


Figure 2. Kaplan-Meier curves illustrating survival in 600 patients with a uveal melanoma. A. Overall survival was 72.2% at 5 years, 63.1% at 10 years and 58.1% at 15 years. Survival was much higher in patients without loops (B) or networks (C). D. Survival in different diameter classes. E. Kaplan-Meier curve showing survival is worst in tumours with many mitoses. F. Survival in function of location of the tumour. G. Tumours containing epithelioid cells had a worse survival. H. Better survival in tumours without necrosis. I. Survival significantly decreased when the tumour invaded vessels.

Variable	Regression coëfficient (standard error)	Likelihood ratio	P	Hazard ratio (95% confidence interval)
Age†	0.415 (0.088)	22.369	<0.001	1.515 (1.275-1.800)
Sex	0.193 (0.139)	1.935	0.164	1.212 (0.924-1.591)
Eye	0.048 (0.138)	0.121	0.728	1.049 (0.800-1.376)
Cell type*	0.590 (0.097)	37.127	<0.001	1.804 (1.492-2.181)
Localisationϒ	0.662 (0.119)	31.145	<0.001	1.938 (1.536-2.445)
Largest basal diameter†	0.760 (0.115)	44.009	<0.001	2.139 (1.708-2.677)
Height†	0.621 (0.119)	27.273	<0.001	1.860 (1.474-2.348)
Scleral invasionß	0.046 (0.039)	1.381	0.240	1.047 (0.970-1.130)
Loops§	1.695 (0.460)	13.572	<0.001	5.448 (2.211-13.426)
Networks§	0.833 (0.369)	5.087	0.024	2.299 (1.115-4.740)
Combined patterns‡	0.695 (0.219)	10.086	0.001	2.005 (1.305-3.079)
P32†	0.333 (0.215)	2.408	0.121	1.395 (0.916-2.174)
Retinal detachment§	0.500 (0.192)	6.804	0.009	1.649 (1.132-2.402)
Mitotic rate†	0.684 (0.091)	56.316	<0.001	1.981 (1.657-2.369)
Necrosis§	0.568 (0.144)	15.459	<0.001	1.764 (1.329-2.341)
Pigmentation (referenced to total pigmentation)α		20.505	<0.001	
No pigmentation	1.221 (0.414)	8.723	0.003	3.392 (1.508-7.629)
Mild pigmentation	1.350 (0.395)	11.659	0.001	3.857 (1.777-8.369)
Severe pigmentation	0.806 (0.394)	4.183	0.041	2.239 (1.034-4.846)
Prior treatment§	0.085 (0.247)	0.118	0.731	1.089 (0.670-1.769)
Bruch's membrane invasion§	0.555 (0.125)	19.834	<0.001	1.741 (1.364-2.223)
Vessel invasionγ	0.252 (0.054)	22.156	<0.001	1.286 (1.158-1.429)
TNM classification	0.627 (0.096)	42.529	<0.001	1.872 (1.550-2.260)

† : continuous variables
* : spindle, mixed, epithelioid cell type
α : categorical variable
§ : categories : no, yes
‡ : no loops, loops without networks, networks
ϒ : before equator, after equator, disk involvement
ß : scleral invasion, episcleral invasion, no scleral involvement
γ : no vascular ingrowth, within tumor, outside tumor, both

Table 3. Univariate analysis of clinicopathological variables of 600 uveal melanoma patients

Multivariate Analysis

In stepwise multivariate Cox regression, the presence of PAS-positive loops entered the model as the most predictive univariate variable (Table 4a), and the significance for the other significant variables was examined.

Networks and combined patterns did not reach significance in a multivariate analysis after entering PAS-positive loops. The next most predictive univariable, the largest basal tumour diameter retained its statistical significance. Of the other variables, only cell type and the invasion of Bruch's membrane could add significance to the model. All other prognostic univariate factors lost their statistical significance once loops and the largest basal diameter had been entered in the model.

The multivariate analysis was also studied in different groups (Tables 4b and 4c). Largest basal diameter and cell type entered the multivariate analysis in the total group and subgroups. Also, since the introduction of PAS positive staining, the presence of loops is a very important prognostic value. Other introduced values were the invasion of Bruch's membrane, age at enucleation and tumour height, but these values had less significance in the model.

Variable	Regression coëfficient (standard error)	Likelihood ratio	P	Hazard ratio (95% confidence interval)
Final model, -2likelihood = 228.045				
Largest basal diameter†	0.221 (0.051)	18.685	<0.001	1.248 (1.129-1.380)
Loops§	1.865 (0.481)	15.035	<0.001	6.454 (2.515-16.563)
Bruch's membrane invasion§	0.574 (0.218)	6.924	0.009	1.776 (2.725-1.158)
Cell type*	0.654 (0.294)	4.941	0.026	1.924 (1.080-3.425)
Model 2, -2likelihood = 232.913				
Largest basal diameter†	0.241 (0.051)	22.167	<0.001	1.272 (1.131-1.406)
Loops§	1.805 (0.470)	14.761	<0.001	6.082 (2.421-15.276)
Bruch's membrane invasion§	0.521 (0.203)	6.591	0.010	1.683 (1.131-2.506)
Model 3, -2likelihood = 235.311				
Largest basal diameter†	0.193 (0.052)	13.979	<0.001	1.213 (1.096-1.342)
Loops§	1.659 (0.469)	12.518	<0.001	5.256 (2.096-13.147)
Cell type*	0.657 (0.296)	4.917	0.027	1.929 (1.079-3.447)

† : continuous variables
* : spindle, mixed, epithelioid cell type
§ : categories : no, yes

Table 4a. Multivariate analysis for clinicopathological variables of 600 enucleated uveal melanoma eyes.

Variable	Regression coëfficient (standard error)	Likelihood ratio	P	Hazard ratio (95% confidence interval)
Final model, -2likelihood = 804.891				
Largest basal diameter†	0.191 (0.032)	35.732	<0.001	1.210 (1.137-1.288)
Mitotic rate†	0.028 (0.007)	16.618	<0.001	1.029 (1.014-1.042)
Height†	0.110 (0.036)	9.434	0.002	1.117 (1.041-1.198)
Cell type*	0.655 (0.145)	20.391	<0.001	1.926 (1.449-2.559)

† : continuous variables
* : spindle, mixed, epithelioid cell type

Table 4b. Multivariate analysis of clinicopathological variables in 250 enucleated eyes before 1982.

Variable	Regression coëfficient (standard error)	Likelihood ratio	P	Hazard ratio (95% confidence interval)
Final model, -2likelihood = 804.891				
Largest basal diameter†	0.194 (0.054)	12.921	<0.001	1.214 (1.042-1.349)
Loops§	1.628 (0.478)	11.591	0.001	5.092 (1.995-12.998)
Cell type*	0.745 (0.308)	5.860	0.015	2.107 (1.152-3.853)
Mitotic rate†	0.053 (0.027)	3.938	0.047	1.055 (1.001-1.112)

† : continuous variables
* : spindle, mixed, epithelioid cell type
§ : categories : no, yes

Table 4c. Multivariate analysis of clinicopathological variables in 350 enucleated eyes after 1982.

DISCUSSION

The purpose of this and other retrospective uveal melanoma survival studies is to identify tumour characteristics that will help in deciding the most appropriate treatment and to allow an accurate prognosis. We also investigated whether these variables changed after the introduction of brachytherapy. The 5-year survival rate of 72.2% is similar to those reported in the literature (69-78%).[2,14,31,32]

PAS-positive patterns

This study on a very large group of enucleated uveal melanoma eyes, confirms the significance of the PAS-positive patterns for prognosis in uveal melanoma. Recent studies revealed the presence of loops[21,23] or the presence of networks[20,33] to be one of the most important prognostic factor. Although the importance of networks has also described, most studies find a higher significance for the presence of loops. There is still discussion on the nature of these patterns. Clarijs et al. showed them to be fluid-conducting connective tissue sheets.[34]

Largest basal diameter

One of the most consistent prognostic factors is largest basal diameter (LBD),[35] and is the basis of the current TNM classification for uveal melanoma. The evaluation of tumour volume or computer-assisted quantification of cross-sectional tumour area are other ways to measure tumour size.[36] However, the prognostic value of LBD has been sufficiently demonstrated, and is easy to use clinically. Table 1 showes that most tumours were part of TNM class 2, reducing the prognostic importance of this classification for uveal melanoma.

Retinal detachment

Several studies also investigated the prognostic role of a retinal detachment.[37,38] The presence of a retinal detachment has been shown to be of prognostic value in a univariate analysis by Kivela et al., and is confirmed here.[38] However, retinal detachment lost its statistical significance in multivariate analysis. Retinal detachment is mainly associated with tumour dimensions.

Cell type

G.R. Callender established the classification of uveal melanoma cells, mainly in spindle and epithelioid cells.[13] The presence of epithelioid cells was also significantly correlated with bad prognosis (Figure 2G) in our study. This may lead to classifying the presence of even a single epithelioid cell in the tumour to the non-spindle type.[39]

Necrosis

An interesting finding was the significant correlation of the presence of necrosis (present in 68.2% of the tumours) with poor prognosis. To our knowledge, this has not been reported in the literature. As necrosis also lost its statistical significance in the multivariate model, after entering largest basal diameter, we may conclude that necrosis is correlated with tumour dimension ($P<0.001$, Table2).

Mitotic count

Mitotic count is a measure of cell proliferation.[14] A high rate of mitosis means a high rate of cell proliferation, and this indicates that the cell is biochemically very active. Tumour cells that are very active are assumed to be more aggressive and therefore more malignant. Our study confirms the prognostic value of mitotic count,[11] although not in a multivariate model.

^{32}P uptake

Although the ^{32}P test was not a predictive factor for the development of metastases, it clearly helped to differentiate non-malignant from malignant tumours. Thomas started testing ^{32}P (1952) as he assumed that nuclear proteins are synthesised more rapidly by tumour cells than normal cells, resulting in a differential uptake of radiolabeled phosphorus.[17] The ^{32}P uptake by tumours was not of prognostic value in our total group of 600 enucleations, although various associations were reported earlier.

Conclusion

The only factors that retained their prognostic value in the overall multivariate model after entering the PAS positive loop pattern and tumour diameter were cell type and invasion of Bruch's membrane. Largest basal diameter and loop patterns were significant in uni- and

multivariate analysis of the total group and in subgroups. Fortunately, the most important prognostic factors can be assessed already prior to enucleation, as imaging techniques evaluating PAS patterns have been reported.[37,40]
Bruch's membrane breakthrough was not of prognostic value in other studies,[14] and did not enter the multivariate models of the subgroups in our study.
In the future, genomic or proteomic expression profiling may discriminate different types of uveal melanoma,[41] as already described with monosomy three. In a limited number of tumours, Onken et al. showed that based on a number of genes two classes of uveal melanoma could be defined.[41]

We conclude that in a large series of enucleated uveal melanomas, tumour size and PAS positive patterns were the most prognostic histopathological parameters. The introduction of brachytherapy did not change the importance of known prognostic factors. Clinical evaluation of these parameters before therapy may also be useful in patients that receive eye-sparing therapies.

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References

1 Parkin DM, Bray F, Ferlay J, Pisanti P. *Estimating the world cancer burden. GLOBOCAN 2000.* Int J Cancer 2000; 94: 153-156.
2 Jensen OA. *Malignant melanomas of the human uvea: 25-year follow-up of cases in Denmark, 1943-1952.* Acta Ophthalmol (Copenh). 1982; 60: 161-182.
3 McLean IW, Foster WD, Zimmerman LE. *Uveal menaoma : locations size, cell type, and enucleation as risk factors in metastasis.* Hum Pathol 1982; 13: 123-132.
4 Noter SL, Rothbarth J, Pijl ME, Keunen JE, Hartgrink HH, Tijl FG, Kuppen PJ, van de Velde CJ, Tollenaar RA. *Isolated hepatic perfusion with high-dose melphalan for the treatment of uveal melanoma metastases confined to the liver.* Melanoma Res 2004; 14: 67-72.
5 Pfohler C, Cree IA, Ugurel S, Kuwert C, Haass N, Neuber K, Hengge U, Corrie PG, Zutt M, Tilgen W, Reinhold U. *Treosulfan and gemcitabine in metastatic uveal melanoma patients :results of a multicenter feasibility study.* Anticancer Drugs 2003; 13: 337-340.
6 Piperno-Neumann S, Dorval T, Lantz O, Desjardins L, Salmon R, Pouillart P, Brichard V. *Phase I/II study of frequent and prolonged immunization with NA17 and differentiation peptides in HLA-A2 paitents with metastatic uveal melanoma: preliminary results.* Ocular Oncology Task Force at EVER 2004; 26th Septembre. Ophthalmic Research 2994; 36, S1: 149.
7 Mudhar HS, Parsons MA, Sisley K, Rundle P, Singh A, Rennie IG. *A critical appraisal of the prognostic and predictive factors for uveal malignant melanoma.* Histopathology 2004; 45, 1-12.
8 Mooy CM, De Jong PTVM. *Prognostic parameters in uveal melanoma: a review.* Surv Ophthalmol 1996; 41: 1894-1899.
9 Coleman K, Baak JP, van Diest P, Mullaney J, Farrell M, Fenton M. *Prognostic factors following enucleation of 111 uveal melanomas.* Br J Ophthalmol 1993; 77: 688-692.
10 Seregard S, Kock E. *Prognostic indicators following enucleation for posterior uveal melanoma. A multivariate analysis of long-term survival with minimized loss to follow-up.* Acta Ophthlamol Scand 1995; 73: 340-344.
11 McLean IW, Foster WD, Zimmerman LE. *Prognostic factors in small malignant melanomas of choroids and ciliary body.* Arch Ophthalmol 1977; 95: 48-58.
12 Miller MV, Herdson PB, Hitchcock GC. *Malignant melanoma of the uveal tract – a review of the Auckland experience.* Pathology 1985; 17: 281-284.
13 Callender GR. *Malignant melanotic tumours of the eye. A study of histologic types in 111 cases.* Trans Am Acad Ophthalmol Otolaryngol 1931; 36: 131-142.
14 Seddon JM, Albert DM, Lavin PT, Tobinson N. *A prognostic factor study of disease-free interval and survival following enucleation for uveal melanoma.* Arch Ophthalmol 1983; 101: 1894-1899.
15 Augsburger JJ, Gonder JR, Amsel J, Shields JA, Donoso LA. *Growth rates and doubling times of posterior uveal melanomas.* Ophthalmology 1984; 91: 1709-1715.
16 McLean IW, Sibug ME, Becker RL, McCurdy JB. *Uveal melanoma : the importance of large nucleoli in predicting patient outcome: an automated image analysis study.* Cancer 1997; 79: 982-988.
17 Thomas CI, Krohmer JS, Storaasli JP. *Detection of intraocular tumours with radioactive phosphorus.* Arch Ophthalmol 1952; 47: 276-286.
18 Shields JA. *Accuracy and limitations of the 32P test in the diagnosis of ocular tumours: an analysis of 500 cases.* Ophthalmology 1978; 85: 950-966.
19 Overkleeft ENM, Zuidervaart W, Hurks HMH, Eilers PHC, de Wolff-Rouendaal D, Jager MJ. *Prognostic value of the Disodium Phosphate 32P uptake test in uveal melanoma. A long-term study.* Arch Ophthalmol 2003; 121: 1398-1403.
20 Folberg R, Rummelt V, Parys-van Ginderdeuren R, Hwang T, Woolson RF, Pe'er J, Gruman LM. *The prognostic value of tumour blood vessel morphology in primary uveal melanoma.* Ophthalmology 1993: 100; 1389-1399.

21 Makitie T, Summanen P, Tarkkanen A, Kivela T. *Microvascular loops and networks as prognostic indicators in choroidal and ciliary body melanomas.* J Natl Cancer Insti 1999; 91: 359-367.

22 Foss AJ, Alexander RA, Hungerford JL, Harris AL, Cree IA, Lightman S. *Reassesment of the pAS patterns in uveal melanoma.* Br J Ophthalmol 1997; 81: 240-246.

23 McLean IW, Keefe KS, Burnier MN. *Uveal melanoma. Comparison of the prognostic value of fibrovascular loops, mean of the ten largest nucleoli, cell type, and tumour size.* Ophthalmology 1997; 104: 777-780.

24 Makitie T, Summanen P, Tarkkanen A, Kivela T. *Microvascular density in predicting survival of patients with choroidal and ciliary body melanoma.* Invest Ophthalmol Vis Sci 1999; 40: 2471-2480.

25 Lane AM, Egan KM, Yang J, Saornil MA, Alroy J, Albert D, Gragoudas ES. *An evaluation of tumour vascularity as a prognostic indicator in uveal melanoma.* Melanoma Res 1997; 7: 237-242.

26 Schaling DF, vand der Pol JP, Schlingemann RO, Parys-van Ginderdeuren R, Ruiter DJ, Jager MJ. *Vascular density and vascular patterns in the prognosis of choroidal melanoma.* In : Thesis Schaling D.F.: Radionuclides and radiolabelled antibodies in choroidal melanoma (diagnosis and therapy). Leiden: Rijksuniversiteit te Leiden; 1996:43-54.

27 Prescher G, Bornfeld N, Hirche H, Horsthemke B, Jockel KH, Becher R. *Prognostic implications of monosomy 3 in uveal melanoma.* Lancet 1996; 347: 1222-1225.

28 Sisley K, Rennie IG, Parsons MA, Jacques R, Hammond DW, Bell SM, Potter AM, Rees RC. *Abnormalities of choromosomes 3 and 8 in posterior uveal melanoma correlate with prognosis.* Genes Chromosomes Cancer 1997; 19: 22-28.

29 Scholes AG, Damato BE, Nunn J, Hiscott P, Grierson I, Field JK. *Monosomy 3 in uveal melanoma : correlation with clinical and histologic predictors of survival.* Invest Ophthalmol Vis Sci 2003; 44: 1008-1011.

30 Tschentscher F, Husing J, Holter T, Kruse E, Dresen IG, Jockel KH, Anastassiou G, Schilling H, Bornfeld N, Horsthemke B, Lohmann DR, Zeschnigk M. *Tumour classification based on gene expression profiling shows that uveal melanomas with and without monosomy 3 represent two distinct entities.* Cancer Res 2003; 63: 2578-2584.

31 Kujala E, Makitie T, Kivela T. *Very long-term prognosis in patients with malignant uveal melanoma.* Invest Ophthalmol Vis Sci 2003; 44: 4651-4659

32 Paul EV, Parnell BL, Fraker M. *Prognosis of malignant melanomas of the choroids and ciliary body.* Int Ophthalmol Clin 1962; 2: 387-402.

33 Seregard S, Spanberg B, Juul C, Oskarsson M. *Prognostic accuracy of the mean of the largest nucleoli, vascular patterns, and PC-10 in posterior uveal melanoma.* Ophthalmology 1998; 105: 485-491.

34 Clarijs R, Ruiter DJ, De Waal RM. *Pathophysiological implications of stroma pattern formation in uveal melanoma.* J Cell Physiol 2003; 194: 267-271.

35 Diener-West M, Hawkins BS, Markowitz JA, Schachat AP. *A review of mortality from choroidal melanoma, II: a meta-analysis of 5-year mortality rates following enucleation, 1966 through 1988.* Arch Ophthalmol 1992; 110: 245-250.

36 Mehaffey MG, Folberg R, Meyer M, Bentler SE, Hwang T, Woolson R, Moore KC. *Relative importance of quantifying area and vascular patterns in uveal melanomas.* Am J Ophthalmol 1997; 123: 798-809.

37 Coleman DJ, Silverman RH, Rondeau MJ, Lloyd HO, Lizi FL, Weingeist TA, Chen X, Vangveeravong S, Folberg R. *Noninvasive in vivo detection of prognostic indicators for high-risk uveal melanoma: ultrasound parameter imaging.* Ophthalmology 2004; 111: 558-564.

38 Kivela T, Eskelin S, Makitie T, Summanen P. *Exsudative retinal detachment from malignant uveal melanoma: predictors and prognostic significance.* Invest Ophthalmol Vis Sci 2001; 42: 2085-2093.

39 Fuchs U, Kivela T, Summanen P, Immonen I, Tarkkanen A. *An immunohistochemical and prognostic analysis of cytokeratin expression in malignant uveal melanoma.* Am J Pathol 1992; 1954: 497-502.

40 Silverman RH, Folberg R, Rondeau MJ, Boldt HC, Lloyd HO, Chen X, Lizzi FL, Weingeist TA, Coleman DJ. *Spectral parameter imaging for detection of prgonostically significant histologic features in uveal melanoma.* Ultrasound Med Biol 2003; 29: 951-959.

41 Onken MD, Worley LA, Ehlers JP, Harbour JW. *Gene expression profiling in uveal melanoma reveals two molecular classes and predicts metastatic death.* Cancer Res 2004; 64: 7205-7209.

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Vascular endothelial growth factor-A in eyes with uveal melanoma

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