New approaches to imaging and treatment of ocular melanoma

Brouwer, N.J.

2.2

Lack of Tumour Pigmentation in Conjunctival Melanoma is Associated with Light Iris Colour and Worse Prognosis

Niels J. Brouwer[1], Marina Marinkovic[1], Gregorius P.M. Luyten[1], Carol L. Shields[2]*, Martine J. Jager[1]*
*CLS and MJJ share senior authorship

Br J Ophthalmol 2019; 103(3):332-337.

1. Department of Ophthalmology, Leiden University Medical Center, Leiden, The Netherlands
2. Ocular Oncology Service, Wills Eye Hospital, Thomas Jefferson University, Philadelphia, Pennsylvania, U.S.A.

ABSTRACT

Aim: To investigate whether differences in iris colour, skin colour and tumour pigmentation are related to clinical outcome in conjunctival melanoma.

Methods: Data of 70 patients with conjunctival melanoma from the Leiden University Medical Centre (Leiden, The Netherlands) and 374 patients from the Wills Eye Hospital (Philadephia, USA) were reviewed. The relation between iris colour, skin colour, and tumour pigmentation versus clinical parameters and outcome was investigated using univariate and multivariate regression analyses.

Results: A light iris colour (blue, grey, green) was present in 261 (59%) of all patients and a dark colour (hazel, brown) in 183 (41%). A low tumour pigmentation was detected in 130 (40%) and a high pigmentation in 197 (60%) patients. Low tumour pigmentation was associated with light iris colour (p=0.021) but not related to skin colour (p=0.92). In univariate analysis, neither iris nor skin colour was related to clinical outcome, while a low tumour pigmentation was related to metastasis formation (HR 2.37, p=0.004) and death (HR 2.42, p=0.020). In multivariate analysis, low tumour pigmentation was related to the development of recurrences (HR 1.63, p=0.043), metastasis formation (HR 2.48, p=0.004) and death (HR 2.60, p=0.014).

Conclusion: Lightly pigmented tumours occurred especially in individuals with lightly coloured irises. While iris colour or skin colour was not significantly related to clinical outcome, a low tumour pigmentation was related to a worse outcome in patients with conjunctival melanoma. The amount and type of melanin in conjunctival melanocytes may be involved in the pathogenesis and behaviour of selected conjunctival melanoma.

INTRODUCTION

Conjunctival melanoma (CoM) is a rare but lethal malignancy of the eye, with a 10-year melanoma-related mortality of approximately 30%.[1-3] As few treatment options exist for advanced stages of CoM, it is important to investigate the mechanisms contributing to this disease and the development of metastases.

A pathway of interest is that of pigment and melanin synthesis. The role of these factors in tumour development and metastasis formation has been investigated in cutaneous and uveal melanoma (UM), and various pathways have been proposed to be involved.[4] Ocular melanin consists of two types: eumelanin and pheomelanin, which have different biological characteristics:[5] eumelanin has a brown/black colour and helps to protect against ultraviolet (UV) radiation-mediated damage, while pheomelanin has a yellow/red colour and has been associated with the induction of genotoxic stress, which is associated with DNA damage.[6-8] The colour of the iris and the skin is determined by the amount and type of melanin.[5,9]

Cutaneous and uveal melanomas typically occur in light-skinned people, and the incidence of both malignancies is higher in individuals with light-coloured irises.[10,11] A light iris colour has been associated with a higher risk of metastatic death in UM, but the mechanisms responsible need to be elucidated.[12] No conclusive results have been published on the association between iris colour, skin colour and the development of CoM. Pigmentation of the tumours themselves was investigated as well: amelanotic cutaneous melanoma has a significantly worse survival compared with melanotic cutaneous melanoma,[13] and one analysis found that low tumour pigmentation was similarly associated with a worse clinical outcome in UM.[14] Low tumour pigmentation has been associated with a worse prognosis in CoM,[15] but this was investigated in a limited number of patients as the disease is so rare.

The aim of this study is to evaluate the association between iris colour, skin colour, tumour pigmentation and clinical outcome in patients with CoM. We hypothesize that the presence of (dark-coloured) eumelanin, as opposed to (light-coloured) pheomelanin, may protect against the development of CoM recurrences and metastases. We therefore expect to find that light iris colour, light skin colour and low tumour pigmentation are associated with a worse clinical outcome in patients with CoM.

METHODS

Patient data

A retrospective analysis on data sets from the Leiden University Medical Center (LUMC, Leiden, The Netherlands) and the Wills Eye Hospital (WEH, Philadelphia, USA) was performed. The Leiden group consisted of 70 patients with histopathologically confirmed primary CoM, diagnosed between 2001 and 2014.[16] The WEH group consisted of 374 patients with histopathologically confirmed primary CoM, diagnosed between 1970 and 2003. The WEH group is part of a larger study group described earlier by Shields *et al*,[17] from which the patients with available data on eye colour were selected.

Statistics

The two data sets were analysed together to obtain enough cases for statistical analysis. Descriptive statistics of both the separate and combined data sets are provided. Categorical data were analysed with Pearson's X^2 tests. Numerical data were analysed with the Mann-Whitney U test. Outcome variables (local recurrence, distant metastasis, melanoma-related death, exenteration) were analysed with univariate and multivariate regression analyses. Two multivariate models were tested. In the first multivariate model, we investigated if the pigment-related variables (iris colour, skin colour, tumour pigmentation) were independently related to the outcome. The variables were entered without any selection criteria. A variable for institution was added to adjust for (unmeasured) differences between the two data sets. In the second multivariate model, we entered all variables with a $p<0.10$ from the univariate analysis using forward selection, to identify a model of significant parameters with a $p<0.05$. The HRs and 95% CIs were provided for all regression analyses.

Clinical characteristics

Iris colour was categorised as either 'light' (blue/grey/green) or 'dark' (hazel/brown). This division is based on the published melanin content of iridal melanocytes in different iris colours, with significantly higher eumelanin, a higher eumelanin/pheomelanin ratio and more total melanin in darker irises compared with lighter irises.[5] Tumour pigmentation was categorised visually as 'low pigmented' (non-pigmented/mixed) or 'high pigmented' (pigmented) (figure 1). Skin colour was categorised as 'fair' (fair/white) or 'non-fair' (tinted/olive/dark). Tumour location on the eye was categorized as 'epibulbar' for CoM only affecting the cornea, limbus or epibulbar conjunctiva, and 'non-epibulbar' for CoM affecting other areas on the eye. As this is a secondary analysis of two data sets, all parameters had been recorded earlier based on patient medical files including available medical photographs.

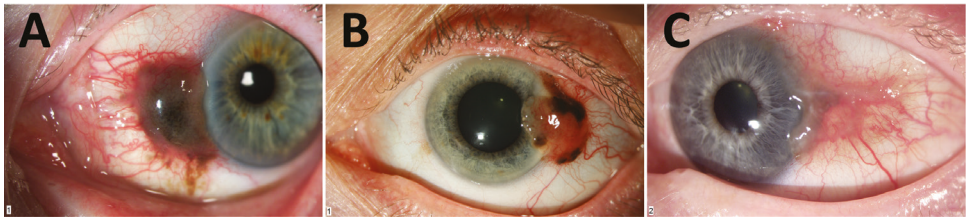

Figure 1. Three conjunctival melanomas with various degrees of pigmentation: (A) Pigmented lesion, (B) mixed lesion and (C) Non-pigmented lesion.

RESULTS

Patient characteristics

A total of 444 patients were included in this study, with 374 patients coming from the WEH, and 70 from the LUMC (table 1). Data on iris colour and skin colour were available for all patients, tumour pigmentation was known in 327 (74%) cases. Mean age at diagnosis was 59.5 years. The mean tumour thickness was 1.77 mm. Most tumours were epibulbar (63%). Patients from the Leiden group presented more often with an epibulbar tumour location compared with the WEH group (p=0.005), but they were similar with regard to other clinical parameters (online supplementary table 1).

Eye colour and skin colour

Light iris colour was detected in 59% of all patients, with a fair skin tone in 88%. Patients from the Leiden group more often had light-coloured irises (p<0.001) and a fair skin (p=0.035) compared with the WEH group (online supplementary table 1).

In the WEH group, a larger maximum basal diameter of the melanoma was associated with darker eye colour (p=0.02), and a non-epibulbar location was observed more frequently in patients with non-fair skin (p=0.005), while this could not be detected for the Leiden patients.

Tumour pigmentation

No or mixed tumour pigmentation was found in 40% of all patients. There was no significant difference in the percentage of lightly pigmented versus highly pigmented tumours between the Leiden group and the WEH group (p=0.31). Overall, there were no differences in clinical characteristics at baseline between lightly pigmented versus highly pigmented tumours. Low tumour pigmentation was related to light iris colour (p=0.022), but not to skin colour (p=0.92) (table 1).

Table 1. Patient and tumour characteristics of the total study group, consisting of 70 cases from the LUMC (Leiden, The Netherlands) and 374 cases from the WEH (Philadelphia, USA)

	Total	Light eye colour	Dark eye colour		Low pigmentation	High pigmentation	
Parameters	**Cases (%)**	**Cases (%)**	**Cases (%)**	**P value**	**Cases (%)**	**Cases (%)**	**P value**
Total	**444 (100)**	**261 (59)**	**183 (41)**		**130 (40)**	**197 (60)**	
Sex							
Male	217 (49)	131 (50)	86 (47)	0.51	56 (43)	98 (50)	0.24
Female	227 (51)	130 (50)	97 (53)		74 (57)	99 (50)	
Age at diagnosis (year)							
<60	202 (45)	115 (44)	87 (48)	0.47	59 (45)	75 (38)	0.19
≥60	242 (55)	146 (56)	96 (52)		71 (55)	122 (62)	
Age at diagnosis (year)							
Mean (SD)	59.5 (17.5)	60.1 (17.5)	58.5 (17.4)	0.37	60.6 (16.7)	62.0 (16.9)	0.38
Side							
Right (OD)	239 (54)	137 (52)	81 (44)	0.50	77 (59)	106 (54)	0.33
Left (OS)	205 (46)	124 (48)	102 (56)		53 (41)	91 (46)	
Location							
Epibulbar	215 (63)	138 (66)	77 (57)	0.08	80 (63)	119 (61)	0.75
Non-epibulbar	128 (37)	70 (34)	58 (43)		48 (38)	77 (39)	
Thickness (mm)							
Mean (SD)	1.77 (2.1)	1.80 (2.4)	1.73 (1.5)	0.30	1.69 (2.5)	1.90 (2.0)	0.50
Tumour LBD (mm)							
Mean (SD)	10.6 (8.1)	9.75 (7.7)	11.9 (8.5)	0.016	10.4 (7.8)	10.8 (8.3)	0.98
Iris colour							
Blue/green/grey	261 (59)	NA	NA	NA	86 (66)	105 (53)	0.021
Hazel/brown	183 (41)				44 (34)	92 (47)	

Table 1. Continued.

Parameters	Total	Light eye colour	Dark eye colour	P value	Low pigmentation	High pigmentation	P value
	Cases (%)	Cases (%)	Cases (%)		Cases (%)	Cases (%)	
Skin colour							
Fair	392 (88)	250 (96)	142 (78)	<0.001	113 (87)	172 (87)	0.92
Non-fair	52 (12)	11 (4)	41 (22)		17 (13)	25 (13)	
Institution							
WEH	374 (84)	202 (77)	172 (94)	<0.001	113 (87)	163 (83)	0.31
LUMC	70 (16)	59 (23)	11 (6)		17 (13)	34 (17)	
Recurrence							
Yes	177 (40)	106 (41)	71 (39)	0.70	67 (52)	81 (41)	0.064
Metastasis							
Yes	62 (14)	34 (13)	28 (15)	0.50	31 (24)	23 (12)	0.004
Melanoma-related death							
Yes	36 (8)	20 (8)	16 (9)	0.68	19 (15)	13 (7)	0.017
Exenteration							
Yes	50 (11)	28 (11)	22 (12)	0.67	24 (19)	23 (12)	0.09

LBD, largest basal diameter; LUMC, Leiden University Medical Center; NA, Not Applicable; OD, oculus dexter; OS, oculus sinister; WEH, Will's Eye Hospital.

Outcome analysis

The mean overall follow-up time was 56.3 months. A total of 177 patients (40%) developed a recurrence, 50 patients (11%) had an exenteration performed during follow-up, 62 patients (14%) developed a metastasis, and 36 patients (8%) died of a melanoma-related cause (table 1). Patients from the Leiden group less frequently developed a recurrence compared with patients from the WEH group (p=0.035), but the groups were similar for other clinical outcome measures (online supplementary table 1).

With univariate analysis (table 2), iris colour and skin colour were not significantly associated with the outcome measures, while low tumour pigmentation was significantly associated with the development of metastases (HR 2.37, p=0.004), and more melanoma-related deaths (HR 2.42, p=0.020); low pigmented tumours tended to have more frequent recurrences (HR 1.52, p=0.064) and a greater number of exenteration (HR 1.71, p=0.089). Follow-up time of lightly pigmented versus highly pigmented tumours was equal, with a mean of 57.9 and 55.2 months, respectively (p=0.42).

In the first multivariate model, the parameters of pigmentation (iris colour, skin colour, tumour pigmentation) were analysed together with an adjustment variable for institution (table 3). Low tumour pigmentation was related to more metastases (HR 2.45, p=0.004), and more melanoma-related deaths (HR 2.76, p=0.010), while there were trends for more recurrences (HR 1.51, p=0.082), and a greater number of exenteration (HR 1.80, p=0.068). Iris colour was not related to any of the outcome measures, but light skin colour showed a trend with more melanoma-related deaths (HR 6.19, p=0.082).

In the second multivariate model, we included parameters with a p<0.10 from the univariate analysis, using forward selection (table 2). As iris colour and skin colour were not related with p<0.10 to any of the outcome measures in univariate analysis, they were not analysed in the second multivariate model. Low tumour pigmentation was significantly related to more recurrences (HR 1.63, p=0.043), metastases (HR 2.48, p=0.004) and melanoma-related deaths (HR 2.60, p=0.014), but not to exenteration.

Table 2. Univariate and multivariate analyses of clinical outcome

	Recurrence			Metastasis			Melanoma-Related Death			Exenteration		
	HR	**95% CI**	**P value**	**HR**	**95% CI**	**P value**	**HR**	**95% CI**	**P value**	**HR**	**95%CI**	**P value**
Univariate analysis												
Iris colour*	1.08	0.73 to 1.59	0.70	0.83	0.48 to 1.42	0.50	0.87	0.44 to 1.72	0.68	0.88	0.49 to 1.59	0.67
Tumour pigmentation†	1.52	0.98 to 2.38	0.064	2.37	1.31 to 4.29	0.004	2.42	1.15 to 5.10	0.020	1.71	0.92 to 3.19	0.089
Skin colour‡	1.07	0.59 to 1.94	0.83	1.05	0.45 to 2.44	0.91	5.00	0.67 to 37.3	0.12	0.66	0.29 to 1.50	0.32
Institution§	0.55	0.32 to 0.97	0.037	0.89	0.42 to 1.91	0.77	1.90	0.85 to 4.23	0.12	1.60	0.78 to 3.30	0.20
Location on eye¶	1.67	1.07 to 2.59	0.023	3.53	1.93 to 6.46	<0.001	4.06	1.91 to 8.65	<0.001	4.44	2.30 to 8.60	<0.001
Age**	1.89	1.28 to 2.79	0.001	1.50	0.86 to 2.60	0.15	1.74	0.85 to 3.58	0.13	1.72	0.93 to 3.19	0.09
Thickness	1.03	0.87 to 1.22	0.76	1.01	0.79 to 1.30	0.91	1.03	0.78 to 1.35	0.84	1.48	1.14 to 1.92	0.003
LBD	1.02	0.99 to 1.05	0.22	0.99	0.96 to 1.03	0.79	0.96	0.91 to 1.02	0.19	1.04	1.00 to 1.08	0.03
Multivariate analysis (forward selection, variables with p<0.10 in univariate analysis)												
Tumour Pigmentation †	1.63	1.02 to 2.60	0.043	2.48	1.33 to 4.64	0.004	2.60	1.22 to 5.57	0.014	NA		
Location¶	1.67	1.05 to 2.67	0.031	3.43	1.83 to 6.46	<0.001	3.61	1.66 to 7.85	0.001	NA		
Age**	1.80	1.12 to 2.88	0.015	NA			NA			NA		
Institution§	0.32	0.16 to 0.66	0.002	NA			NA			NA		

** Light versus dark (ref).*

† Lightly/mixed pigmented versus highly pigmented (ref).

‡ Fair versus non-fair (ref).

§ Leiden versus Philadelphia (ref).

¶ Non-epibulbar versus epibulbar (ref).

*** Age ≥60 years versus <60 year (ref).*

LBD, largest basal diameter; NA, not applicable, ref, reference.

Table 3. Multivariate analysis of pigment-related variables with clinical outcome

Univariate analysis	Recurrence			Metastasis			Melanoma-Related Death			Exenteration		
	HR	95% CI	P value	HR	95% CI	P value	HR	95% CI	P value	HR	95%CI	P value
Multivariate analysis (pigment-related variables, adjusted for institution)												
Iris Colour*	0.88	0.55-1.43	0.61	0.75	0.40-1.42	0.38	0.56	0.25-1.25	0.16	0.80	0.41-1.59	0.53
Tumour Pigmentation†	1.51	0.95-2.39	0.082	2.45	1.34-4.48	0.004	2.76	1.28-5.95	0.010	1.80	0.96-3.40	0.068
Skin Colour‡	1.33	0.66-2.66	0.42	1.44	0.55-3.80	0.46	6.19	0.79-48.4	0.082	0.71	0.29-1.73	0.45
Institution§	0.29	0.14-0.59	0.001	0.71	0.28-1.80	0.47	1.87	0.72-4.85	0.20	1.55	0.68-3.56	0.30

** Light versus dark (ref).*

† Lightly/mixed pigmented versus highly pigmented (ref).

‡ Fair versus non-fair (ref).

§ Leiden versus Philadelphia (ref).

95% CI=95% Confidence Interval; HR, Hazard Ratio; Ref, reference

DISCUSSION

We investigated the association between tumour pigmentation, iris colour, skin colour and clinical outcome in CoM. Low tumour pigmentation was significantly associated with a greater risk for recurrence, metastasis and melanoma-related death, even after adjustment for other clinical parameters and hospital. Iris colour or skin colour were not significantly related to outcome. Low tumour pigmentation was significantly related to light iris colour in patients with CoM, but not to skin colour.

To our knowledge, only one study reported on iris colour and clinical outcome in CoM.[18] Our current study is an extension of that study, increasing the number of patients from 150 to 444. Similar to the observation in the smaller group, we did not detect an independent relation between iris colour and outcome in CoM. This differs from findings in UM, where patients with blue/grey iris colour had a significantly increased risk of metastatic death compared with patients with darker irises.[12,19]

Our study showed an association between low tumour pigmentation and a greater risk for recurrences, metastases and metastatic deaths. The association between low tumour pigmentation and recurrence has been reported before in a smaller study, unadjusted for other parameters,[15] and a trend for this association was reported to occur in a Danish study.[20] The association between a low tumour pigmentation and the development of metastasis or death was shown earlier with univariate analysis for a set including the WEH patients,[17] but was not detected by the two other studies, which could relate to the considerably smaller sample sizes, having 69 and 127 patients, respectively.[15,20]

We did not observe a significant relation between low tumour pigmentation and risk of exenteration, which had been observed previously in a smaller case series.[21] As that study was adjusted for different variables and included fewer cases (n=151), this may explain why we currently have a different observation. As the decision to perform an exenteration is based on various (clinical) factors, it might be difficult to identify unbiased prognostic parameters.

Interestingly, tumour pigmentation was still related to clinical outcome in a multivariate analysis which included the three investigated pigment-related parameters (iris colour, skin colour, tumour pigmentation) (table 3).

To our knowledge, this is the first report to find an association between low tumour pigmentation and light iris colour in CoM, as has already been observed in choroidal and iris melanoma.[19 22,23]

Interestingly, we did not detect a relationship between low tumour pigmentation and skin colour in CoM, which we had expected because of the functional similarity of the conjunctiva and the skin.

Clinical outcome was also not related to skin colour in our study group. It may be concluded that a possible relationship is absent or too weak to be of clinical relevance, or that the (lack of) variation in our population did not allow a proper analysis.

In the study of different types of melanocytes, it is important to recall that conjunctival, uveal (including iridal) and cutaneous melanocytes are all assumed to be derived from the same embryonic cells.[24] They originate in the neural crest, with precursor cells following different migration routes to the distinct anatomical locations. Conjunctival melanocytes migrate to the surface ectoderm-derived epithelium and show functional similarities to melanocytes in the skin, such as transferring melanin to surrounding cells. Uveal melanocytes migrate to deeper, mesoderm-derived tissues and are adjacent to the neuro-ectoderm-derived retinal pigment epithelium (RPE) cells.[24,25] Although conjunctival, uveal and cutaneous melanomas are all derived from the neural crest, it is as yet unknown how the genetic differences – with e.g. different mutations in CoM compared with UM - originate.

We propose that the amount and type of melanin present in the melanocytes of the conjunctiva relate to the development and behaviour of CoM. Melanin can be divided into two types: brown/black eumelanin and yellow/red pheomelanin, with different characteristics.[6] Dark-coloured irises have uveal melanocytes that contain more total melanin, and have a higher eumelanin/pheomelanin ratio, compared with melanocytes of light-coloured irises.[5] A similar effect has been found in skin melanocytes, with more total melanin and relatively less pheomelanin in darker skin.[9] Conjunctival melanocytes were similarly found to contain more melanin in eyes with dark irises, although the eumelanin/pheomelanin ratio is unknown.[24]

By the design of the study, lacking a good comparison, we cannot conclude if eye colour or skin colour predisposes to the development of CoM. However, light-coloured eyes could be more prone to development of CoM because of the relatively large amount of pheomelanin in the melanocytes, together with a lack of total pigment to protect against UV damage. Larsen *et al*[20,26] demonstrated recently that UV-induced *BRAF* mutations occurred more frequently in CoM in sun-exposed (epibulbar or caruncular) sites, and in mixed or non-pigmented lesions. Skin colour or iris colour was not investigated in relation to *BRAF* mutations, however. It would be of interest to study the mechanisms in pigmentation and (UV-induced) mutations to investigate a potential causality.

This study does allow to elaborate on the mechanisms of CoM behaviour once it has developed. First, a disbalance of pheomelanin and eumelanin may promote aggressive outgrowth leading to worse clinical behaviour. This is with the assumption that low tumour pigmentation reflects a low eumelanin/pheomelanin ratio, as is suspected by the dark colour of eumelanin compared with the lightly coloured pheomelanin.

A second mechanism to be considered is that changes in melanin relate to other, genetic, aberrations of the tumour. As such, the pigmentation is not causative of behaviour, but indicative of other mechanisms. Following further malignant changes in melanocytes, the ability to produce pigment may be lost, resulting in amelanotic lesions.

A third factor that should be considered, is that external factors are involved. It is harder to determine the tumour margins in lightly pigmented lesions, making it more difficult to identify and treat affected areas of the conjunctiva. As primary tumour treatment is an important prognostic parameter in CoM,[16,18] this could have led to suboptimal treatment of lightly pigmented lesions, and residual melanoma cells might have caused the higher recurrence and metastasis rates.

A clinical implication of our findings is that clinicians must be more aware of the worse prognosis of lightly pigmented CoM. With tumour margins more difficult to assess in the absence of pigment, a wider surgical approach could be justified in removing such lesions, with more extensive (adjuvant) treatment. As lightly pigmented lesions develop more often in patients with a light iris colour, this calls for even more caution in patients with lightly coloured eyes.

A strength of this study is the large sample size, allowing multivariate analysis. Two models could be presented, with adjustment for other parameters. Also, we were able to investigate eye colour, tumour pigmentation and skin colour together, which is interesting as these parameters all depend on similar pathways of melanin production. Recently, a study was published on clinical parameters that were associated with outcome in CoM in the Leiden group, identifying the hospital of initial treatment and the type of treatment as prognostically important for the development of recurrences.[16] Prior to this, an analysis of patients that included the WEH group, identified other parameters such as tumour origin and location as being related to metastasis development.[17] We compared as many of the different parameters as possible with tumour pigmentation, but not all parameters were available. Unfortunately, we were not able to test for genetic aberrations or determine the cellular contents of melanin in our cases; our findings warrant further investigation, however.

In conclusion, we found that low tumour pigmentation is related to light iris colour and a worse clinical outcome in CoM. Iris colour or skin colour was not related to clinical outcome. Our findings suggest a role for the amount and type of melanin present in the melanocytes of the conjunctiva in the behaviour of CoM. Future research should elucidate the exact – and sequential – molecular pathways that relate pigmentation to tumour behaviour.

Contributors: Design of the study: all authors. Data acquisition: NJB, MM, CLS. Data interpretation: all authors. Drafting and critical revision and approval of the manuscript: all authors.
Funding: NJB received an MD/PhD programme grant from the LUMC. The sponsor or funding organization had no role in the design or conduct of this research.
Competing interests: None declared.
Patient consent: Not required.
Ethics approval: The institutional medical ethics committee of the Leiden University Medical Center decided that this retrospective study did not require their approval under Dutch law. The study adhered to the tenets of the Declaration of Helsinki.
Provenance and peer review: Not commissioned; externally peer reviewed.

REFERENCES

1. Missotten GS, Keijser S, De Keizer RJ, De Wolff-Rouendaal D. Conjunctival melanoma in the Netherlands: a nationwide study. *Invest Ophthalmol Vis Sci.* 2005;46(1):75-82.
2. Paridaens AD, Minassian DC, McCartney AC, Hungerford JL. Prognostic factors in primary malignant melanoma of the conjunctiva: a clinicopathological study of 256 cases. *Br J Ophthalmol.* 1994;78(4):252-259.
3. Werschnik C, Lommatzsch PK. Long-term follow-up of patients with conjunctival melanoma. *Am J Clin Oncol.* 2002;25(3):248-255.
4. Slominski RM, Zmijewski MA, Slominski AT. The role of melanin pigment in melanoma. *Exp Dermatol.* 2015;24(4):258-259.
5. Wakamatsu K, Hu DN, McCormick SA, Ito S. Characterization of melanin in human iridal and choroidal melanocytes from eyes with various colored irides. *Pigment Cell Melanoma Res.* 2008;21(1):97-105.
6. Ito S, Wakamatsu K. Quantitative analysis of eumelanin and pheomelanin in humans, mice, and other animals: a comparative review. *Pigment Cell Res.* 2003;16(5):523-531.
7. Mitra D, Luo X, Morgan A, et al. An ultraviolet-radiation-independent pathway to melanoma carcinogenesis in the red hair/fair skin background. *Nature.* 2012;491(7424):449-453.
8. de Lange MJ, Razzaq L, Versluis M, et al. Distribution of GNAQ and GNA11 Mutation Signatures in Uveal Melanoma Points to a Light Dependent Mutation Mechanism. *PLoS One.* 2015;10(9):e0138002.
9. De Leeuw SM, Smit NP, Van Veldhoven M, et al. Melanin content of cultured human melanocytes and UV-induced cytotoxicity. *J Photochem Photobiol B.* 2001;61(3):106-113.
10. Olsen CM, Carroll HJ, Whiteman DC. Estimating the attributable fraction for melanoma: a meta-analysis of pigmentary characteristics and freckling. *Int J Cancer.* 2010;127(10):2430-2445.
11. Weis E, Shah CP, Lajous M, Shields JA, Shields CL. The association between host susceptibility factors and uveal melanoma: a meta-analysis. *Arch Ophthalmol.* 2006;124(1):54-60.
12. Schmidt-Pokrzywniak A, Kalbitz S, Kuss O, et al. Assessment of the effect of iris colour and having children on 5-year risk of death after diagnosis of uveal melanoma: a follow-up study. *BMC Ophthalmol.* 2014;14:42.
13. Moreau JF, Weissfeld JL, Ferris LK. Characteristics and survival of patients with invasive amelanotic melanoma in the USA. *Melanoma Res.* 2013;23(5):408-413.
14. Shields CL, Kaliki S, Cohen MN, et al. Prognosis of uveal melanoma based on race in 8100 patients: The 2015 Doyne Lecture. *Eye (Lond).* 2015;29(8):1027-1035.
15. Anastassiou G, Heiligenhaus A, Bechrakis N, et al. Prognostic value of clinical and histopathological parameters in conjunctival melanomas: a retrospective study. *Br J Ophthalmol.* 2002;86(2):163-167.
16. Brouwer NJ, Marinkovic M, van Duinen SG, et al. Treatment of conjunctival melanoma in a Dutch referral centre. *Br J Ophthalmol.* 2017:Published online first: 9 November 2017. doi:2010.1136/bjophthalmol-2017-311082.
17. Shields CL, Markowitz JS, Belinsky I, et al. Conjunctival melanoma: outcomes based on tumor origin in 382 consecutive cases. *Ophthalmology.* 2011;118(2):389-395 e381-382.
18. Shields CL. Conjunctival melanoma: risk factors for recurrence, exenteration, metastasis, and death in 150 consecutive patients. *Trans Am Ophthalmol Soc.* 2000;98:471-492.
19. Regan S, Judge HE, Gragoudas ES, Egan KM. Iris color as a prognostic factor in ocular melanoma. *Arch Ophthalmol.* 1999;117(6):811-814.
20. Larsen AC, Dahmcke CM, Dahl C, et al. A Retrospective Review of Conjunctival Melanoma Presentation, Treatment, and Outcome and an Investigation of Features Associated With BRAF Mutations. *JAMA Ophthalmol.* 2015;133(11):1295-1303.
21. Shields JA, Shields CL, Gunduz K, Cater J. Clinical features predictive of orbital exenteration for conjunctival melanoma. *Ophthal Plast Reconstr Surg.* 2000;16(3):173-178.

22. Rootman J, Gallagher RP. Color as a risk factor in iris melanoma. *Am J Ophthalmol.* 1984;98(5):558-561.
23. Wierenga APA, Dogrusöz M, Kroes W, et al. Tumor pigmentation in Uveal melanoma is related to eye color [abstract]. *Investigative Ophthalmology & Visual Science.* 2017;58(8):4417-4417.
24. Hu DN, McCormick SA, Seedor JA, Ritterband DC, Shah MK. Isolation, purification and cultivation of conjunctival melanocytes. *Exp Eye Res.* 2007;84(4):655-662.
25. Iwamoto S, Burrows RC, Grossniklaus HE, et al. Immunophenotype of conjunctival melanomas: comparisons with uveal and cutaneous melanomas. *Arch Ophthalmol.* 2002;120(12):1625-1629.
26. Larsen AC, Dahl C, Dahmcke CM, et al. BRAF mutations in conjunctival melanoma: investigation of incidence, clinicopathological features, prognosis and paired premalignant lesions. *Acta Ophthalmol.* 2016;94(5):463-470.

SUPPLEMENTARY MATERIAL

Supplementary table 1. Patient and tumour characteristics of the two analysed groups of patients with histologically-proved conjunctival melanoma.

	Total	Leiden	Philadelphia	
Parameter	**Cases (%)**	**Cases (%)**	**Cases (%)**	**P-value**
Total	**444 (100)**	**70 (16)**	**374 (84)**	
Sex				
male	217 (49)	35 (50)	182 (49)	0.84
female	227 (51)	35 (50)	192 (51)	
Age at diagnosis (year)				
<60	202 (45)	35 (50)	167 (45)	0.41
≥60	242 (55)	35 (50)	207 (55)	
Age at diagnosis (year)				
mean (SD)	59.5 (17.5)	60.3 (18.3)	59.3 (17.3)	0.71
Side				
right (OD)	239 (54)	37 (53)	202 (54)	0.86
left (OS)	205 (46)	33 (47)	172 (46)	
Location				
epibulbar	215 (63)	54 (77)	161 (59)	0.005
non-epibulbar	128 (37)	16 (23)	112 (41)	
Thickness (mm)	(n=130)	(n=54)	(n=76)	
mean (SD)	1.77 (2.1)	2.25 (2.8)	1.43 (1.3)	0.14
Tumour LBD (mm)	(n=320)	(n=50)	(n=270)	
mean (SD)	10.63 (8.1)	8.97 (6.1)	10.94 (8.4)	0.30
Pigmentation				
non/mixed pigmented	130 (40)	17 (33)	113 (41)	0.31
pigmented	197 (60)	34 (67)	163 (59)	
Iris colour				
blue/green/grey	261 (59)	59 (84)	202 (54)	<0.001
hazel/brown	183 (41)	11 (16)	172 (46)	
Skin colour				
fair	392 (88)	67 (96)	325 (87)	0.035
non-fair	52 (12)	3 (4)	49 (13)	
Recurrence				
yes	177 (40)	20 (29)	157 (42)	0.035
Metastasis				
yes	62 (14)	9 (13)	53 (14)	0.77
Melanoma-related death				
yes	36 (8)	9 (13)	27 (7)	0.11
Exenteration				
yes	50 (11)	11 (16)	39 (10)	0.20

LBD, largest basal diameter; SD, Standard Deviation.