New approaches to imaging and treatment of ocular melanoma

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Treatment of Conjunctival Melanoma in a Dutch Referral Centre

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Br J Ophthalmol 2018; 102(9):1277-1282

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ABSTRACT

Aims: To evaluate the treatment of conjunctival melanoma at a large Dutch referral centre and to make recommendations for clinical management.

Methods: A retrospective review was performed of clinical and histological data of 70 patients treated for a primary conjunctival melanoma between 2001 and 2014 at the LUMC, Leiden, The Netherlands. Detailed follow-up data were available for all patients.

Results: The mean follow-up time was 70.2 months. The overall 5-year recurrence rate was 29%, the 5-year metastasis rate 12%, and the 5-year melanoma-related survival 90%. Treatment with excision alone had a significantly higher 5-year recurrence rate than (the combination of) other treatments (HR 3.73, 95% CI 1.19 to 11.6, p=0.02). Initial treatment in an ocular oncology centre was associated with fewer recurrences compared with initial treatment by a local ophthalmologist of a referring centre (HR 0.32, 95% CI 0.11 to 0.94, p=0.04), despite similar tumour baseline characteristics.

Conclusion: Conjunctival melanoma is a rare disease with a high recurrence rate. A treatment strategy with local excision and adjuvant therapy gave a good clinical outcome, excision alone as a treatment should be considered obsolete. Initial treatment in a large referral centre improves clinical outcome, and patients should be referred to a specialised centre as soon as possible.

INTRODUCTION

Conjunctival melanoma (CM) is a rare ocular malignancy with an incidence of 0.3-0.8 per million in Caucasians.[1-4] Although the disease remains uncommon in other ethnicities, a rising incidence in Caucasians has been reported.[1,2,4,5] CM originates from melanocytes of the conjunctiva and can develop in association with primary acquired melanosis (PAM) (in up to 74% of cases), nevi (7%) or de novo (19%).[6] The clinical presentation of CM may vary, and melanoma may be localised in any part of the bulbar, forniceal, or tarsal conjunctiva. The presenting lesion may be amelanotic, brownish or black.

Primary CM is generally treated with wide local excision, followed by adjuvant treatment, such as cryotherapy, brachytherapy or topical chemotherapy.[7] More extensive surgical procedures such as exenteration are used as a last resort therapy. Other treatments such as electron beam radiotherapy and proton beam irradiation are used but the available literature regarding their use is limited.[7] Newer treatments as targeted therapy and immunotherapy for metastases are under investigation, although no proven treatment for distant metastasis is available yet. The local recurrence rate – despite treatment – is high (61% of patients after 5 years), and a melanoma-related death of up to 14% after 5 years has been reported.[8] Different factors affect clinical outcome, most of which are related to tumour location, thickness and histopathological characteristics.[6,8-10]

In this study, we describe the clinical outcome of 70 patients with CM seen at a national referral centre for ocular malignancies in The Netherlands. The study group consists of first-presenting primary tumours, with a complete follow-up. We set out to determine which treatments had the best outcome and to make recommendations for clinical management based on our data and experience.

METHODS

Patient selection

We identified patients with CM seen or treated at our institution for a first presentation of CM between January 2001 and December 2014 by searching the institutional cancer registration system and institutional pathology reports. Patients referred for a recurrence were not included. In total, 70 patients with histologically proven invasive primary CM were included in this study. Patients with only non-invasive in situ melanoma of the conjunctiva were excluded. The pathological examination was performed by an experienced ophthalmological pathologist; material obtained in other centres was reviewed in our institution.

Clinical and histological data

A retrospective review of clinical records, pathology reports and photographic images was performed. Baseline characteristics collected at presentation included patient age at diagnosis, tumour size and localisation. Based on data from pathology reports, medical files, and (colour) photographs, tumour size and localisation were identified. CM of the cornea, limbus or bulbar conjunctiva was categorised as 'epibulbar', with CM at other sites being categorised as 'non-epibulbar'. Cell type (spindle/epithelioid), presence of mitoses, ulceration, and extension into lateral or deep margin were obtained from pathology reports and by review of pathology samples. Follow-up data included type and number of received treatments and clinical outcome (local recurrence, metastasis, death). The location of the first received treatment was categorised as 'ocular oncology centre' (our institution) or 'local ophthalmologist of a referring centre' (elsewhere). Local recurrence was defined as the recurrence of histologically proven invasive CM. Metastases were identified with imaging techniques, including ultrasound (US), MRI, CT, or pathological analysis of suspected lesions. The seventh edition of the American Joint Committee on Cancer tumour, node, metastases (AJCC TNM) staging was used to stage all tumours.[11]

Statistical analysis

Univariate Cox regression analyses were done and Kaplan-Meier (KM) survival curves were generated to analyse clinical outcome. HRs with corresponding 95% CIs were provided. KM analyses were tested for significance with log-rank tests.

Differences between categorical data were evaluated using Pearson's x^2 test or Fisher exact test. Differences between numerical data were analysed with the Mann-Whitney U test.

A P-value < 0.05 was considered statistically significant for all analyses. Data analyses were performed with SPSS software V.23.0.

RESULTS

Clinical presentation

Baseline characteristics of the included patients are presented in table 1. Seventy patients (35 males, 35 females) with a mean age at diagnosis of 60.3 years were included (median: 60.3 years). Tumour location was epibulbar in 54 cases (77%), and non-epibulbar in 16 cases (23%). The mean 'largest basal diameter' at presentation was available in 50 cases with a mean of 9.0 mm (median: 7.1 mm). Tumour thickness was available in 54 cases with a mean of 2.3 mm (median: 1.2 mm). According to the seventh edition of the AJCC TNM classification, 77% of the cases were graded as T1 and 23% as T2. No lymph node metastases (N1) or distant metastases (M1) were present at baseline.

Table 1. Patient and tumour characteristics.

	Overall	Recurrence		Metastasis		Melanoma-Related Death		Exenterations	
Item	Cases (%)	Cases (%)	p	Cases (%)	p	Cases (%)	p	Cases (%)	p
Overall	**70 (100)**	**20 (29)**		**9 (13)**		**9 (13)**		**11 (16)**	
Sex									
Male	35 (50)	10 (29)	1.00	6 (17)	0.48*	5 (14)	1.00*	7 (20)	0.32
Female	35 (50)	10 (29)		3 (9)		4 (11)		4 (11)	
Age at diagnosis									
<60 years	35 (50)	8 (23)	0.29	2 (6)	0.15*	2 (6)	0.15*	4 (11)	0.32
≥60 years	35 (50)	12 (34)		7 (20)		7 (20)		7 (20)	
Side									
Left (OS)	33 (47)	10 (30)	0.76	4 (12)	1.00*	5 (15)	0.73*	6 (18)	0.59
Right (OD)	37 (53)	10 (27)		5 (14)		4 (11)		5 (14)	
Location									
Epibulbar	54 (77)	17 (32)	0.53*	6 (11)	0.42*	5 (9)	0.20*	3 (6)	<0.001
Non-epibulbar	16 (23)	3 (19)		3 (19)		4 (25)		8 (50)	
cTNM									
T1	54 (77)	17 (32)	0.53*	6 (11)	0.42*	5 (9)	0.20*	3 (6)	<0.001
T2	16 (23)	3 (19)		3 (19)		4 (25)		8 (50)	
PAM									
Present	65 (93)	20 (31)	0.31*	8 (12)	0.51*	8 (12)	0.51*	11 (17)	1.00*
Absent	0 (0)	0 (0)		0 (0)		0 (0)		0 (0)	
Unknown	5 (7)	0 (0)		1 (20)		1 (20)		0 (0)	
Initial treatment									
Our institution	48 (69)	10 (21)	0.03	6 (13)	1.00*	7 (15)	0.71*	10 (21)	0.15*
Elsewhere	22 (31)	10 (46)		3 (14)		2 (9)		1 (5)	
Period									
2001 to 8/2012	53 (76)	17 (32)	0.36*	8 (15)	0.44*	9 (17)	0.10*	6 (11)	0.12*
9/2012 to 2014	17 (24)	3 (18)		1 (6)		0 (0)		5 (29)	
Thickness (mm)									
<2	36 (51)	10 (28)	0.41	4 (11)	1.00*	4 (11)	0.67*	2 (6)	0.004*
≥2	18 (26)	7 (39)		2 (11)		3 (17)		7 (39)	

*P values are calculated with Pearson's x^2 tests, unless indicated with * for Fisher's exact tests.*
cTNM, clinical tumour, node, metastases stage; PAM, primary acquired melanosis.

Treatments

Data on initial treatment following diagnosis of the CM was available for all patients (table 2). In total, 48 patients (69%) received the first treatment for their CM in an ocular oncology centre, and 22 patients (31%) received their first treatment from the local ophthalmologist of the referring

centre. Patient characteristics did not differ between the two groups in mean age, tumour size or thickness; a trend was observed for more stage 1 (epibulbar) melanoma in the referred patients (p=0.063) (supplementary table 1). Treatment for the primary CM consisted most often of surgical excision with adjuvant therapy, being cryotherapy (10%, n =7), chemotherapy (1%, n=1,), ruthenium plaque (16%, n=11), strontium brachytherapy (30%, n=21) or iridium brachytherapy (3%, n=2). Other treatments were excision alone (26%, n=18), exenteration (9%, n=6), or external beam radiotherapy (6%, n=4). Patients who received their first treatment elsewhere all underwent local excision (without other treatment) before they were referred to our institution. After intake, 15 patients received adjuvant re-excision, cryotherapy, brachytherapy or a combination of those. Seven patients received no further treatment as already months had passed without clinical changes, or as the exact location of the primary lesion could not be determined any more. Treatments for first recurrences were most often excision with ruthenium (21%, n=4) or excision with strontium (27%, n=5). Last resort therapy for recurrences was external beam irradiation (n=1, 5%) or exenteration (n=3, 16%). At the end of follow-up, an exenteration was performed in 11 cases (16%).

Table 2. Initial treatments for conjunctival melanoma in respect to period and clinical outcome.

	Total	2001 to August 2012	September 2012 to 2014	Recurrence	Metastasis	Melanoma-Related Death	Exenteration
Item	**Cases (%)**	**Cases (%)**	**Cases (%)**	**Cases (%)**	**Cases (%)**	**Cases (%)**	**Cases (%)**
Overall	**70 (100)**	**53 (100)**	**17 (100)**	**20 (29)**	**9 (13)**	**9 (13)**	**11 (16)**
Excision alone	18 (26)	16 (30)	2 (12)	9 (50)	1 (6)	2 (11)	0 (0)
Excision + cryotherapy	7 (10)	7 (13)	0 (0)	1 (14)	2 (25)	2 (25)	1 (13)
Excision + mitomycin	1 (1)	1 (2)	0 ()	1 (100)	0 (0)	0 (0)	0 (0)
Excision + ruthenium	11 (16)	0 (0)	11 (65)	3 (27)	1 (9)	0 (0)	1 (9)
Excision + strontium	21 (30)	21 (40)	0 (0)	4 (19)	2 (10)	2 (10)	1 (5)
Excision + iridium	2 (3)	2 (4)	0 (0)	1 (50)	1 (50)	1 (50)	1 (50)
External beam radiation	4 (6)	4 (8)	0 (0)	1 (25)	2 (50)	2 (50)	1 (25)
Exenteration	6 (9)	2 (4)	4 (24)	0 (0)	0 (0)	0 (0)	6 (100)

Clinical outcome

Follow-up data was available for all patients with a mean of 70.2 months (median 56.7, range 3.3-172.3). No patient was lost to follow-up, and follow-up data of >1 year were available for 68 patients (97%). In total, 20 patients developed a local recurrence (29%), with a 5-year recurrence rate of

29%. Distant metastases were found in nine cases (13%), which were located in the liver (n=4), lung (n=3), brain (n=1) or elsewhere (n=4), with an overall 5-year metastasis rate of 12% (10-years: 23%). Regional (lymph node) metastases were detected in seven patients (10%), of whom 5 (71%) developed distant metastases later on; four patients (6% of all patients) developed distant metastases without (known) prior lymph node involvement. At the end of follow-up, 21 patients had died (30%) of whom 9 had died from melanoma-related causes, 4 from other causes, and 8 due to an unknown cause. Proven melanoma-related mortality at the end of follow up is therefore 13%, with a 5-year melanoma-related survival of 90% (10 years: 74%). The 5-year overall survival is 72% (10 years: 58%). With KM analysis, at 5-years, the exenteration rate was 14% (10 years: 20%).

Median visual acuity (VA, Snellen value) at baseline was 1.00 (mean: 0.96). The VA at the end of follow-up had remained the same in 54 cases (77%), had decreased due to treatment in 13 cases (19%) and due to other causes in 3 cases (4%). Overall, median VA at the end of follow up was 1.00 (mean: 0.84). Without exenterations (n=11), median VA was 1.00 (mean 0.99) (supplementary table 2).

Outcome analysis

We analysed the value of several parameters as potential predictive factors for the four main endpoints of this study (local recurrence, metastasis, melanoma-related survival and exenteration), as demonstrated in table 3. A higher stage according to the TNM classification was associated with an increased risk for exenteration (HR 17.0, 95% CI 3.7 to 77.9, p<0.001). Treatment with excision alone had a significantly higher 5-year recurrence rate than (the combination of) other treatments (HR 3.73, 95% CI 1.19 to 11.6, p=0.02). The recurrence rate was less for patients treated directly at our ocular oncology centre compared with patients receiving their first treatment elsewhere (HR 0.32, 95% CI 0.11 to 0.94, p=0.04). Tumour thickness >2.0 mm was significantly associated with the risk of eventual exenteration (HR 10.8, 95% CI 2.0 to 59.9, p=0.006). Patients with a local recurrence were at higher risk of death due to melanoma (HR 6.71, 95% CI 1.49 to 30.4, p=0.013), and a trend towards a higher risk for metastasis was observed (HR 3.83, 95% CI 0.91 to 16.1, p=0.067). Cell type (spindle/epithelioid), mitoses (no/yes), ulceration (no/yes), extensions into lateral margin (no/yes) and extension into deep margin (no/yes) were not significantly related to the outcome in our cohort.

DISCUSSION

We evaluated the clinical outcome of 70 patients with CM treated at our institution and obtained follow-up data of all patients. The mean follow-up time was 70.2 months. We observed a 5-year local recurrence rate of 29%, a 5-year metastasis rate of 12% and a 5-year melanoma-related survival of 90%. Patients receiving their first treatment at an ocular oncology centre had significantly fewer recurrences than patients receiving their first treatment by the local ophthalmologist of the referring centre, despite similar baseline characateristics.

Our results compare favourably to other reports with regard to the main clinical outcome parameters. The 5-year recurrence rate of 29% is favourable compared with the ranges of 26-61% reported by other groups[6,8,10,12,13] while the 5-year metastasis rate of 12% is comparable to the rates in other reports (11-16%).[12,14] Our 10-year metastasis rate of 23% is within the range of 18-26% reported in the literature,[12,14] as is the 5-year melanoma-related survival of 90% (reported ranges of 68-93%[1,8,9,12,13]). The rate of initial exenterations is somewhat low (9%), though wide ranges have been reported of 3-17%.[8-10,15,16] The eventual rate (11%) is comparable to others, ranging from 10% to 37%, with various follow-up times.[1,6,12,17]

A variety of treatments was available for our patients. A comparison between treatment options for clinical outcome is hampered by the small numbers in certain treatment groups, but a favourable outcome was detected for patients treated with adjuvant brachytherapy (either strontium or ruthenium plaque therapy) compared with the other groups (table 2). A clear worse recurrence rate was found for patients treated with excision alone compared with patients receiving other treatments (table 3). Although CM has the reputation of a sight-threatening disease,[18] VA remained good for all patients treated with non-exenteration (supplementary table 2). This quantifies an earlier suggestion by Damato.[19]

Table 3. Clinical outcome and prognostic values.

	Local Recurrence		Metastasis		Melanoma-Related Death		Exenteration	
Item	**HR (95% CI)**	**P value**	**HR (95% CI)**	**P value**	**HR (95% CI)**	**P value**	**HR (95% CI)**	**P value**
Sex								
Male	Ref	1.00	Ref	0.29	Ref	0.72	Ref	0.33
Female	1.00 (0.35 to-2.82)		2.21 (0.51-9.64)		1.29 (0.32-5.28)		1.94 (0.51-7.33)	
Age								
<60 years	Ref	0.29	Ref	0.09	Ref	0.09	Ref	0.33
≥60 years	1.76 (0.61-5.05)		4.13 (0.79-21.48)		4.13 (0.79-21.5)		1.94 (0.51-7.33)	
Location								
Epibulbar	Ref	0.33	Ref	0.43	Ref	0.11	Ref	<0.001
Non-epibulbar	0.50 (0.13-2.00)		1.85 (0.41-8.40)		3.27 (0.76-14.0)		17.0 (3.7-77.9)	
cTNM								
T1	Ref	0.33	Ref	0.43	Ref	0.11	Ref	<0.001
T2	0.50 (0.13-2.00)		1.85 (0.41-8.40)		3.27 (0.76-14.0)		17.0 (3.7-77.9)	
Initial location								
Our Institution	0.32 (0.11-0.94)	0.04	0.91 (0.20-4.01)	0.90	1.71 (0.33-8.98)	0.53	5.5 (0.66-46.21)	0.12
Elsewhere	Ref		Ref		Ref		Ref	
Period								
2001 to August 2012	Ref	0.26	Ref	0.34	NA		Ref	0.09
Sept2012 to 2014	0.45 (0.12-1.79)		0.35 (0.04-3.04)				3.3 (0.85-12.5)	
Treatment								
Excision alone	3.73 (1.19-11.6)	0.02	0.32 (0.04-2.79)	0.30	0.80 (0.15-4.28)	0.80	NA	
Other	Ref		Ref		Ref			
Thickness (mm)								
<2	Ref	0.41	Ref	1.00	Ref	0.57	Ref	0.006
≥2	1.66 (0.50-5.47)		1.00 (0.17-6.05)		1.60 (0.32-8.07)		10.8 (2.0-59.9)	
Recurrence								
No	NA		Ref	0.07	Ref	0.01	Ref	0.19
Yes			3.83 (0.91-16.1)		6.71 (1.49-30.4)		2.44 (0.65-9.18)	

cTNM, clinical tumour, node, metastases system; NA, not applicable; Ref, reference value.

This study shows a better outcome for patients receiving their first treatment in an ocular oncology centre compared with patients first treated elsewhere (figure 1). This is interesting since no significant differences in maximum tumour size (p=0.36), thickness (p=0.96) or stage (p=0.063) were observed between these two groups, and all patients were referred because of a primary tumour, not a recurrence (supplementary table 1). Iatrogenic tumour seeding may be the cause of this observation, as noticed by Damato and Coupland after an audit of CM patients at their institution in Liverpool,[19] since less experienced surgeons may be less knowledgeable in their approach to this rare disease. A second cause that we propose may be treatment delay and information loss during the referral. Without extensive (photographic) documentation prior to surgery, it is generally difficult to plan appropriate adjuvant therapy and follow-up. By the design of this study – retrospectively including patients who were referred to our institution - we could not rule out a selection bias in patients who were treated elsewhere first, but as the (estimated) majority of Dutch patients with CM will be seen in our centre, we feel that this bias is limited. Like Damato and Coupland, we advise that patients with a lesion suspicious of CM are referred to a specialised centre, preferably without any prior surgery or biopsy. This referral should be accompanied with extensive documentation of the original lesion, and, if applicable, with presurgery photographs.

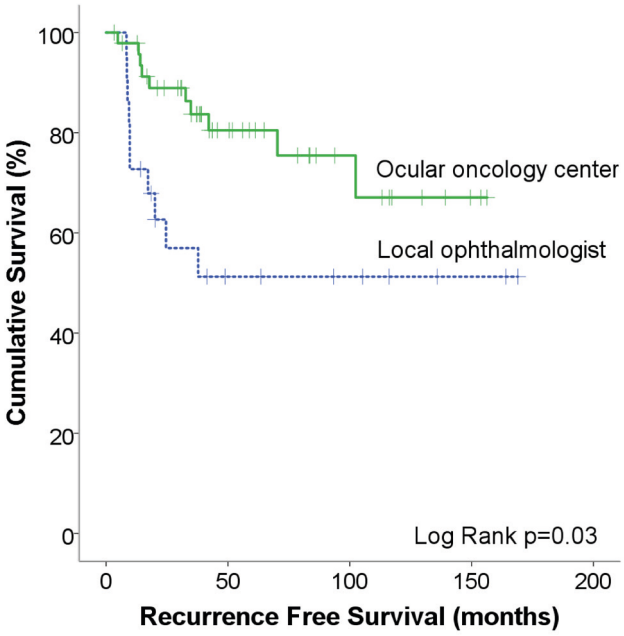

Figure 1. Kaplan-Meier analysis of the recurrence-free survival according to the institute of initial treatment; our institution (ocular oncology centre) versus elsewhere (local ophthalmologist).

In our institution, all patients with suspected ocular malignancies are seen by an ocular oncologist. The mainstay of our current treatment of smaller CM is wide local excision (margins of 2 mm) with adjuvant brachytherapy. During surgery, we apply formalin in a 4% solution with a cotton tip for 20 seconds to the lesion prior to excision. This is believed to cause fixation and to prevent tumour seeding, performed in our institution for many years.[20] Removal of the melanoma is performed using a no-touch technique. At the end of the procedure, the wound is closed when possible, especially if brachytherapy is planned. Larger wound surfaces and especially those in the nasal angle or fornices are covered with amniotic membrane to prevent the development of symblepharon; in smaller wounds, this is not necessary.The excised material is reviewed with immunohistochemical stainings by an experienced ophthalmo-pathologist to confirm diagnosis and assess tissue margins. Brachytherapy is usually applied in a second procedure with several days in between, after pathology has confirmed the diagnosis and has shown all conjunctival surgical margins to be free of tumour. For brachytherapy, we use Ruthenium-106 plaques (BEBIG, Berlin, Germany). Treatment aim is 100Gy at 2mm; this depth is default since no tumour thickness is left after surgery. Larger CM - not fully coverable with a Ruthenium-106 plaque - is treated with exenteration or external radiotherapy, though the latter should be considered as palliative procedure only. For cryotherapy, the double freeze-thaw procedure is used both on the conjunctival and/or limbal margins and the scleral bed. Excision alone should be considered obsolete. Excision with only cryotherapy or mitomycin C has become less common in our institution. In September 2012, strontium brachytherapy was largely abandoned in our centre for logistical reasons. Proton beam irradiation is currently not available in the Netherlands allthough a specialised centre will open shortly. We do not perform sentinel lymph node biopsies as a regular procedure, common greyscale US examination of the cervical/neck lymph nodes is performed every six months, however. A cytological puncture of the lymph nodes is performed if US examination reveals suspicious nodes.

At diagnosis, our systemic work up consists of X-ray imaging of the chest to detect possible pulmonary metastases and analysis of liver function and enzymes for hepatic metastases, in addition to the earlier mentioned US of the cervical/neck lymph nodes. We did not perform systemic screening as a regular procedure during follow-up of the studied period, but have since changed our protocol. Our follow-up regimen now consists of an outpatient clinic visit after 2, 4, 6, and 8 weeks, then once every 3 months for the first year, and once every 6 months thereafter. This is as suggested by Westekemper *et al*, though more frequent in the first visits.[21] Key points of the visit are slit-lamp examination with evertion of the eyelid, and clinical (ocular) photography; photography is always performed at intake, after treatment and at multiple moments during follow-up. Preferably, patients are seen by the same ophthalmologist at every visit enabling detection of small changes in appearance of the conjunctiva, and we would recommend that this follow-up is performed in the tertiary centre. All patients are discussed in a multi-disciplinary meeting with the ocular oncologists

and a radiotherapist. Currently, if PAM is present in areas besides the CM, topical treatment with mitomycin C (drops of 0.04%, four times daily, in two consecutive series of 14 days with 1 week in between) is applied. This was not yet part of the regular protocol during this study however.

This study describes the most recent cohort of patients with CM in The Netherlands. Availability of detailed follow-up data is a strong feauture of this study, as no patient was lost to follow-up. This can be explained by the relatively small size of the Netherlands and the dense, organised structure of healthcare with a national cancer registry. Although we describe one of the larger cohorts of CM patients, sample sizes are still small and this urges a critical view of the data analysis. It should be also noted that our cohort only contained T1 and T2 CM, although no selection regarding tumour stage was applied. Together with the high percentage of co-occurring PAM in our cohort (93%), a known precursor of CM, these issues might have hampered our statistical power to detect prognostic factors. The incidence of CM in the Netherlands could not be determined by this study, but is estimated to be in the range of 0.3-0.8/million, based on data from Scandinavian countries and the USA.[1-4]

In conclusion, CM is a rare ocular malignancy and continues to have a high local recurrence rate and a high mortality. With a current treatment strategy of local excision and adjuvant brachytherapy as the mainstay, we achieved a good clinical outcome comparable to other groups. VA is unthreatened in CM, apart from cases where there is a need for exenteration. Our study confirms the recommendation that patients with a lesion suspicious for CM should be referred as soon as possible to a reference centre for diagnosis and treatment, as this significantly improves clinical outcome. If patients are treated elsewhere first, we stress the importance of presurgery documentation, with photography, to allow proper adjuvant treatment and follow-up.

ACKNOWLEDGEMENTS

The authors thank all employees of their institution involved in the care and scientific research of ocular oncology patients for the contribution to this study.
Contributors: Design of the study: NJB, MM, MJJ. Data acquisition: NJB, MM, SGD. Data interpretation and critical revision and approval of the manuscript: all authors. Drafting the manuscript: NJB.
Funding: NJB received a MD/PhD programme grant from the Leiden University Medical Center.
Competing interests: None declared.
Ethics approval: The institutional medical ethics committee agreed with this retrospective study; the study adhered to the tenets of the declaration of Helsinki.
Provenance and peer review: Not commissioned; externally peer reviewed.

REFERENCES

1. Tuomaala S, Eskelin S, Tarkkanen A, Kivela T. Population-based assessment of clinical characteristics predicting outcome of conjunctival melanoma in whites. *Invest Ophthalmol Vis Sci.* 2002;43(11):3399-3408.
2. Yu GP, Hu DN, McCormick S, Finger PT. Conjunctival melanoma: is it increasing in the United States? *Am J Ophthalmol.* 2003;135(6):800-806.
3. Isager P, Osterlind A, Engholm G, et al. Uveal and conjunctival malignant melanoma in Denmark, 1943-97: incidence and validation study. *Ophthalmic Epidemiol.* 2005;12(4):223-232.
4. Triay E, Bergman L, Nilsson B, All-Ericsson C, Seregard S. Time trends in the incidence of conjunctival melanoma in Sweden. *Br J Ophthalmol.* 2009;93(11):1524-1528.
5. Hu DN, Yu G, McCormick SA, Finger PT. Population-based incidence of conjunctival melanoma in various races and ethnic groups and comparison with other melanomas. *Am J Ophthalmol.* 2008;145(3):418-423.
6. 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.
7. Wong JR, Nanji AA, Galor A, Karp CL. Management of conjunctival malignant melanoma: a review and update. *Expert Rev Ophthalmol.* 2014;9(3):185-204.
8. 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.
9. 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.
10. 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.
11. Edge SB BD, Compton CC. Malignant melanoma of the conjunctiva. In: Edge SB BD, Compton CC, ed. *AJCC Cancer Staging Manual*. 7 ed. New York: Springer; 2010:539-546.
12. Shields CL. Conjunctival melanoma: risk factors for recurrence, exenteration, metastasis, and death in 150 consecutive patients. *Trans Am Ophthalmol Soc.* 2000;98:471-492.
13. Werschnik C, Lommatzsch PK. Long-term follow-up of patients with conjunctival melanoma. *Am J Clin Oncol.* 2002;25(3):248-255.
14. Tuomaala S, Kivela T. Metastatic pattern and survival in disseminated conjunctival melanoma: implications for sentinel lymph node biopsy. *Ophthalmology.* 2004;111(4):816-821.
15. Shields CL, Shields JA, Gunduz K, et al. Conjunctival melanoma: risk factors for recurrence, exenteration, metastasis, and death in 150 consecutive patients. *Arch Ophthalmol.* 2000;118(11):1497-1507.
16. 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.
17. Seregard S, Kock E. Conjunctival malignant melanoma in Sweden 1969-91. *Acta Ophthalmol (Copenh).* 1992;70(3):289-296.
18. Lim LA, Madigan MC, Conway RM. Conjunctival melanoma: a review of conceptual and treatment advances. *Clin Ophthalmol.* 2013;6:521-531.
19. Damato B, Coupland SE. An audit of conjunctival melanoma treatment in Liverpool. *Eye (Lond).* 2009;23(4):801-809.
20. Oosterhuis JA, de Wolff-Rouendaal D. Local metastasis in conjunctival melanoma. *Doc Ophthalmol.* 1983;56(1-2):55-59.
21. Westekemper H, Schallenberg M, Tomaszewski A, et al. [Malignant epibulbar tumours: new strategies in diagnostics and therapy]. *Klin Monbl Augenheilkd.* 2011;228(9):780-792.

SUPPLEMENTARY MATERIAL

Supplementary Table 1. Location of initial treatment.

	Overall	Initial location: Other	Initial location: Our Institution	
Item	**Cases**	**Cases (%)**	**Cases (%)**	**P value**
Overall	**70 (100)**	**22 (31)**	**48 (69)**	
Sex				0.61
-Male	35 (50)	10 (45)	25 (52)	
-Female	35 (50)	12 (55)	23 (48)	
Age at diagnosis				1.00
-<60 years	35 (50)	11 (50)	24 (50)	
-≥60 years	35 (50)	11 (50)	24 (50)	
Side				0.85
-OS	33 (47)	10 (45)	23 (48)	
-OD	37 (53)	12 (55)	25 (52)	
Location				0.063
-Epibulbar	54 (77)	20 (91)	34 (71)	
-Non-epibulbar	16 (23)	2 (9)	14 (29)	
cTNM				0.063
-T1	54 (77)	20 (91)	34 (71)	
-T2	16 (23)	2 (9)	14 (29)	
PAM				0.32*
-Present	65 (93)	19 (86)	46 (96)	
-Absent	0 (0)	0 (0)	0 (0)	
-Unknown	5 (7)	3 (14)	2 (4)	
Period				0.42
2001 - 08/2012	53 (7)	18 (82)	35 (73)	
-09/2012 - 2014	17 (24)	4 (18)	13 (27)	
Thickness				0.68
-Less 2mm	36 (51)	12 (71)	24 (65)	
-2mm or more	18 (26)	5 (29)	13 (35)	
LBD (mean, SD)	9.0 (6.1)	6.3 (3.3)	9.3 (6.3)	0.19**
Thickness (mean, SD)	2.3 (2.80)	2.1 (1.9)	2.3 (3.1)	0.59**

cTNM, clinical TNM stage; LBD, largest basal diameter; SD, Standard Deviation.
*P values are calculated with Pearson Chi-Square tests, unless indicated with * for Fisher Exact tests and ** for Mann-Whitney U tests.*

Supplementary Table 2. Visual Acuity at baseline and end of follow-up.

Item	Overall Cases (%)	Initial location: Elsewhere	Initial location: Our Institution	P value
VA initial		N=22	N=48	
-Mean, Snellen [SD]	0.96 [0.30]	0.92 [0.16]	0.98 [0.34]	0.15
-Median, Snellen	1.00	0.95	1.00	
VA at end of Follow-Up - overall				
-Mean, Snellen [SD]	0.84 [0.47]	0.93 [0.34]	0.79 [0.51]	0.57
-Median, Snellen	1.00	1.00	1.00	
VA at end of Follow-Up – excl exenterations		N=21	N=38	
-Mean, Snellen, [SD]	0.99 [0.32]	0.97 [0.28]	1.00 [0.35]	0.51
-Median, Snellen	1.00	1.00	1.00	
VA loss at end of Follow-Up				
-No loss	54 (77)	19 (86)	35 (73)	
-Loss by exenteration	11 (15)	1 (5)	10 (21)	
-Loss by other treatment	2 (3)	2 (9)	0 (0)	
-Loss by other cause	3 (4)	0 (0)	3 (6)	

VA, Visual Acuity.
P values were obtained by Mann-Whitney U test.