Conjunctival Melanoma in Finland from 1967 to 2000: a population-based study

By
Seppo Tuomaala

Academic Dissertation
To be publicly discussed, by permission of
The Medical Faculty of the University of Helsinki,
In the Auditorium of the Haartman Institute,
Haartmaninkatu 3, Helsinki
On March 19th, 2008, at 12 o’clock noon.
To my Parents
TABLE OF CONTENTS

ORIGINAL PUBLICATIONS ................................................................. 7

ABBREVIATIONS ................................................................................. 8

1 ABSTRACT ........................................................................................................ 9

2 INTRODUCTION ................................................................................................. 10

3 REVIEW OF LITERATURE .................................................................................. 13

3.1 Clinical Aspects ............................................................................................. 13

3.1.1 Epidemiology ............................................................................................. 13

3.1.2 Predisposing Factors .................................................................................. 13

3.1.2.1 Age and Gender ...................................................................................... 13

3.1.2.2 Race ......................................................................................................... 13

3.1.2.3 PAM with Atypia .................................................................................... 14

3.1.2.4 Nevus and the Atypical Mole Syndrome .................................................... 15

3.1.2.5 Ultraviolet Radiation .............................................................................. 16

3.1.3 Diagnosis .................................................................................................... 16

3.1.3.1 Clinical Diagnosis .................................................................................. 16

3.1.3.2 Histopathologic Diagnosis .................................................................... 17

3.1.4 Treatment ................................................................................................... 18

3.1.4.1 Local Resection with or without Cryoocoagulation .................................. 18

3.1.4.2 Topical Mitomycin C .............................................................................. 18

3.1.4.3 Enucleation and Exenteration ................................................................. 19

3.1.4.4 Radiotherapy .......................................................................................... 19

3.1.5 Metastatic Disease ...................................................................................... 20

3.1.5.1 Metastatic Pattern and Sites of Metastases .............................................. 20

3.1.5.2 Staging of Conjunctival Melanoma ........................................................ 20

3.1.5.3 Treatment of Locoregional Metastases .................................................... 22

3.1.5.4 Treatment of Systemic Metastases ........................................................ 22

3.2 Prognostic Factors ......................................................................................... 23

3.2.1 Prognostic Clinical Factors of Recurrence .................................................. 23

3.2.1.1 Tumor Location and Multifocality ........................................................... 23

3.2.1.2 Tumor Thickness and Largest Basal Tumor Diameter ......................... 23

3.2.1.3 Adjuvant Therapy .................................................................................. 23

3.2.2 Prognostic Histopathologic Factors of Recurrence .................................... 24

3.2.2.1 Incomplete Removal of Primary Tumor .................................................. 24

3.2.2.2 Invasion Deeper than Substantia Propria .............................................. 24

3.2.2.3 Cell Type .............................................................................................. 24

3.2.2.4 Grade of Pigmentation ......................................................................... 24

3.2.2.5 Tumor Origin from PAM with Atypia .................................................... 24

3.2.3 Prognostic Genetic Factors of Recurrence ................................................. 24

3.2.4 Prognostic Clinical Factors of Survival ...................................................... 25

3.2.4.1 Age ....................................................................................................... 25

3.2.4.2 Tumor Location and Multifocality ........................................................ 25

3.2.4.3 Local Recurrence of the Primary Tumor ................................................. 26

3.2.4.4 Tumor Thickness and Largest Basal Tumor Diameter ....................... 26

3.2.5 Prognostic Histopathologic Factors of Survival ......................................... 26

3.2.5.1 Incomplete Removal of Primary Tumor ................................................ 26

3.2.5.2 Invasion Deeper than Substantia Propria .............................................. 27

3.2.5.3 Growth Pattern .................................................................................... 27

3.2.5.4 Tumor Cell Proliferation ..................................................................... 27

3.2.5.5 Cell Type ............................................................................................ 27

3.2.5.6 Lymphatic Invasion ............................................................................. 28

3.2.5.7 Inflammatory Response ...................................................................... 28

3.2.5.8 Grade of Pigmentation ....................................................................... 28

3.2.5.9 Histopathologic Characteristics of the Associated PAM .................. 28

3.2.6 Prognostic Genetic Factors of Survival ...................................................... 28

3.3 Comparison of Conjunctival Melanoma with Cutaneous and Uveal Melanoma ......................................................................................... 29

3.3.1 Cutaneous Melanoma .............................................................................. 29
This dissertation is based on the following publications on primary conjunctival melanoma. The original publications in the text will be referred to by their Roman numerals I-IV:


<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AJCC</td>
<td>American Joint Committee on Cancer</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>CM</td>
<td>Conjunctival melanoma(s)</td>
</tr>
<tr>
<td>CD</td>
<td>Cluster of differentiation</td>
</tr>
<tr>
<td>CMM</td>
<td>Cutaneous malignant melanoma(s)</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>FDA</td>
<td>United States Food and Drug Administration</td>
</tr>
<tr>
<td>HE</td>
<td>Hematoxylin and Eosin</td>
</tr>
<tr>
<td>HR</td>
<td>Hazard ratio</td>
</tr>
<tr>
<td>HUCH</td>
<td>Helsinki University Central Hospital</td>
</tr>
<tr>
<td>HPF</td>
<td>High power field</td>
</tr>
<tr>
<td>IL-2</td>
<td>Interleukin-2</td>
</tr>
<tr>
<td>LBD</td>
<td>Largest basal tumor diameter</td>
</tr>
<tr>
<td>MLN</td>
<td>Mean diameter of the ten largest nucleoli</td>
</tr>
<tr>
<td>MMC</td>
<td>Mitomycin C</td>
</tr>
<tr>
<td>MVD</td>
<td>Microvascular density</td>
</tr>
<tr>
<td>MVP</td>
<td>Microvascular patterns</td>
</tr>
<tr>
<td>N/A</td>
<td>Not applicable</td>
</tr>
<tr>
<td>NS</td>
<td>Not significant</td>
</tr>
<tr>
<td>PAD</td>
<td>Pathologic-anatomical diagnosis</td>
</tr>
<tr>
<td>PAM</td>
<td>Primary acquired melanosis</td>
</tr>
<tr>
<td>SLNB</td>
<td>Sentinel lymph node biopsy</td>
</tr>
<tr>
<td>TNM</td>
<td>Tumor, node, metastasis (classification)</td>
</tr>
<tr>
<td>UICC</td>
<td>International Union Against Cancer</td>
</tr>
<tr>
<td>UM</td>
<td>Uveal melanoma(s)</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet (radiation)</td>
</tr>
<tr>
<td>UV-A</td>
<td>Ultraviolet A</td>
</tr>
<tr>
<td>UV-B</td>
<td>Ultraviolet B</td>
</tr>
</tbody>
</table>
1 ABSTRACT

This study was carried out to better understand the clinical behavior of CM in order to improve the treatment and eventually the prognosis of patients. The first of the four studies (I) describes the clinical determinants of prognosis and the incidence of CM in Finland. The second study (II) describes a clinical entity that I named “corneally displaced conjunctival melanoma”. These rare tumors, formerly referred to as “corneal melanomas”, probably originated in the conjunctival melanocytes that have migrated to the cornea. The third study (III) analyses the histopathologic characteristics of CM and their association with recurrence and survival. The last study (IV) concentrates on the metastatic pattern of CM to gain insights into the possible usefulness of SLNB in detection of regional lymph node metastases.

I. Eighty-five new cases of CM were diagnosed in Finland between 1967 and 2000. The annual crude incidence of CM was 0.51 per million inhabitants. The average age-adjusted incidence of 0.54 doubled during the study period, analogous to the increase in the incidence of CMM during this period. Nonlimbal tumors were more likely than limbal ones to recur and they were associated with decreased survival. Increasing tumor thickness and recurrence of the primary tumor were other independent clinical factors related to death from CM.

II. Four (5%) patients had a CM limited to the cornea without evidence of a tumor other than PAM of the conjunctiva. Because there are no melanocytes in the cornea, the origin of these melanomas most likely is the limbal conjunctiva. All four corneally displaced CM were limited to the epithelium, and none of the patients developed metastases. An anatomic sub-classification based on my patients and world literature was developed.

III. The histopathologic specimens of 85 patients with primary CM were studied for cell type, mitotic count, tumor-infiltrating lymphocytes and macrophages, MVD, MVP, and MLN. The absence of epithelioid cells, increasing mitotic count and small MLN were associated with shorter time to recurrence according to the Cox univariate regression. None of the histopathologic variables was associated with mortality from CM. According to the Kaplan-Meier analysis, the location of the primary tumor was also related to local recurrence. Palpebral tumors recurred more often than other types.

After four additional years of follow-up, the clinical prognostic factors identified in the first study remained significant predictors of death from CM. At the end of this study in 2004, 26 patients had developed metastases, and all of them had died of CM.
IV. In 20 patients the metastatic pattern could be determined. Ten patients had initial systemic metastases detected, nine had initial regional metastases, and in one case the two types were detected simultaneously. The patients most likely to develop either type of initial metastases were those with nonlimbal CM, those with a primary CM more than 2 mm thick, and those with recurrent CM.

During the past three decades the incidence of CM has increased at the same pace as CMM, suggesting a possible role for UV radiation in its pathogenesis.

Approximately two thirds of the patients had limbal CM, a location associated with good prognosis. One third, however, had a primary CM originating outside the limbus. In these patients the chance of developing local recurrences as well as systemic metastases was significantly higher than in patients with limbal CM. Each recurrence accompanies an increased risk of developing metastases, and recurrences contribute to death along with increasing tumor thickness and nonlimbal tumor location.

In my data, an equal number of patients with initial locoregional and systemic metastasis existed. Patients with limbal primary tumors less than 2 mm in thickness rarely experienced metastases, unless the tumor recurred. Consequently, the patients most likely to benefit from SLNB are those who have nonlimbal tumors, CM that are over 2 mm thick, or recurrent CM.

The histopathology of CM differs from that of UM. Microvascular factors did not prove to be of prognostic importance, possibly due to the fact that CM at least as often disseminates first to the regional lymph nodes, unlike UM that almost always disseminates hematogenously.

2 INTRODUCTION

The conjunctiva is a thin layer covering the anterior part of the eye, except the cornea, as well as the posterior surface of the eyelids. The conjunctiva is covered by two or more layers of stratified columnar epithelium, except at the limbus and palpebral margins where stratified squamous epithelium is present. The conjunctival stroma is composed of fibrovascular connective tissue of varying thickness; it is thin in the palpebral portion and thicker in the fornices and in the bulbar part. The bulbar conjunctiva is loosely attached to the sclera allowing free eye movements whereas the palpebral part the conjunctiva is strictly attached to the underlying tarsus. Melanocytes, which are identical to the dendritic melanocytes in the skin, are found in the basal cell layer of the epithelium and stroma.62,86
CM is a relatively rare tumor. Its incidence in various, mainly Caucasian, population-based studies has varied from 0.24 to 0.8 per million inhabitants per year (Table 1). In more pigmented races it is extremely uncommon. The incidence of CM in Caucasian populations is approximately one tenth of that of UM\textsuperscript{13} and about one percent of the incidence of CMM.\textsuperscript{153}

**Table 1. Incidence of CM according to population-based studies.**

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study Period</th>
<th>Number of Patients</th>
<th>Mean Age</th>
<th>Crude Annual Incidence per 1 000 000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norregaard\textsuperscript{136}</td>
<td>1960-1980</td>
<td>55 (49/51)</td>
<td>65</td>
<td>0.45</td>
</tr>
<tr>
<td>Lommatzsch\textsuperscript{120}</td>
<td>1960-1985</td>
<td>196 (39/61)</td>
<td>55</td>
<td>0.80</td>
</tr>
<tr>
<td>Seregard\textsuperscript{169}</td>
<td>1969-1991</td>
<td>45 (51/49)</td>
<td>60</td>
<td>0.24</td>
</tr>
<tr>
<td>Missotten\textsuperscript{132}</td>
<td>1950-2002</td>
<td>194 (45/55)</td>
<td>57</td>
<td>0.50</td>
</tr>
</tbody>
</table>

*The incidence in this study is based on data from The National Cancer Registry of the German Democratic Republic spanning the years 1960-1985, when 196 new cases of CM were diagnosed in the population averaging 16.7 million. The mean age is for 81 patients analyzed in detail in that study.
† This study is intended to be population-based, estimated to include approximately 70% of patients diagnosed with CM in the Netherlands during the study period.

More than 50% of conjunctival melanomas arise from PAM with atypia.\textsuperscript{44,46,64,143,169} Clinically PAM presents as a variably pigmented flat lesion of the conjunctival epithelium. About one out of five CM originate from a pre-existing nevus and about the same number originate de novo.\textsuperscript{98,101} It is very common for a CM to recur. According to various population-based studies, 35% to 60% of the primary tumors recur at least once during the first 5 years after treatment of the primary tumor (Table 2).\textsuperscript{132,136,199}

**Table 2. Local recurrence and melanoma-specific survival of patients with CM according to population-based studies.**

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study Period</th>
<th>Number of Patients</th>
<th>5 and 10 Year Local Recurrence Rate</th>
<th>5 and 10 Year Survival Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norregaard\textsuperscript{136}</td>
<td>1960-1980</td>
<td>55 (49/51)</td>
<td>35% / 43%</td>
<td>86%/73%</td>
</tr>
<tr>
<td>Lommatzsch\textsuperscript{120}</td>
<td>1960-1988</td>
<td>81 (36/64)</td>
<td>N/A</td>
<td>87%/76%</td>
</tr>
<tr>
<td>Seregard\textsuperscript{169}</td>
<td>1969-1991</td>
<td>45 (51/49)</td>
<td>N/A</td>
<td>82%/70%</td>
</tr>
<tr>
<td>Missotten\textsuperscript{132}</td>
<td>1950-2002</td>
<td>194 (45/55)</td>
<td>61%/67%‡</td>
<td>86%/72%</td>
</tr>
</tbody>
</table>

* This study is intended to be population-based, estimated to include approximately 70% of patients diagnosed with CM in the Netherlands during the study period.
‡ Patients with primary CM treated with exenteration were excluded from the analysis of local recurrence.
The bulbar conjunctiva mainly receives its blood supply from the branches of the anterior ciliary artery, while palpebral conjunctiva is mainly supplied by the terminal branches of the ophthalmic artery, supplemented by branches of the facial artery. The bulbar conjunctival veins drain into the episcleral venous plexuses and the palpebral conjunctival veins join the posttarsal veins of the eyelid and communicate with the orbital veins as well as the deep facial branches of the anterior facial vein and pterygoid plexuses. Lymphatic channels, which are present in all parts of conjunctival stroma, join the lymphatics of the eyelid and drain medially to the submandibular, and laterally to the preauricular lymph nodes.62

CM can metastasize both locally to the tumor bed and regional lymph nodes as well as hematogenously.58,64 According to population-based studies from Denmark, Germany, Sweden and the Netherlands, the 5 and 10 year melanoma specific mortality has been between 13% to 18%, and 24% to 30% (Table 2). 120,132,136,169

Local excision with cryocoagulation has been the treatment of choice during the last decades.44,46,177 Formerly some authors, considering CM to be the most malignant of ocular tumors, suggested that the entire eye and orbital tissue should be exenterated.157 Nowadays, local excision is often combined with topical MMC following primary surgery.60,61 The treatment of recurrent lesions varies from a simple excision to exenteration according to the size and invasion of the tumor. Affected lymph nodes may in some cases be removed by lymph node dissection. SLNB is common practice in patients with CMM and is now also under investigation in patients with CM as well.54 No generally effective treatment for systemic metastases exists.

The purpose of this study was to estimate the incidence, and possible changes in it, of CM in the mainly Caucasian population of Finland from 1967 to 2000. The analysis included all 85 patients diagnosed CM during the study period and enabled the evaluation of various clinical and histopathologic prognostic factors in a population-based setting. Likewise, the metastatic pattern of CM was studied, especially with the aim of identifying tumor characteristics of the patients most likely to benefit from SLNB.

The aim of clarifying the clinical and histopathologic characteristics of this disease was ultimately to provide tools for individualized treatment and follow-up of patients in order to improve survival.
3 REVIEW OF LITERATURE

3.1 Clinical Aspects

3.1.1 Epidemiology

In different, mainly Caucasian population-based studies the crude incidence of CM has varied from 0.24 to 0.8 per year per million inhabitants (Table 1). The lowest crude incidence for a Caucasian population was reported in Sweden from 1969 to 1991 and the highest is based on the German Cancer Registry data from 1960 to 1985. In Denmark an incidence of 0.45 per million, derived from a study comprising the years 1960 to 1980, can be estimated. Recent data from the United States suggests a steady increase in the incidence of CM in Caucasians during the last three decades, similar to that of CMM.

In non-Caucasian, especially in African and Asian, populations CM is extremely rare.

3.1.2 Predisposing Factors

3.1.2.1 Age and Gender

CM is more common in the elderly. The mean age at diagnosis of patients with CM in different population-based and other larger studies has ranged from 55 to 65 years (Table 1). According to the available population-based studies, CM appears to be equally common in men and women.

3.1.2.2 Race

CM is a disease affecting mainly Caucasians. The incidence of CM in people of African origin has been estimated to be roughly one tenth of that in those of European origin. In a literature review from 2003, no more than 36 documented cases of CM in blacks were found in the world literature. It is also rare in Orientals. In the Singapore Cancer Registry only 16 new cases of CM were recorded from 1968 to 1995 in a population that roughly averaged 3 million people during that period.
3.1.2.3 **PAM with Atypia**

PAM is the term used for a pigmented lesion of the conjunctival epithelium that is not present at birth but appears later in life, most commonly in the fifth or sixth decade of life.\(^\text{64}\) Histopathologically, PAM can further be divided into PAM with atypia, which progresses into invasive CM in about half of the cases, and PAM without atypia that possibly never progresses into invasive melanoma.\(^\text{66}\) Approximately 60% to 75% of CM originate from PAM with atypia.\(^\text{46,64,169,44,143}\) Clinically, PAM presents as a unilateral, superficial lesion of the conjunctiva that seems to be composed of small, variably pigmented intraepithelial spots.\(^\text{64,98}\)

Clinically PAM with atypia should be suspected especially when the lesion is large, multifocal or it grows during follow-up. It is not possible by clinical examination to exclude the presence of PAM with atypia even in case of a small pigmented lesion, however, because it has been detected in lesions as small as 4 mm in diameter.\(^\text{62}\) Histopathology may also reveal “acquired melanosis sine pigmento” in areas that clinically seem to be unaffected, making it difficult to clinically determine the exact extent of the lesion.\(^\text{99,140}\)

PAM must be differentiated from complexion-associated conjunctival (“racial”) melanosis, congenital ocular melanocytosis and pigmentation related to systemic disease (e.g. Addison’s disease), as well as from conditions that may mimic melanosis pigmentation, such as deposits from mascara.

Histopathologically, PAM without atypia is typically composed of increased numbers of melanocytes restricted to the basilar region of epithelium without nuclear hyperchromasia or prominence of the nucleoli.\(^\text{98}\) PAM with atypia is characterized by the proliferation of small polyhedral cells, spindle cells, large dendritiform melanocytes, or epithelioid cells that may remain restricted to the basilar region, form nests at all levels of the epithelium, spread individually to all levels of the epithelium (pagetoid extension) or proliferate in a sheet-like fashion corresponding to melanoma in situ.\(^\text{98}\)

When atypia is detected by histopathology, the lesion should be completely removed and the patient must be followed at short intervals to detect any recurrence. Cryotherapy\(^\text{66,98}\) and, more recently, topical MMC are used as adjunctive treatment after removal of PAM, or in some cases even as the only post-biopsy treatment. In most centers, 0.02%-0.04% MMC is administered four times a day for two weeks and then the course is repeated after a few weeks break.\(^\text{47,61,73}\)
3.1.2.4 Nevi and the Atypical Mole Syndrome

A nevus of the conjunctiva is quite common. It typically presents as a variably pigmented, flat lesion on the limbal or bulbar conjunctiva, about 30% being almost entirely nonpigmented. Most nevi become visible by the age of ten and remain stable decades after puberty. Conjunctival nevi can further be divided into junctional, compound, and subepithelial nevi. In one study analyzing the histopathology of 71 conjunctival nevi, 2 (3%) were classified as junctional, 54 (76%) as compound, and 15 (21%) as subepithelial nevi. This classification probably describes different stages of evolution of a nevus from junctional to subepithelial, rather than distinctive entities. Junctional nevus may histopathologically be indistinguishable from PAM. Junctional nevi are seen in children, however, and an adult whose conjunctival pigmented lesion contains nests of cells or a basilar hyperplasia pattern has PAM, not a junctional nevus.

In different studies the proportion of CM thought to develop from a pre-existing nevus has varied from less than 10% to 20% of all cases. In a series of 69 patients from Germany, however, up to 39% of the CM were thought to have originated from a nevus.

In a significant proportion of CM with a history of pre-existing nevus, the simultaneous existence of histopathologic nevus and PAM with atypia make the determination of actual origin from a nevus uncertain.

The risk of patients suffering from the atypical mole syndrome developing a CMM during their lifetime is known to be much higher than in the general population. A lot of controversy exists regarding the diagnostic criteria and the definition of this syndrome, which can be either sporadic or hereditary. The atypical mole syndrome has also been referred to as the dysplastic nevus syndrome, the “classic” atypical mole syndrome (CAMS), the large atypical nevus syndrome, Clark’s nevus syndrome, B-K mole syndrome, and the familial atypical multiple mole syndrome (FAMMM). The last two describe hereditary forms of the syndrome. CAMS is defined by the presence of (1) 100 or more melanocytic nevi, (2) one or more melanocytic nevi 8 mm or larger in diameter, and (3) one or more melanocytic nevi with clinically atypical features. This definition is rather representative of what is usually meant by atypical mole syndrome, some definitions emphasize more the hereditary or histopathologic characteristics of the syndrome. The patients with hereditary forms of this syndrome are at the highest risk of developing CMM during their lifetime.

Because of the rarity of both atypical mole syndrome and, especially, CM, it is difficult to estimate the exact risk of CM developing in individuals with this syndrome, but
the general belief is that individuals with atypical mole syndrome are overrepresented among patients with CM.\footnote{13,168}

3.1.2.5 Ultraviolet Radiation

Development of CMM has been linked to the exposure to UV radiation. Solar UV radiation exposure is estimated to account for over 90\% of melanomas in North America and Australia.\footnote{7} Solar ultraviolet radiation predisposes to malignant change in the skin by direct mutagenic effects on DNA, by stimulating the cellular constituents of the skin to produce growth factors, by reducing cutaneous immune defences, and by promoting reactive oxygen species of melanin that cause DNA damage and suppress apoptosis.\footnote{131}

The limbal and bulbar conjunctiva, unlike the uvea that is protected by two natural filters, the cornea and the lens,\footnote{205} is directly exposed to UV-A and UV-B radiation just like the melanocytes of the skin.

It is well demonstrated that a light skin complexion, which has increased vulnerability to UV radiation, is associated with an increased risk of CMM.\footnote{89} UM, which is not linked with UV-radiation, however, appears also to be more common in individuals with light skin complexion and light colored eyes.\footnote{197} Contrary to the striking increase in the incidence of CMM, the incidence of UM has been stable, or perhaps even decreased, during the last decades\footnote{13,69,182} In one study from the United States the skin complexion of patients with CM was judged to be light (fair) in 148 (99\%) and dark (olive) in 1 (1\%) of 149 consecutive Caucasian patients treated in a large ocular oncology center.\footnote{173}

Patients with xeroderma pigmentosum, who are very sensitive to short UV wavelengths, are believed to develop not only CMM but also CM more frequently than individuals without this condition.\footnote{5,103,141}

More evidence comes from recent epidemiological studies that have found a two-fold increase in the incidence of CM during the last three decades, an increase very similar to that of CMM.\footnote{94,204} According to cancer registry data from Denmark, however, the age-adjusted incidence of CM remained stable between 1943 and 1997 in that country.\footnote{95}

3.1.3 Diagnosis

3.1.3.1 Clinical Diagnosis

CM can grow on any site of the conjunctiva.\footnote{6,46,98,157} It can also grow on the cornea without evidence of invasive conjunctival tumors.\footnote{42,72,163,194} These tumors have historically been referred to as "corneal melanomas", although they probably develop as a result of contiguous
spread from a CM or as a result of malignant change in melanocytes that have migrated into the corneal epithelium from the limbus, often in conjunction with PAM with atypia.\textsuperscript{183,194,203}

The color of CM varies from pink (amelanotic) to dark brown or black. When the tumor appears on the bulbar conjunctiva and is readily visible, normally little delay in seeking medical advice occurs. These tumors are therefore diagnosed earlier than those growing on palpebral conjunctiva. When the tumor is palpebral it may grow large without causing symptoms. Some tumors are diagnosed when precursory lesions, PAM and nevus, are being routinely followed up by an ophthalmologist, or when the patient notices a change in such a lesion. In cases where a history of a growing, pigmented lesion on the conjunctiva exists, the clinical diagnosis of melanoma is easily suspected. When the tumor is amelanotic, however, it may be confused with squamous cell carcinoma, lymphoma, papilloma, a benign lymphoproliferative lesion, pyogenic granuloma or even pterygium when it extends over the cornea.\textsuperscript{64}

3.1.3.2 Histopathologic Diagnosis

In several studies on the histopathology of CM, an attempt to classify CM using the classification developed for CMM, namely lentigo maligna melanoma, superficial spreading melanoma, and nodular melanoma, was made in the 1970s and 1980s.\textsuperscript{14,65} This classification, however, has not proved useful in CM since most tumors present characteristics of various subtypes or their histopathology does not fall in any of these categories.\textsuperscript{65,97} According to one study, almost all tumors would be classified as superficial spreading melanomas if this system was to be applied to CM. Consequently, the authors simply advice the use of the term “malignant melanoma” for any lesion in which tumor cells have invaded from the overlaying conjunctival epithelium through the basement membrane to the substantia propria.\textsuperscript{65}

CM is typically composed of a mixture of some or all of four types of cells: small polyhedral cells, large epithelioid cells, spindle cells, and balloon cells.\textsuperscript{65} Thus, classification of the cell type according to the Callender classification used for UM, based on the presence or absence of epithelioid and spindle cells is not directly applicable.

Important histopathologic features that differentiate CM from a benign nevus include: intraepithelial PAM with atypia that shows evidence of pagetoid spread, PAM with atypia that extends substantially beyond the lateral margin of the tumor, mitotic activity, patchy or band-like inflammation at the base of the lesion in the substantia propria, the absence of maturation into smaller cells with delicate intercellular stroma at the base of the subepithelial component, and the production of melanin in the deepest part of the lesion.\textsuperscript{98}
3.1.4 Treatment

3.1.4.1 Local Resection with or without Cryocoagulation

Local resection with a 3-5 mm free conjunctival margin, combined with cryocoagulation of the conjunctival margins has been the most widely used form of treatment for CM during the recent decades.44,46,99,177 When the CM is located liminally or on the bulbar conjunctiva, the tumor is usually excised under local anesthesia without difficulty. Because the Bowman’s layer is a natural barrier, care should be taken not to break it during excision of a corneally extending tumor unless stromal infiltration is strongly suspected.139,143,177 In most cases, the corneal part of the tumor is easily peeled off the Bowman’s layer.177

After excision of a limbal or bulbar CM, cryotherapy is applied to the margins of the remaining bulbar conjunctiva. This is normally done by freezing the margin of the conjunctiva from underneath as it is lifted away from the sclera with a nitrous oxide cryoprobe. According to one technique, the ice ball of the probe is left to reach a size of 4-5 mm, it is then allowed to thaw, and the cycle repeated once. The probe is then moved to an adjacent area of conjunctiva and the treatment is repeated until all margins have been treated by this method.176,177

Local resection of a diffuse or palpebral CM is not as straightforward as that of limbal melanomas. Palpebral tumors tend to grow undiagnosed longer, be bigger than limbal ones, and to be associated with extensive PAM that may be impossible to remove completely. The surgery must then be tailored according to the extent and size of each tumor. When large areas of conjunctiva need to be resected, full thickness mucosal grafts from the buccal mucosa or contralateral eye, or an amniotic membrane transplant is needed.142,144

3.1.4.2 Topical Mitomycin C

Topical MMC has become a widely used adjunctive treatment after surgical resection of the primary or recurrent tumor, as well as in the treatment of PAM with atypia.47,73 Mitomycin is an alkylating agent and binds to DNA during any phase of the cell cycle, leading to irreversible cross-linking and the inhibition of DNA synthesis and function. It has been used in ophthalmic surgery both intraoperatively and postoperatively to prevent pterygium recurrence and scarring after glaucoma filtration surgery.108

MMC is most often administered in two repeated two-week courses of topical 0.02% to 0.04% MMC four times a day.73 The treatment is relatively well tolerated. Keratoconjunctivitis due to local toxicity of MMC is a universal finding, however, and
sometimes leads to discontinuation of the therapy. Histopathologically, conjunctival atrophy and thinning, nuclear changes, and subconjunctival inflammation have been described in patients treated with MMC for CM. 

Even though small case series show that MMC is effective in destroying atypical melanocytes in PAM and CM, larger studies with a longer follow-up are required to estimate its actual treatment benefit in decreasing recurrences and mortality from CM.

3.1.4.3 Enucleation and Exenteration

Previously, exenteration of the orbit was recommended as the primary treatment for CM, and even for PAM with atypia, by Reese who considered CM to be the most malignant ophthalmic tumor. Technically, exenteration consists of the surgical removal of the entire conjunctiva, the eye, and the orbital contents. Because no evidence exists of improved prognosis by early exenteration, it is nowadays reserved for advanced stages of CM with orbital and intraocular invasion, often after several recurrences. In different essentially population-based series, the proportion of patients undergoing exenteration at some stage of the disease has varied widely from 10% to 40%. Enucleation of the globe has occasionally been used as a treatment of primary or recurrent CM. Because most of the conjunctiva is left behind in enucleation and the eyeglobe is seldom directly involved, however, it is a rare procedure for the treatment of CM.

3.1.4.4 Radiotherapy

Radiotherapy has gained little popularity in the treatment of CM. It was first described in the late 1950s when a combination of surface beta and gamma irradiation with low and high voltage external beam therapy was used to treat CM. 

Later, beta irradiation with $^{90}$Sr/$^{90}$Y applicators has been used in some centers, mostly as an adjuvant treatment after local excision. In a series of 19 patients with extensive primary or recurrent CM was treated by combining a surgical excision with proton beam irradiation, $^{60}$Co plaque brachytherapy and cryotherapy. During a relatively short follow-up no exenterations were needed due to further recurrences in this group of patients. 

A retrospective study from the Netherlands included 11 patients treated with radiotherapy as an alternative to orbital exenteration. Radiotherapy was successful in preventing further recurrences and distant metastases in only 3 patients. Moreover, 2 patients went blind because of radiation-related ocular complications. In the same series, 4 patients
were treated with combined local excision and radiotherapy. One developed 3 recurrences and 2 patients developed distant metastases.\(^4^6\)

A recent series from Germany reported 20 patients with extensive CM treated with proton beam radiotherapy as an alternative to exenteration. During a mean follow-up of 3 years, 6 patients experienced further recurrence and 2 of them underwent exenteration. Six patients had metastatic disease diagnosed during the follow-up. Complications of radiation included dry eyes in 19 patients, cataract in 7 patients, and vascularization of the cornea in 4 patients. The best corrected visual acuity was reported to be stable in 12 patients however.\(^2^0^2\)

To date, no large randomized studies on radiotherapy in CM exist, making it impossible to evaluate the real benefit of this type of treatment.

### 3.1.5 Metastatic Disease

**3.1.5.1 Metastatic Pattern and Sites of Metastases**

CM can metastasize through both lymph vessels and hematogenously. It has been reported that in one half\(^5^8,6^4\) to two thirds of the cases\(^1^3^2,1^7^3\) regional lymph node metastases are detected prior to systemic ones. This corresponds to the metastatic pattern of CMM, in which about two thirds of patients initially develop locoregional (local in-transit or lymph node metastases) rather than systemic hematogenous metastases.\(^1^3^0\)

The earlier detection of locoregional compared to systemic metastases in patients with CM and CMM can be interpreted in at least two ways. First, it can be hypothesized that melanoma progression is an orderly process involving the local lymph nodes prior to systemic dissemination. Secondly, locoregional dissemination can be seen as a first manifestation of systemically disseminated disease that is not yet detectable elsewhere.

In CM the regional lymph node metastases are found in the submandibular, preauricular, and cervical lymph nodes. The most common sites for systemic metastases include the liver, the lungs, the brain and the skin.\(^5^8,6^4,1^3^2\)

**3.1.5.2 Staging of Conjunctival Melanoma**

The Tumor, Node, Metastasis (TNM) System is an international classification system developed in collaboration by AJCC and UICC to aid in the management, research, and assessment of prognosis for different forms of cancer.\(^1^8^4\) For conjunctival melanoma TNM Classification is summarized in Table 3.
Table 3. TNM classification for CM.\textsuperscript{184}

TNM Clinical Classification

\textbf{T-Primary Tumor}

<table>
<thead>
<tr>
<th>T-stage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>TX</td>
<td>Primary tumor cannot be assessed</td>
</tr>
<tr>
<td>T0</td>
<td>No evidence of primary tumor</td>
</tr>
<tr>
<td>T1</td>
<td>Tumor(s) of bulbar conjunctiva occupying one quadrant or less</td>
</tr>
<tr>
<td>T2</td>
<td>Tumor(s) of bulbar conjunctiva occupying more than one quadrant</td>
</tr>
<tr>
<td>T3</td>
<td>Tumor(s) of conjunctival fornix and/or palpebral conjunctiva and/or caruncle</td>
</tr>
<tr>
<td>T4</td>
<td>Tumor invades the eyelid, cornea, and/or orbit</td>
</tr>
</tbody>
</table>

\textbf{N-Regional lymph nodes}

<table>
<thead>
<tr>
<th>N-stage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>NX</td>
<td>Cannot be assessed</td>
</tr>
<tr>
<td>N0</td>
<td>No lymph node metastasis</td>
</tr>
<tr>
<td>N1</td>
<td>Lymph node metastasis present</td>
</tr>
</tbody>
</table>

\textbf{M-Distant metastasis}

<table>
<thead>
<tr>
<th>M-stage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>MX</td>
<td>Cannot be assessed</td>
</tr>
<tr>
<td>M0</td>
<td>No distant metastasis</td>
</tr>
<tr>
<td>M1</td>
<td>Distant metastasis present</td>
</tr>
</tbody>
</table>

\textit{pTNM Pathological Classification}

\textbf{T-Primary Tumor}

<table>
<thead>
<tr>
<th>pT-stage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>pTX</td>
<td>Primary tumor cannot be assessed</td>
</tr>
<tr>
<td>pT0</td>
<td>No evidence of primary tumor</td>
</tr>
<tr>
<td>pT1</td>
<td>Tumor(s) of bulbar conjunctiva occupying one quadrant or less and 2 mm or less in thickness</td>
</tr>
<tr>
<td>pT2</td>
<td>Tumor(s) of bulbar conjunctiva occupying more than one quadrant and 2 mm or less in thickness</td>
</tr>
<tr>
<td>pT3</td>
<td>Tumor(s) of conjunctival fornix and/or palpebral conjunctiva and/or caruncle or tumor of the bulbar conjunctiva more than 2 mm in thickness</td>
</tr>
<tr>
<td>pT4</td>
<td>Tumor invades the eyelid, cornea and/or orbit</td>
</tr>
</tbody>
</table>

The pN and pM categories correspond to the N and M categories, respectively.

\textit{G Histopathological Grading}

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>GX</td>
<td>Grade cannot be assessed</td>
</tr>
<tr>
<td>G0</td>
<td>Primary acquired melanosis</td>
</tr>
<tr>
<td>G1</td>
<td>Malignant melanoma arising from a nevus</td>
</tr>
<tr>
<td>G2</td>
<td>Malignant melanoma arising from primary acquired melanosis</td>
</tr>
<tr>
<td>G3</td>
<td>Malignant melanoma arising de novo</td>
</tr>
</tbody>
</table>

\textit{Stage Grouping}

No stage grouping is presently recommended.
3.1.5.3 Treatment of Locoregional Metastases

Large studies report a relatively favorable prognosis after the surgical removal of clinically involved lymph nodes for patients with limited regional metastasis in patients with CMM. Likewise, successful management of metastatic disease confined to regional lymph nodes has been reported in some patients with CM.

Traditionally, clinical suspicion of enlarged lymph nodes in the clinical examination led to either radical or selective lymphadenectomy in patients with CM or CMM. More recently, SLNB has become routine in patients with CMM, and in most centers it is performed routinely on all patients except those with very thin (less than 1 mm in thickness) CMM.

If one or more sentinel lymph nodes contain metastatic melanoma, regional lymphadenectomy (selective lymph node dissection) and, sometimes, adjuvant therapy are offered. At the present SLNB appears to be a useful procedure in staging clinical stage II CMM, but so far no proven survival benefit is associated with this procedure.

In CM patients, SLNB has been experimentally used during the recent years in some ocular oncology centers, including ours. SLNB consists of localizing the sentinel nodes in a nodal basin draining from the site of a melanoma by radioactive $^{99}$Tc and a blue dye, followed by their surgical removal. It is a technically feasible and potentially useful procedure in patients with CM. Future series on this technique on patients with CM will hopefully provide a reliable tool for earlier detection of clinically occult regional nodal involvement.

Whether SLNB is used or not, it is very important to study the local lymph nodes during the follow-up of a patient with CM very carefully at every visit to detect locoregional metastases as early as possible.

3.1.5.4 Treatment of Systemic Metastases

No specific treatment protocols for systemically disseminated CM have been published. Patients with disseminated disease are being treated like those with stage IV (systemically disseminated) CMM. Chemotherapy with single-agent dacarbazine is the only FDA approved chemotherapy agent for metastatic CMM.

Immunological approaches have yielded the only newly FDA approved agent for metastatic CMM in 30 years, high-dose bolus IL-2, based on durable responses in some patients, but with associated high toxicity rate and cost. Treatments combining chemotherapy and biotherapy with interferon alfa-2a and IL-2 are also used and result in good response and durable remission in some patients with disseminated melanoma.
A wide range of new therapeutic approaches, including chemotherapy, biochemotherapy, nonspecific immune adjuvants, cancer-specific vaccines, cytokines, monoclonal antibodies, and specific immunostimulants, are undergoing clinical investigations and trials.\cite{188}

### 3.2 Prognostic Factors

#### 3.2.1 Prognostic Clinical Factors of Recurrence

It is very common for a CM to recur (Table 2). In a Danish population-based study, $35\%$ and $43\%$ of the patients had experienced at least one recurrence after a follow-up of 5 and 10 years.\cite{136} In a recent population-based study from the Netherlands, the 5 and 10 year recurrence rates were $61\%$ and $67\%$. In that particular study, 14 out of 194 (7\%) patients who had primary exenteration were excluded from the analysis of recurrence.\cite{132} In a retrospective study series from Germany, 44 of 85 (52\%) patients developed at least one local recurrence during a median follow-up of 13 years.\cite{199} In other larger studies,\cite{2,44,173} the recurrence rates have been comparable to these most recent population-based studies.

##### 3.2.1.1 Tumor Location and Multifocality

Location of the primary tumor outside the limbus\cite{173} or the epibulbar conjunctiva\cite{132,199} has been associated with a shorter time to local recurrence. Likewise, multifocality of the primary tumor is associated with an increased risk of local recurrence.\cite{99}

##### 3.2.1.2 Tumor Thickness and Largest Basal Tumor Diameter

Thickness of the primary tumor was statistically significantly associated with the first local recurrence in a North American referral-based series comprising 150 patients with CM,\cite{173} and was of borderline significance in a recent, essentially population-based, analysis of 194 patients from the Netherlands.\cite{132} LBD is not a predictor of local recurrence according to the available data.\cite{132,199}

##### 3.2.1.3 Adjuvant Therapy

Whether some form of treatment is more effective than others in preventing local recurrences can only reliably be evaluated by randomizing patients into groups receiving different forms of initial treatment. No such studies on patients with CM exist. The few nonrandomized retrospective series suggest a superior outcome, in regards to local recurrence for patients receiving either brachytherapy or cryotherapy as adjuvant treatments after local excision,\cite{44,132} while other retrospective series were not able to demonstrate such a benefit.\cite{2}
3.2.2 Prognostic Histopathologic Factors of Recurrence

3.2.2.1 Incomplete Removal of Primary Tumor
Incomplete excision (at least one margin not free of tumor histopathologically) of the primary CM is expectedly associated with increased risk of local recurrence.\textsuperscript{2,65,102}

3.2.2.2 Invasion Deeper than Substantia Propria
In CM the extent of stromal invasion is more difficult to assess histopathologically than in CMM because the substantia propria is not stratified into layers analogous to the papillary and reticular dermis of the skin. Data from Germany, however, indicates a higher risk of recurrence for those patients with a primary tumor invading the underlying sclera or ocular muscle.\textsuperscript{2}

3.2.2.3 Cell Type
Presence of epithelioid cells was associated with a short time to local recurrence in one study.\textsuperscript{2}

3.2.2.4 Grade of Pigmentation
In a referral-based study, tumors with irregular pigmentation recurred more often than those that did not show such a pigmentation pattern.\textsuperscript{2} In a study from the United States, tumors having a red appearance (amelanotic) had an increased risk of recurrence compared to primary melanomas with a darker pigmented appearance.\textsuperscript{173}

3.2.2.5 Tumor Origin from PAM with Atypia
Some studies report increased risk of recurrence for tumors originating from PAM with atypia compared to those originating from a pre-existing nevus or de novo,\textsuperscript{46,64} while others have not found such a relationship.\textsuperscript{2,132,173}

3.2.3 Prognostic Genetic Factors of Recurrence
To my knowledge, no studies on genetic factors related to local recurrence in CM exist.
3.2.4 Prognostic Clinical Factors of Survival

According to population-based studies from Denmark, Germany, Sweden, and the Netherlands, the 5 and 10 year melanoma specific mortality has been between 13% to 18% and 24% to 30% (Table 2).120,132,136,169

3.2.4.1 Age

Most studies on prognostic factors in patients with CM have not taken into account competing risks that may be associated with a patient’s age, such as the size of the tumor. Two large studies, however, exist that have taken these possible confounding factors appropriately into account. First, a recent population-based study from the Netherlands showed a significantly better survival for younger patients compared to those over 68. The difference in survival was explained by the significantly greater thickness and LBD of tumors in the group of older patients.132 Second, a study from the United Kingdom, comprising 256 patients with CM, was unable to show a statistically significant association between the age of the patient and survival when competing risks were taken into account.143

A retrospective study from Germany, comprising 85 patients, found that patient age over 55 along with primary tumor location were the only predictors of mortality from CM using the Cox multivariate regression.199 A theoretical source of bias in that study was that the thickness of the primary tumor was not assessed, giving the possibility that older patients had thicker tumors and therefore had worse survival than younger ones. Other studies have suggested either worse46, better36, or equal prognosis2 for older patients compared to younger ones.

3.2.4.2 Tumor Location and Multifocality

A location of the primary tumor outside limbal74 or bulbar (including limbal)2,46,64,132,143,178 conjunctiva is probably the prognostic factor most consistently associated with adverse outcome in patients with CM. Even if limbal and bulbar primary tumors are analyzed together as bulbar tumors, the results of analyses are likely mainly to reflect the prognosis of limbal melanomas because 4 out of 5 bulbar (according to definitions used in some studies) primary tumors touch the limbus.132 The risk of patients with CM outside the bulbar conjunctiva dying from melanoma has been estimated to be 2 to 6 times higher compared to those with limbal and bulbar melanomas together.2,132,143 Large series from the UK and Germany reported the
5-year survival of patients in the former group to be 72% and 71% compared to 92% and 95% for patients in the latter group.\textsuperscript{120,143} A minority of studies have not been able to find an association between primary tumor location and survival.\textsuperscript{169}

Multifocality of the primary tumor was found to be associated with a fivefold increase in mortality among patients with favorable (epi)bulbar melanomas, but not in patients with an unfavorable location in a referral-based study including 256 patients.\textsuperscript{143} In a recent population-based study from the Netherlands, multifocality was associated with significantly worsened survival.\textsuperscript{132}

### 3.2.4.3 Local Recurrence of the Primary Tumor

To most accurately evaluate the effect of local recurrence on survival, recurrence of the primary tumor should be analyzed as a time-varying covariate that is not present at the time of diagnosis of the primary tumor, but appears later, possibly as a repeated event during the follow-up.\textsuperscript{147,190} Studies using these newer statistical methods on CM could not be found in the literature.

Two studies treating local recurrence statistically incorrectly as a base-line variable have associated local recurrence with increased risk of melanoma related death.\textsuperscript{44,65}

### 3.2.4.4 Tumor Thickness and Largest Basal Tumor Diameter

An association between the thickness of the primary tumor,\textsuperscript{46,120,132,178,187,209} or in some studies that of the thickest melanoma,\textsuperscript{65} primary or recurrent, and survival has been found in several prognostic studies concerning patients with CM. Increasing evidence suggests that 1.5 – 2.0 mm could be close to the critical thickness between clinically significantly better and worse prognosis.\textsuperscript{15,46,120,132,178}

Likewise, the largest basal diameter of the primary tumor has been found to be related to survival in some studies.\textsuperscript{169}

### 3.2.5 Prognostic Histopathologic Factors of Survival

#### 3.2.5.1 Incomplete Removal of Primary Tumor

An incompletely excised (at least one margin not histopathologically free of tumor) primary CM is likely to recur.\textsuperscript{2,65,102} Evidence from retrospective studies also supports, not surprisingly, an increased risk of death from melanoma of patients with histopathologic evidence of incompletely removed primary melanoma.\textsuperscript{2,173} In a German study, a mortality
three times higher was found for patients with histopathologic evidence of incompletely removed primary CM.2

3.2.5.2 Invasion Deeper than Substantia Propria

As previously stated, the extent of stromal invasion is difficult to assess histopathologically in CM because substantia propria is not stratified into layers analogous to the skin. This probably makes the thickness of the primary tumor, measured in millimetres, a more reliable indicator of the vertical extent of invasion. When histopathology reveals invasion deeper than the substantia propria into the underlying sclera or ocular muscle, prognosis is guarded. Referral-based data from Germany indicates that 11 patients with a primary tumor invading deeper than the substantia propria had a 5.5 times higher risk of dying from CM compared to 55 patients with more superficial primary tumors.2

3.2.5.3 Growth Pattern

A nodular or mixed (superficial and nodular) growth pattern of the primary tumor, compared to superficial growth pattern only, predicted a 1.2 times higher mortality from CM in a study comprising 68 patients.2

3.2.5.4 Tumor Cell Proliferation

A high mitotic count has been the histopathologic variable most consistently associated with adverse outcome in patients with CM.36,64,169,187 In addition to the standard method of counting mitoses from HE stained specimens, one study assessed the amount of proliferating cells by immunoreactivity for the proliferating cell nuclear antigen, a DNA polymerase delta auxiliary protein, by using the PC10 antibody. In that study, comprising 20 patients with CM, the patients who subsequently died of metastatic disease had significantly higher counts of cells that were positive for proliferating cell nuclear antigen per square millimeter.167

3.2.5.5 Cell Type.

The cell type in UM is assessed according to the Callender classification, originally described by G.R. Callender in 1931,24 and its modified versions.127 Patients with a tumor containing spindle-like, cohesive cells have a better prognosis compared to those with an epithelioid tumor, characterized by less cohesive round cells.68,165,170 Presence of epithelioid cells has been found to predict mortality from CM in some,143,169 but not in all,65,74 studies.
3.2.5.6 Lymphatic Invasion
A large study, including 256 patients, found that patients with CM and histopathologic evidence of lymphatic invasion had a four times greater mortality. The increased mortality with lymphatic invasion was most evident in the group of patients with favorably located bulbar tumors, in which patients with lymphatic involvement had an eight times higher mortality compared to those without such an invasion. In the group of patients with an unfavorable location, a twofold risk of death was estimated, but the higher risk in this group of patients was of borderline significance.\textsuperscript{143}

3.2.5.7 Inflammatory Response
The first response to a malignant tumor is lymphocytic infiltration around and inside the tumor. Absence or weakness of this primary inflammatory response (none or few tumor- infiltrating lymphocytes) has been associated with increased mortality from CM in two histopathologic studies.\textsuperscript{64,178}

3.2.5.8 Grade of Pigmentation
Grade of pigmentation can be assessed as a clinical or a histopathologic variable. It probably reflects the quantity of melanin inside the atypical melanocytes forming the tumor. One study associated a higher grade of pigmentation with an increased risk of death from melanoma,\textsuperscript{15} while in two separate studies it was not a significant predictor of mortality.\textsuperscript{2,44}

3.2.5.9 Histopathologic Characteristics of the Associated PAM
Evidence from population-based and other larger studies indicate that there is no difference in survival between CM originating from PAM compared to those originating de novo, or from a pre-existing nevus.\textsuperscript{65,132} A referral-based histopathologic study, including 98 patients with a primary tumor originating from PAM with atypia, established presence of atypical melanocytes within the epithelium of the PAM component of the lesion (common pagetoid growth pattern) and the presence of moderate to severe atypia in the PAM component as predictors of death from CM.\textsuperscript{64}

3.2.6 Prognostic Genetic Factors of Survival
To my knowledge, no studies on genetic factors related to the survival of patients with CM have been published.
3.3 Comparison of Conjunctival Melanoma with Cutaneous and Uveal Melanoma

3.3.1 Cutaneous Melanoma

The incidence of CMM in mainly Caucasian populations has grown steadily worldwide during the last few decades, most likely due to increased (intermittent) exposure to UV radiation.\textsuperscript{45,109,114,186}

Overall mortality from CMM has also increased, but not at the same pace as CMM incidence because of earlier diagnosis and improved survival. In the United States, the 5-year survival rate for patients with CMM was 40\% in 1940, whereas in 1975 it was already 67\%.\textsuperscript{38} A recent epidemiological study from Germany reported an approximately 90\% 5-year survival for patients diagnosed with CMM between 1996 and 2000.\textsuperscript{20} The earlier diagnosis is due to increased awareness of the condition by medical professionals and the public alike. In the German study, the median tumor thickness was 1.81 mm in 1976 compared to 0.53 mm in 2000.\textsuperscript{20}

In about 70\% of patients with CMM locoregional metastases are detected prior to systemic ones. Of these, most are regional lymph node metastases, but some, about 20\% of all metastases, are called in-transit or satellite metastases which appear on the skin close to the primary tumor, or between the tumor bed and regional lymph nodes. These metastases are thought to be formed by melanoma cells that have escaped the primary tumor via lymphatic channels but not reached the sentinel lymph node.\textsuperscript{130}

The most important prognostic factor in patients with CMM is the presence of metastases. Distant metastases indicate poor prognosis, but patients with limited regional lymph node metastases may enjoy prolonged survival.\textsuperscript{200} SLNB and dissection have become the standard procedure for evaluation of the local lymph nodes, and sentinel lymph node status assessed by SLNB is included as a part of the AJCC staging system for patients with CMM.\textsuperscript{9}

According to a large North American study, the 5-year survival for patients with one or more than four melanoma-positive lymph nodes, was 53\% and 25\%. In the same study, the estimated 15-year survival for all patients with at least one melanoma-positive sentinel lymph node was 28\%, indicating that that selective lymphadenectomy probably is a curative procedure in some patients with CMM.\textsuperscript{200}

In patients with Stage I or II CMM (no evidence of metastases) at the time of diagnosis, measurement of tumor thickness by the method of Breslow\textsuperscript{18} remains the single
most accurate prognostic factor. The presence of ulceration, determined as loss of epidermis overlying an invasive melanoma, is the other clinical prognostic factor that is graded for Stage I and II CMM in the current AJCC staging system for CMM. In some studies, the effect of ulceration on prognosis has been independent of tumor thickness, whereas in others these two factors have been interrelated. It has been speculated that the method used for measuring thickness of ulcerated melanomas may lead to underestimation of the thickness of such melanomas, and explains the apparently independent association between ulceration and prognosis.

The level of invasion of CMM as recorded by histopathologic examination has a strong association with prognosis, but is not independent of tumor thickness.

A high mitotic rate has been constantly associated with an increased risk of metastasis in patients with CMM. An epithelioid cell type, which is related to increased mortality in UM, has been found to predict mortality from CMM in some, but not all studies, and is not regarded as a reliable prognostic factor in CMM. Factors currently under investigation are tumor vascularity, vascular invasion, tumor regression, and tumor-infiltrating lymphocytes. Based on these, new therapeutic approaches such as cancer vaccines are being investigated, and many are currently being tested in clinical trials.

### 3.3.2 Uveal Melanoma

UM is the most common intraocular tumor in adults. According to recently published data from the European Cancer Registry, its age-standardized incidence varied from 2 to 8 new cases per million per year in different European countries between 1983 and 1994. The lowest incidence was reported for Spain and southern Italy and the highest for the Nordic countries and Scotland. In non-Caucasian populations UM is very rare. The mean age at the time of diagnosis is 50 to 60 years.

UM develops from melanocytes of the uvea, which is divided into choroid that covers the posterior segment of the eye between the retina and the sclera and the more anteriorly located ciliary body and iris. The uvea, especially the choroid, is the most vascularized part of the eye. The ciliary body supports the lens and secretes aqueous humor and the iris, through its sympathetic and parasympathetic action, regulates the amount of light entering the eye through the pupil. According to various studies, 64% to 90% of UM originate in the choroid, and about 10% and 3% in the ciliary body and the iris. UM limited to the iris (iris melanoma) is normally discussed as a separate entity because of its favorable prognosis compared to ciliochoroidal melanomas.
The etiology of UM remains unknown. Even though lightly pigmented skin and light iris color are established risk factors, the role of ultraviolet light exposure in the pathogenesis is minor and uncertain. Uvea, unlike skin and limbal and bulbar conjunctiva, which are directly exposed to UV radiation, is mostly protected by the lens that is an effective filter of UV radiation. Data from the European Cancer Registry showed a much higher incidence of UM in the Nordic countries where the population is less exposed to sunlight compared to southern Europe. This same geographical pattern is seen in CMM as well, and it has been speculated that the susceptibility of individuals with lightly pigmented skin to develop CMM overshadows the opposing effect of decreasing UV intensity at higher latitudes. The incidence of UM has also remained stable during the last few decades, contrary to that of CMM. Large studies on UM report a slightly higher incidence rates for males. At least one tenth of UM develop from a previously diagnosed choroidal nevus.

Enucleation was the treatment historically offered to all patients with ciliochoroidal melanomas. With increasing evidence showing that eye preserving forms of treatment are not associated with increased mortality, enucleation has been largely replaced by radiotherapy, especially brachytherapy using $^{106}\text{Ru}$ and $^{125}\text{I}$ plaques. Since the late 1970s in institutions with access to a nuclear accelerator, charged particle radiation using proton beam and helium ion irradiation have been used. Other forms of eye-preserving treatment of UM include, stereotactic radiotherapy, endoresection, local transscleral resection, laser photocoagulation, and transpupillary thermotherapy.

UM differs from both CMM and CM in that it always metastasizes hematogenously, because there are no lymph vessels inside the eye. Only in the rare case that the anterior sclera is penetrated by UM cells, it may disseminate through conjunctiva and lymphatics to the regional lymph nodes. In more than 90% of cases, the liver is involved when metastatic disease is diagnosed.

About 40% of patients with UM die of metastatic disease within 10 years of the diagnosis, irrespective of the type of treatment.

The clinical factor most consistently associated with outcome in patients with UM is the size of the tumor, whether measured as largest basal diameter, height, or volume. The location of primary tumor is, likewise, an independent prognostic factor. Ciliary body melanomas have a worse survival rate than those involving only the choroid. Iris melanomas very rarely metastasize.
The histopathology of UM has been extensively studied, even though with the current widespread use of irradiation therapy, histopathologic specimens are rarely obtained nowadays. The tumors can be classified according to their cell type into spindle cell, mixed cell, and epithelioid cell melanomas.\textsuperscript{24,127} The prognostic implications of this classification have been known since 1931.\textsuperscript{24} Tumors containing epithelioid cells grow faster and have a worse prognosis.\textsuperscript{24,68,122,165}

Extravascular matrix patterns, formerly known as microvascular patterns, are functionally extracellular vascular-like structures that are formed around nests of uveal melanoma cells. These patterns can further be divided into several categories. The best established patterns related to adverse prognosis include closed loops and, especially, networks.\textsuperscript{68,123}

High microvascular density (MVD), a morphologic measure of intratumor vascularization predicts mortality in several types of cancer including UM.\textsuperscript{122}

Other histopathologic markers of prognosis include MLN,\textsuperscript{1,75} cell proliferation,\textsuperscript{105,146} and the number of tumor-infiltrating macrophages.\textsuperscript{192}

Probably the greatest advance in the understanding of UM during the last decade regards its genetics. Loss of chromosome 3, which correlates with aggressive behavior and large size of the tumor, is now considered one of the most reliable prognostic factors predicting death from UM.\textsuperscript{91,150,164} It has been suggested that UM cells with two fully functional chromosome 3 may not develop metastases at all.\textsuperscript{180} Other common chromosomal anomalies involve chromosomes 8 and 6.\textsuperscript{91}
4 AIMS OF THE PRESENT STUDY

The purpose of this study was to:

1. Estimate the incidence of CM in Finland between 1967 and 2000 and to study whether the incidence has been increasing similarly to CMM or been stable like for UM.
2. Describe the clinical characteristics of “corneally displaced CM”.
3. Study the relative importance of different clinical and histopathologic factors predicting local recurrence and outcome of CM in a population-based material.
4. Analyze the metastatic pattern of CM and the theoretical applicability of SLNB in detecting early locoregional metastases.
5. Provide evidence for efficient treatment of primary and recurrent CM.
5 PATIENTS AND METHODS

5.1 Patients (I-IV)

5.1.1 Eligibility Criteria and Enrollment
All patients diagnosed to have a primary CM in Finland between January 1967 and December 2000 were eligible to this study. The earliest available histopathologic specimen of a patient with CM in the files of the Ophthalmic Pathology Laboratory of the Helsinki University Central Hospital dated back to 1967, hence the first year of the study.

The patients were identified from The Finnish Cancer Registry and double checked from the records of the Helsinki University Central Hospital and its Ophthalmic Pathology Laboratory, a national referral laboratory where the diagnosis of most patients treated elsewhere had previously been confirmed. In Finland, it is the duty of both the clinician and the pathologist to independently inform the Cancer Registry when a new primary or recurrent cancer is diagnosed. According to files of the Cancer Registry, 20 patients carried the diagnosis of ‘melanoma of the eye’ with unspecified location, and presence of CM was excluded by reviewing charts from the hospitals where they had been treated; all proved to be UM. Similarly, those patients with PAM who had been registered as having invasive CM were excluded from the present study after review of their charts. All patients identified from the Ophthalmic Pathology Laboratory were found to have been registered in the Cancer Registry as well. No attempt was made to search for additional patients, not filed in the Cancer Registry, from other hospitals.

The clinical and follow-up data for 85 consecutive patients with primary CM, who were thus identified, were assembled from the Finnish Population Registry, patient charts of hospitals where they had been treated for CM, its metastases, or secondary cancers, from pathology laboratories, and from death certificates. All 85 patients were included in the analysis of clinical and histopathologic features and prognosis of CM (I, III).

To define corneally displaced malignant CM (II), were eligible four patients who had a primary tumor growing on only the cornea.

Likewise, all 20 patients who had local or systemic metastasis diagnosed prior to death by histopathology, clinical examination or imaging were eligible for the analysis of the metastatic pattern of CM (IV).

The study was approved by the Institutional Review Board and it followed the tenets of the Declaration of Helsinki.
5.1.2 **Assessment of Outcome**

The date of diagnosis and date of local recurrence were taken to be the date of the first recorded observation of the tumor by a physician whose clinical diagnosis led to a histopathologic confirmation of the primary and recurrent CM. The date of metastasis was the date on which dissemination was confirmed by biopsy, imaging or clinical examination.

Complete follow-up data up till December 2000 (I,II,IV) and December 2004 (III) were available for 84 of the 85 patients. One patient had been diagnosed as having CM at the age of 82 in 1969. He was free of disease a year later, but was then lost to follow-up and censored from the analysis at this time. The Population Registry was unable to provide the date and cause of his death. The median follow-up time was 6.3 years (range, 0.3-33) for patients who were still alive in December 2000 (I,II,IV) and 7.9 (range, 0.5-37) for patients still alive in December 2004 (III).

5.1.3 **Confirmation of the Diagnosis and Cause of Death**

I was able to retrieve, for review and histopathologic confirmation of diagnosis, specimens from 83 (98%) of the 85 primary tumors. Of the two missing specimens, one had been reviewed earlier in Ophthalmic Pathology Laboratory of HUCH when the original diagnosis was made, and I could retrieve a recurrence from the second tumor and confirmed it to be a CM.

I was able to retrieve histopathologic specimens of metastases from 15/26 (58%) patients judged to die from CM by the end of the follow-up in 2004 and verify the diagnosis. In one case, the cause of death had been registered as primary hepatic carcinoma. Autopsy specimens, however, were consistent with metastatic CM. Autopsies were performed altogether in 9 (35%) of the 26 patients who were coded as having died of CM and all the autopsy specimens were available for re-study. In the third study (III) the proportion of patients with histopathologically confirmed metastases is inadvertently stated as 11/26 (42%). In the remaining patients the diagnosis of metastatic melanoma was mainly based on imaging studies and in some cases, combined with fine needle biopsy specimens that were no longer available for re-study.
5.2 Population Data (I)

Annual population data by age group was obtained from the Statistics Finland. During the study period, Finland had a stable population of 4.6 to 5.2 million inhabitants of Caucasian origin. Immigration from other countries was negligible, and the proportion of people of African and Oriental origin was less than 1%. According to the Statistics Finland, immigration was 0.27% compared to the entire Finnish population in 1990 and 0.33% in 2000. The proportion of people of African and Oriental origin can, with wide safety margin, be assumed to have been less than 1% during the study period based on data on the languages (98 % Finnish or Swedish and approximately 1% Russian or Estonian, according to Statistics Finland) spoken as mother tongue in Finland at the end of the study period.

5.3 Assessment of Tumor Characteristics

5.3.1 Clinical Tumor Characteristics (I-IV)

5.3.1.1 Prior Nevus and PAM

Presence or absence of prior conjunctival nevus and PAM were recorded from patient charts and past photographs. A nevus was coded as being present when a history of a nonprogressive conjunctival tumor at the same location for 10 years or more was elicited. PAM was coded as being present when previous or concurrent nonelevated conjunctival pigmentation outside the invasive tumor was recorded in the chart or could be verified from photographs.

5.3.1.2 Location

The tumors were classified into six categories according to location:
1.) corneally displaced (invasive CM growing on the cornea without evidence of conjunctival tumors other than PAM),
2.) limbal (CM involving the corneoscleral limbal area),
3.) bulbar (CM in bulbar conjunctiva separated from the limbus),
4.) caruncular (CM mainly involving the caruncle),
5.) palpebral (CM involving tarsal and fornical conjunctiva with or without extension to the skin)
6.) diffuse melanoma (CM comprising more than two quadrants of conjunctiva or involving more than one location).
Patients and Methods

To allow comparison with previous reports, I combined in the analysis limbal CM with four corneally displaced CM, which likely originated from atypical melanocytes that had migrated into the cornea from the limbus (I,III,IV).

5.3.1.3 Tumor Thickness
The paraffin blocks of primary tumors, recurrences and metastases were stained with HE, and the thickness of the primary tumor was measured with a calibrated ocular micrometer.

5.3.1.4 Largest Basal Tumor Diameter and Area
The LBD of the tumor was preferably measured from scanned color photographs using image analysis software (Olympus DP-SOFT, Version 3.0, Soft Imaging System GmbH, Hamburg, Germany), which were available from 31 (36%) of 85 primary tumors, assuming that the average cornea was 11 mm in diameter.74 The episcleral contact area of the tumor was also measured from the scanned photographs. When none were available, the LBD was taken from measurements and sketches recorded in the patient chart and histopathology report.

5.3.2 Histopathologic Tumor Characteristics (III)

5.3.2.1 Assessment of Cell Type
The cell type was graded analogous to the modified Callender classification (spindle, mixed, epithelioid) from a HE stained section by one investigator. If no typical spindle or epithelioid cells were present, cell type was coded indeterminate. Most of these tumors consisted of small round or polyhedral cells. The predominant cell type could be evaluated in 67 (79%) of the 85 primary tumors.

5.3.2.2 Assessment of Mitotic Count
Mitoses were counted at 400X magnification from HE stained sections (Olympus WK 10x/20L-H). Ten randomly selected HPF were evaluated for the presence of mitoses by two graders and the mean of these two observations was taken as the final mitotic count. If the tumor was large enough, the mitoses were counted from a single section. When not enough tumor material was available for ten HPF in one section, however, the count was taken from two sections, when available. If there were at least five HPF in the only section available, the mitotic count per ten HPF was estimated by multiplying the mitotic count accordingly. Very small tumors containing four or less HPF were excluded from the analysis. Likewise,
technically unsatisfactory stainings were excluded. The mitotic count could be assessed for 44 (52%) of 85 CM.

5.3.2.3  Assessment of Nucleoli
The sections were deparaffinized and bleached with 0.25% (weight/volume) potassium permanganate for one hour and placed into 5% (weight/volume) oxalic acid for 5 minutes. One-step silver staining was performed according to Moshari and McLean\textsuperscript{133} using two solutions. The first solution was 40 ml of 2.0% (weight/volume) gelatin (Bacto\textsuperscript{®} Gelatin, Difco Laboratories, Detroit, MI) and 0.88% (volume/volume) formic acid. The second was 80 ml of 50% (weight/volume) silver nitrate in distilled water. The solutions were mixed in the dark and poured into a dish to cover the sections for 30 minutes. The sections were finally washed in distilled water, dehydrated, and coverslips were mounted with Mountex\textsuperscript{®} (Histolab Products AB, Gothenburg, Sweden).

Each slide was examined under a light microscope (Olympus BH-2) at 40X magnification for digital photography (Olympus DP10). A series of photographs at 40X optical magnification were then taken to image the nucleoli along the longest axis of the tumor. If shorter than 5 mm, the entire central longest axis of the tumor was photographed, divided into slightly overlapping images (resolution 1280 x 1024 pixels, image area 218 μm x 175 μm).

From each photograph, the largest diameter of the largest nucleoli were measured using the image analysis software (Olympus DP-10 Soft). Strips of 41 μm in height were scanned beginning from the top of each photograph until a linear length of 5 mm or the entire specimen had been evaluated. Measurements of the 10 largest nucleoli were retained from each tumor for statistical analysis. MLN could be assessed in 64 (75%) of the 85 primary tumors.

5.3.2.4  Assessment of Tumor-Infiltrating Lymphocytes and Macrophages
Tumor infiltrating lymphocytes were assessed from HE stained sections. Based on a preliminary study of about 40 slides, the amount of lymphocytes was graded as few (Fig.1A in III, a tumor with none or very few lymphocytes inside the tumor), moderate (Fig.1B in III, a tumor with moderate numbers of lymphocytes inside the tumor or a tumor with a few areas with many lymphocytes), and many (Fig.1C,D in III, tumor nests surrounded by
patients and Methods

lymphocytes). Tumor-infiltrating lymphocytes could be graded in 60 (71%) of the 85 primary CM.

MAb PG-M1 (IgG3; lot 2562; Dakopatts, Klostrup, Denmark; diluted 1:50) to the CD68 epitope, an intracytoplasmic 110-kDa glycoprotein of lysosomal granules, which is expressed by macrophages in most human tissues\(^59,151\) was used in the present study. This antibody immunostains tumor-infiltrating macrophages more consistently in UM than other tested anti-CD68 antibodies.\(^{124}\) The number of immunopositive cells was graded semiquantitatively (few vs. moderate vs. high number of cells) by comparing CD68-immunostained sections to published standard photographs from a previous study of UM.\(^{124}\) Tumor-infiltrating macrophages could be assessed in 58 (68%) of the 85 primary CM.

5.3.2.5  Assessment of Extravascular Matrix Loops and Networks
Closed extravascular matrix loops and networks, consisting of at least three back-to-back loops, were identified according to Folberg et al.\(^{67}\) from sections bleached with potassium permanganate and oxalic acid and stained with periodic acid-Schiff without counterstain. They were viewed under a green filter (Wratten No.58, Kodak, Rochester, NY).\(^{67,123}\) Loops of all sizes were taken into account. Extravascular matrix loops were coded as present when they occurred within sheaths of infiltrating CM, and differentiated from connective tissue surrounding melanoma cells when these grew in a nested pattern.\(^{68}\) The slides were independently examined by three graders. In case of disagreement between graders, the final grading was assigned by consensus under a multi-headed microscope. MVP could be assessed for 55 (65%) of the 85 primary CM.

5.3.2.6  Assessment of Microvascular Density
Microvessels were identified with the monoclonal antibody (mAb) QBEND/10 to the CD34 epitope of endothelial cells (lot 121202; Novocastra Laboratories, Newcastle-upon-Tyne, UK; diluted 1:25).\(^{4,155}\) They were counted at 400X magnification from the most highly vascularized area (“hot spot”, identified under 100 x magnification)\(^{122}\) using an eyepiece with an etched graticule corresponding to 0.313 mm\(^2\) (Olympus WK 10x/20L-H). Any immunolabeled element, clearly separate from adjacent ones and totally inside the graticule or touching its top or left border, was counted as a microvessel.\(^{71}\) Micovessels were counted three times, and the highest count was used in analysis. MVD could be assessed in 56 (66%) of the 85 primary CM.
5.4 Statistical Methods and Data Analysis

5.4.1 General Guidelines (I-IV)
Analyses were performed with the STATA statistical software package (release 7.0, Stata Co., College Station, TX)(I-IV) and R (version 1.4.0, The R Foundation for Statistical Computing, Vienna, Austria; available at http://www.r-project.org) software packages (IV). Contingency tables were analyzed with Fisher’s exact test and Pearson’s chi-square test (I,III,IV), nonparametric test for trend (III), and distributions of continuous variables were compared with the nonparametric Mann-Whitney U test (I and IV) and nonparametric Kruskal-Wallis test (III). Exact probabilities were computed with StatXact-5 (Cytel Software, Cambridge, MA). The difference from unity of the male-to-female ratio was analyzed with the binomial test (I). The Spearman’s rank correlation was used to analyze interrelationships between two independent continuous variables (III).

5.4.2 Analysis of Incidence (I)
The crude, age-adjusted and age-specific incidence rate of CM were calculated, adjusting the incidence for the 2000 United States standard million population by the direct method.\(^3\) Annual incidence data was displayed in a scatterplot and summarized by running line smoothing without weighting.\(^25\) For comparison, data on CMM was obtained from the Finnish Cancer Registry and analyzed accordingly. The age-standardized incidence rate ratio of CMM to CM was calculated and plotted (I). They were also analyzed by linear least squares regression.

The population-based prevalence of Type I and II corneal invasive melanomas with 95% confidence intervals was calculated (II) (InStat 2, Graph Pad Software, San Diego, CA).

5.4.3 Univariate Analysis of Local Recurrence and Survival (I,III)
Univariate analysis of survival time data (I,III) was based on the Kaplan-Meier product-limit method.\(^92,145\)

Location of the primary CM was dichotomized into limbal (with corneally displaced) and nonlimbal (bulbar, palpebral, caruncular and diffuse). Age at diagnosis was divided in tertiles, LBD was arbitrarily divided into three categories, and tumor thickness was divided into three categories based on previous studies which have suggested a thickness of 1 mm and 2 mm to as indicative of a high risk of metastasis (<1 mm vs. 1-2 mm vs. >2
The effect of adjuvant treatment was analyzed by comparing patients who received no adjuvant therapy to those who were treated either with adjuvant cryocoagulation or topical MMC (I). Cell type was analyzed by combining mixed and epithelioid cell tumors. Mitotic count was dichotomized into less than 2 mitoses and 2 or more mitoses per 10 HPF (III). MLN and MVD were divided in tertiles (III). To analyze melanoma-specific survival, patients judged as dying of causes unrelated to CM were censored at their time of death. Unordered and ordered survival curves were compared with the log-rank test and test for trend. The Bonferroni correction was used to correct p-values for multiple comparisons.

5.4.4 Multivariate Analysis of Local Recurrence and Survival (I,III)

Multivariate analysis of time to local recurrence was based on an extension of the Cox proportional hazards model, which allows analysis of ordered multiple events.32,190

The conditional risk set model of Prentice, Williams, and Peterson149 was fitted, which assumes that patients are not at risk for a second recurrence until they have experienced their first recurrence, etc. The analysis was based on time from entry. Standard errors were calculated using the robust variance estimator of Lin and Wei116 and tied survival times were handled with the Efron approximation.92 Location of the primary CM was dichotomized into limbal (including corneally displaced) and nonlimbal (bulbar, palpebral, caruncular and diffuse) based on Kaplan-Meier analysis and previous publications.74,143,173 Age at diagnosis, thickness, LBD, and contact area of the primary tumor were modeled as continuous variables.

Multivariate analysis of melanoma-specific survival was based on an extension of the Cox proportional hazards model which allows internal time-varying covariates.92 In addition to standard analysis of time till the first local recurrence,92,145 the full history of recurrences was analyzed using repeated events, multiple failure-time data.32,190 In the latter analysis, patients were returned to the risk set after each recurrence. To analyze melanoma-specific survival, patients judged as dying from causes unrelated to CM were censored at their time of death. Three alternative strategies for modeling local recurrence were applied. In the first one, the corresponding variable was set to 0 until the first local recurrence and to 1 thereafter. This model would suggest that subsequent recurrences do not additionally affect the prognosis and the risk remains constant after the first recurrence. In the second, this variable was set to 0 and increased by 1 after each local recurrence, giving each recurrence an equal weight. In the third one, the variable was arbitrarily increased by 1, 0.8, 0.6, 0.4, and 0.2 after the first, second, third, fourth, and fifth local recurrence, giving each recurrence progressively less weight. The
last model would suggest that the first recurrence is of greatest significance, e.g. because micrometastases spawned from it might overgrow subsequent ones, but later recurrences still contribute to prognosis.

The assumption of proportional hazards was verified by adding each covariate by a log time interaction to the model and by assessing the significance of the product term using the partial likelihood ratio test, which was also used to compare alternative models. The number of variables in the final model for was restricted to two, based on the rule of having at least 15 to 20 events per each additional variable. The regression coefficients and hazard ratios (HR), with 95% confidence intervals, were calculated.

5.4.5 Analyses of Metastatic Pattern (IV)

Calculation of time to metastasis was based on cumulative incidence analysis using the R-cmprsk-library. Initial regional metastasis, systemic metastasis, and death were modeled as competing risks, and patients who were alive at the end of the follow-up were censored. For comparison with previous publications, the analysis was repeated with the Kaplan-Meier (product-limit) method. The latter method considers patients as being still at risk of the event of interest after they have been censored because of a competing event, and consequently it overestimates the proportion of patients developing the event. Cumulative incidence curves were compared with Gray’s K-sample test.

Overall survival after metastasis was analyzed with the Kaplan-Meier method and the log-rank test.
6 RESULTS

6.1 Incidence of Conjunctival Melanoma (I)
During the study period, 85 new patients with CM were identified in the Finnish population (mean, 4 880 000 inhabitants), corresponding to a mean of 2.5 patients per year (range, 0 to 8). The crude and age-standardized annual incidences were 0.51 and 0.54 per million. The age-specific incidence was 0.06 for those under 30 years old, and 0.48, 1.05, and 1.57 for the age groups of 30-49, 50-70, and over 70.

The smoothed age-standardized incidence remained stable (mean, 0.4 per million) until 1975 and suggested a steady increase thereafter to 0.8 per million (Fig.1A in I).

By linear regression, the increase tended to be statistically significant over the entire study period (Fig. 1A, \( P = 0.081 \)), and the increase was significant from 1975 to 2000 (Fig. 1B, \( P = 0.011 \)). The corresponding smoothed incidence of CMM increased at approximately the same rate from 1967 to 1998 from 41 to 101 per million (Fig.1B in I). The smoothed relative incidence of CMM compared to CM remained stable at around 150:1 (Fig.1C in I).

![Figure 1](image)

*Figure 1. Linear regression with the 95% confidence intervals as dotted lines calculated over the age-standardized incidence of CM (A) from 1967 to 2000 and (B) from 1975 to 2000.*

6.2 Baseline Characteristics (I-IV)
The male-to-female ratio did not differ statistically from unity (\( P=0.39 \), binomial test). Of the 85 patients, 47 (55%) were males. The median age at diagnosis of the primary CM was 60 years (range, 20-90, Table 1 in I).
6.3 **Primary Treatment (I-IV)**

Of the primary tumors, 78 (92%) were removed by local excision, 4 patients underwent primary exenteration (before 1981), and two underwent enucleation (before 1979). One corneally displaced melanoma was removed by penetrating keratoplasty.

The adjacent conjunctiva was cryocoagulated from beneath with a nitrous oxide probe in 16 eyes, 3 eyes received topical mitomycin C (2 cycles of 0.04 mg/ml for 2 weeks), and 2 eyes received both types of adjuvant treatment (Table 1 in I). In 13 eyes, the primary resection was promptly followed by 9 secondary local resections, 3 exenterations and one enucleation (in 1983) because the margins were involved by invasive melanoma. Of the 85 primary surgeries, 51 (60 %) were performed in the Department of Ophthalmology of the HUCH.

6.4 **Corneally Displaced Primary Malignant Melanomas (II)**

Of the 85 consecutive Caucasian patients with malignant CM in my population-based, nationwide study, as their primary manifestation four (5%, 95% confidence interval, 1-12) had an invasive tumor limited to the cornea. I created a classification for these tumors based on the exact anatomic location of the tumor and the presence or absence of PAM (Table 4). Of the four tumors, two were of Type I (separated from the limbus) and two were of Type II (paralleled the limbus). Two tumors belonged to subtype IA and IIA (no PAM) and the others to subtype IB and IIB (Table 2 in II).

All four tumors were managed with local excision, which was combined with penetrating keratoplasty in one patient. Stromal invasion was never observed and all tumors were HMB-45 positive by immunohistochemistry. One of the two melanomas not associated with PAM at diagnosis developed putative corneal PAM during follow-up (Case 1, Table 2 in II). No local or systemic recurrence occurred during a median follow-up of 2 y 5 months (range, 1 y 8 months to 7 y 10 months). The patient with the shortest follow-up died of clinically diagnosed, localized pancreatic cancer.

6.5 **Clinical Tumor Characteristics (I)**

6.5.1 **Location of the Primary Tumor**

Limbal conjunctiva was the most common type of primary tumor site (59%; 95% CI, 48-69%) followed by bulbar conjunctiva, diffuse tumors, palpebral conjunctiva, corneally displaced tumors and caruncle (Table 5). Both eyes were equally affected (right:left, 41:44). None of the patients had bilateral melanoma.
Table 4. Classification of corneally displaced primary malignant conjunctival melanomas

<table>
<thead>
<tr>
<th>Classification</th>
<th>Number of Patients</th>
<th>% (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Tumor is separated from the limbus by clear cornea</td>
<td>4</td>
<td>5 (1-12)</td>
</tr>
<tr>
<td>II Tumor parallels the corneal limbus without conjunctival invasion</td>
<td>50</td>
<td>59 (48-69)</td>
</tr>
<tr>
<td>III The epicenter of the tumor is corneal, but the tumor involves limbal conjunctiva†</td>
<td>11</td>
<td>13 (7-22)</td>
</tr>
<tr>
<td>Caruncle</td>
<td>3</td>
<td>4 (1-10)</td>
</tr>
<tr>
<td>Palpebral conjunctiva</td>
<td>8</td>
<td>9 (4-18)</td>
</tr>
<tr>
<td>Diffuse involvement</td>
<td>9</td>
<td>11 (5-19)</td>
</tr>
</tbody>
</table>

† Corneal origin uncertain

6.5.2 Predisposing Lesions

In 23 of 77 patients (30%; 95% CI, 20-41%) with adequate clinical information, the melanoma had likely developed from a prior conjunctival nevus, and 48 of 79 eyes (61%; 95% CI, 49-72 %) had had clinically detectable PAM (Table 1 in I); 6 patients had a history of both nevus and PAM. In 7 (8%) additional patients, clinically detectable PAM appeared during follow-up, but had not been evident at the time of diagnosis. Limbal tumors tended to be less frequently associated with PAM than nonlimbal tumors (46% vs. 71%, P = 0.086 Pearson’s chi-square test).
6.5.3  **Size of the Primary Tumor**

The median thickness of 72 measurable primary CM was 1.3 mm (range, 0.2-8.8). The LBD of 76 measured tumors ranged from 1 to 19 mm (median, 7, Figure 2). The contact area of 31 photographed tumors ranged from 4 to 132 mm² (median, 29).

Limbal melanomas were thinner (median, 1.1 vs. 3.3 mm, \( P = 0.0003 \) Mann-Whitney \( U \) test), and their diameter (median, 7 vs. 10 mm, \( P = 0.042 \)) and contact area (median, 24 vs. 63 mm², \( P = 0.042 \)) were smaller compared to nonlimbal melanomas (Table 1 in I).

![Figure 2. Tumor thickness and LBD of 67 primary CM with available data.](image)

6.6  **Histopathologic Tumor Characteristics (III)**

6.6.1  **Cell Type**

The cell type was epithelioid in 18 (27%), mixed in 23 (34%), spindle in 17 (25%), and indeterminate in 9 (13%) of the 67 primary CM (Table 1 in III). Epithelioid cells were less frequently found in melanomas that were nonlimbal (\( P = 0.038 \), Fisher’s exact test) and thicker (\( P = 0.009 \), Kruskal-Wallis; Table 1 in III).

6.6.2  **Mitotic Count**

Mitotic count varied from 0 to 19 (median, 0) per 10 HPF in 44 primary tumors. A high mitotic count was associated with large tumor thickness (\( P < 0.001 \), Spearman correlation; Table 1 in III) and tended to be associated with nonlimbal tumor location (\( P = 0.074 \)).
6.6.3 Mean Diameter of the Ten Largest Nucleoli
MLN varied from 2.8 to 7.5 µm (median, 4.3; mean 4.3, SD 0.91) in 64 primary tumors. Tumor location ($P = 0.49$, Kruskal-Wallis) and presence of epithelioid cells ($P = 0.17$) were unassociated with MLN, but it tended to correlate with tumor thickness ($P = 0.058$, Spearman correlation; Table 1 in III).

6.6.4 Tumor-Infiltrating Lymphocytes
Tumor-infiltrating lymphocytes were few in number in 27 (45%), moderate in 17 (28%), and many in 16 (27%) of 60 primary CM. Lymphocytes were more frequent in limbal CM ($P = 0.018$, nonparametric test for trend) and their number decreased with increasing tumor thickness ($P = 0.016$, Spearman correlation; Table 1 in III).

6.6.5 Tumor-Infiltrating Macrophages
The number of CD68-immunopositive cells was few in 7 (12%), moderate in 24 (41%), and high in 27 (47%) of 58 primary CM. Tumor location ($P = 0.19$, nonparametric trend), thickness ($P = 0.58$, Spearman correlation), and presence of epithelioid cells ($P = 0.86$, Kruskal-Wallis) were not associated to the number of CD68-immunopositive cells (Table 1 in III).

6.6.6 Extravascular Matrix Loops and Networks
Extravascular loops without networks were present in 22 (40%), networks in 23 (42%) and neither of the two patterns in 10 (18%) of 55 primary tumors. Three primary tumors were composed of individual nests of tumor cells and were not classifiable and the remaining specimens were technically unsatisfactory. Tumor location ($P = 0.13$, nonparametric test for trend) and thickness ($P = 0.97$, Spearman correlation) were not associated with these matrix patterns (Table 1 in III).

6.6.7 Microvascular Density
MVD varied from 13 to 99 (median, 48) vessels/0.313 mm$^2$ in 56 primary tumors. Tumor location ($P = 0.57$, Kruskal-Wallis) and thickness ($P = 0.55$, Spearman correlation) were unassociated with MVD (Table 1 in III), but MVD was associated with the presence of epithelioid cells (median, 38 vs. 48 vessels/0.313 mm$^2$, $P = 0.037$, Kruskal-Wallis).
6.7 Local Tumor Recurrence (I,III)

The cumulative probability of the first local recurrence was initially analyzed concentrating on clinical prognostic (I) factors and later by including histopathologic data (III), with a longer follow-up. The clinical factors found to be significant predictors of the first local recurrence in the initial analysis were included in the second analysis to test for their independence and to refine the effect size.

Up until November 2004, for those patients who were still alive, the CM recurred locally 64 times in 31 (36%) patients (median, 1; range, 1-6) during the median follow-up of 7.9 years (range, 0.5-37). After recurrence, four patients underwent secondary exenteration and one painful eye was enucleated. One patient refused exenteration for recurrence and was treated with radiotherapy followed by evisceration. The remaining 25 patients with recurrent melanoma were treated with local excision. By Kaplan-Meier analysis, the 5-year and 10-year cumulative probabilities of the first local recurrence were 0.34 (95% CI, 0.25-0.46) and 0.36 (95% CI, 0.27-0.49) (III). The 5 and 10 year estimates of cumulative probability of the first recurrence in the first paper (I) with a median follow-up of 6.3 years (range, 0.3-33) were 0.36 (95% CI, 0.25-0.48), and 0.38 (95% CI, 0.28-0.52). No clinically significant difference existed between the effect size of the two analyses (I and III).

The location of the primary CM was associated with time to the first recurrence (Fig.2A in III; \( P = 0.038 \), log-rank test). Melanomas in palpebral conjunctiva recurred more frequently than other tumors. Tumor thickness was not associated with time to the first local recurrence (\( P = 0.22 \), log-rank test for trend), but I could not exclude a better prognosis of the primary CM that are less than 1 mm in thickness (Fig.2B in III). The gender (\( P = 0.51 \) log-rank test, difference between curves), age at diagnosis of primary CM (Fig.2B in I), and history of previous conjunctival nevus (Fig.2C in I) and PAM (Fig.2D in I), LBD (Fig.2G in I), contact area (\( P = 0.32 \)), and adjuvant treatment (Fig.2H in I) were not significantly associated with local recurrence.

Of the histopathologic factors, predominant cell type (\( P = 0.047 \), log-rank test for trend) and absence of epithelioid cells (Fig.2C in III; \( P = 0.026 \), log-rank test) were associated with a shorter time to the first local recurrence. Primary CM with indeterminate cell type resembled those with no epithelioid cells. Smaller MLN was associated with a shorter time to first local recurrence (Fig.2D in III; \( P = 0.035 \), log-rank test for trend). A higher mitotic count (Fig.2E in III; \( P = 0.34 \)), the presence of tumor-infiltrating lymphocytes (Fig.2F in III; \( P = 0.22 \)) and macrophages (\( P = 0.79 \)), presence of extravascular matrix loops and networks
Results

(Fig. 2G in III; \( P = 0.39 \)), and MVD (Fig. 2H in III; \( P = 0.35 \)) were not associated with first local recurrence.

By univariate Cox regression, mitotic count (HR 1.11), absence of epithelioid cells (HR 0.35) and small MLN (HR 0.56 for each category increase), were associated with shorter time to the first local recurrence (Table 2 in III). MLN and mitotic count remained significant in a bivariate model.

6.8 Melanoma-Related Mortality (I,III)

Of the 85 patients, 26 (30\%) died of metastatic CM during a median follow-up of 7.9 years (range, 0.5-37), 3 (3\%) died of another malignant tumor, and 15 (17\%) from causes not related to cancer. The median time from diagnosis of the primary CM to death from metastatic disease was 4.5 years (range, 1-19). Considering melanoma deaths, the cumulative 5-year and 10-year probabilities of survival were 0.83 (95\% CI, 0.72-0.89) and 0.67 (95\% CI, 0.54-0.77) (III).

The 5 and 10 year estimates of the proportion of surviving in the first analysis (I) were 0.80 (95\% CI, 0.68-0.88) and 0.62 (95\% CI, 0.47-0.74), falling within the 95\% confidence intervals of the later analysis (III).

By Kaplan-Meier analysis, location (\( P = 0.0007 \), log-rank test), especially nonlimbal as compared to limbal location (Fig.3A in III; \( P = 0.0001 \)), thickness (Fig.3B in III; \( P = 0.026 \), log-rank test for trend), and LBD (Fig.3H in I; \( P = 0.003 \)) of the primary CM were associated with melanoma-related mortality. A CM more than 2 mm in thickness had a worse survival than thinner ones (\( P = 0.038 \), log-rank test with Bonferroni correction, III).

Gender (Fig.3B in I), age at diagnosis of primary CM (Fig.3C in I) and history of previous conjunctival nevus (Fig.3D in I) and PAM (Fig.3E in I) did not predict melanoma-related death.

Absence of epithelioid cells (Fig.3C in III; \( P = 0.14 \), log-rank test), the mitotic count (Fig 3D in III; \( P = 0.30 \)), MLN (Fig.3E in III; \( P = 0.51 \), log-rank test for trend), the presence of tumor-infiltrating lymphocytes (\( P = 0.20 \)) and macrophages (Fig.3F in III; \( P = 0.40 \)), the presence of extravascular matrix loops and networks (Fig.3G in III; \( P = 0.87 \)), and MVD (Fig.3H in III; \( P = 0.29 \)) were not associated with the probability of dying from CM. My data, however, do not exclude a better prognosis of patients with primary CM that have few infiltrating macrophages (Fig.3F in III).
By the univariate Cox regression, nonlimbal location (HR 4.60), thickness (HR 1.32 for each mm increase), and local recurrence (HR 2.48) of the primary CM were associated with an increased risk of melanoma-related death (Table 3 in III). The association with local recurrence strengthened (Wald chi-square, 18.8 vs. 2.47) when recurrence was analyzed as a time-varying covariate (HR 1.76; Table 3 in III).

Of the bivariate models including tumor location, tumor thickness, local recurrence modeled as a time-varying covariate, and presence of epithelioid cells, those that included local recurrence with tumor thickness and tumor location fitted best with the data. In the model including tumor location and local recurrence, both variables were independent predictors of melanoma related death (Table 3 in III).

### 6.9 Metastatic Pattern (IV)

Of the 20 patients who had metastases from CM diagnosed before death, 11 (55%) were men and 9 were women. The median age at diagnosis of the primary tumor was 52 years. The primary tumor was limbal in 7 eyes, bulbar in 3 eyes and palpebral and diffuse in 5 eyes each. The median thickness and LBD of the 15 primary tumors for which these data were available were 2.8 mm and 10 mm, respectively (Table 1 in IV).

Local resection was the primary treatment in 16 (80%) patients. Three patients with a diffuse CM underwent primary exenteration. One patient underwent enucleation. Of patients who underwent local resection, 12 (75%) developed a median of three local recurrences (range, 1-6). After recurrence, 3 patients underwent exenteration, one enucleation and one external beam radiotherapy. Other recurrences were managed with local resection.

#### 6.9.1 Diagnosis and Pathway of First Metastases

Metastasis was confirmed by histopathology in 12 (60%) patients, by radiology in 5 (25%), and by clinical examination in 3 (15%). The first clinical metastasis was regional in 9 (45%, 95% CI 23-68) patients. Submandibular lymph nodes were involved in 6, parotid nodes in 2, and preauricular nodes in 1 patient.

The first site of metastases was systemic in 10 patients (50%, 95% CI 27-73). Three patients had metastases detected in two sites and one in three. Four patients had their first metastases detected in the lungs, three in the liver, two patients each, in the subcutaneous
Results
tissue or bone; and 1 patient each, in the stomach or peritoneum. One patient had isolated biliary duct metastasis. An additional patient had pulmonary metastases and preauricular lymph node metastases detected at the same time.

Regional lymphatic metastases was significantly associated with young age at diagnosis of the primary CM (Table 1 in 4; 47 vs. 56 years, $P = 0.030$, Mann-Whitney $U$ test).

6.9.2 Time to First Metastasis

The median time from diagnosis of the primary CM to the diagnosis of metastasis was 2.6 years (range, 0.5-15.8). This interval was 2.3 years (range, 0.5-15.8) for regional metastases and 3.4 years (range, 1.2-9.8) for systemic metastases with or without metachronous regional metastases ($P = 0.46$ Mann-Whitney $U$-test). For five patients who developed both types of metastases sequentially the median time from diagnosis of regional to systemic metastases was 1.0 years (range, 0.4-2.5).

By cumulative incidence analysis, the 10-year incidence of regional and systemic metastases was 0.11 and 0.18 (Fig.1A in IV ). For comparison, the corresponding product-limit (Kaplan-Meier) estimates were 0.12 and 0.21.

Cumulative incidence of regional metastases was comparable for limbal and nonlimbal CM (Fig.1B in IV; $P = 0.43$ Gray’s K-sample test), whereas the incidence of systemic metastasis was higher for nonlimbal CM (0.07 vs. 0.38 at 10 years; Fig.1B in IV ; $P = 0.00023$). Both regional (0.05 vs. 0.18 at 10 years; Fig.1C in IV ; $P = 0.062$) and systemic metastases (0.06 vs. 0.28 at 10 years; Fig.1C in IV; $P = 0.026$) tended to be more frequent when the primary melanoma was more than 2 mm in thickness as compared to those that were 2 mm or less.

Patients who experienced no local recurrence tended to less frequently have initial regional (0.06 vs. 0.19 at 10 years; Fig.1D in IV ; $P = 0.062$) as well as initial systemic metastases (0.12 vs. 0.28 at 10 years; Fig.1D in IV; $P = 0.062$) than patients who had one or more local recurrences.

6.9.3 Treatment of Metastases

Three of 10 patients with regional metastasis underwent radical neck dissection, and one patient had intestinal metastasis removed by surgery. Seven patients received radiation therapy and the same number were treated with chemotherapy, in two cases with interferon.
6.9.4 Survival After Metastases

The median overall survival after metastasis was 15 months (95% CI 3-30; range, 1-44 months). Patients who first developed regional rather than systemic with or without metachronous regional metastases survived longer (30 vs. 8 months, \( P = 0.012 \) log-rank test; Fig.2 in IV). One patient who was alive without residual disease four years after removal of a solitary lymph node metastasis at the end of the follow-up of the study (IV) died of systemic metastases in 2003, having survived 6 years and 1 month after the removal of the initial lymph node metastasis.
7 DISCUSSION

My aim was to study the clinical and histopathologic characteristics of CM in a population-based setting. For this kind of research, Finland offered several advantages. First, the Finnish population during the study period was relatively stable with little migration, and it was composed almost completely of people of Caucasian origin, the group most at risk of getting CM. Secondly, Finland has had an efficient Cancer Registry since 1953. According to a nation wide audit published in 1994, only 0.9% of the more than 60 000 solid malignant tumors that could be identified from the hospital discharge registries between 1985 and 1988 were missing in the Cancer Registry. Thirdly, the diagnosis of most of the patients had been confirmed in the Ophthalmic Pathology Laboratory of the Department of Ophthalmology of HUCH, a tertiary referral center, making it possible to double-check the information retrieved from the Cancer Registry. Despite the retrospective nature and a relatively long study period, most follow-up information, patient charts, photographs and histopathologic specimens were available for reanalysis. I am confident that my study is representative in regards to the clinical and histopathologic characteristics of CM in Finland from 1967 to 2000.

Assessment of the correct cause of death is vital in cancer research. A histopathologic audit of 145 patients who died of UM showed that from 7% to 10% of original histopathologic cancer diagnoses that had not been based on immunohistochemistry were incorrect. More than half of my patients who were classified as having died of CM had histopathologic confirmation of their metastasis and the specimen was still available for re-examination. Otherwise, the diagnosis of metastatic melanoma was based on imaging studies and clinical examination. An autopsy was performed on only 35% of my patients judged to die of CM, probably based on the clinically evident fact that the patients were known to be suffering from systemically disseminated melanoma. While every attempt to ascertain the right cause of death of each patient was made, possible bias as regard to both false positive and negative tumor deaths is obviously always present in a retrospective study, especially one spanning a long time period.

The crude incidence of CM during the study period in Finland was comparable to the other essentially population-based studies (Table 1). This was the first study to report an increase in the age-standardized incidence of CM over the last few decades, especially during the last 20 years of the study-period. This increase was comparable to the increase in the incidence of CMM (Figure 1 in I). While I believe that the observed increase in the incidence
of CM was not due to inefficient reporting of incident cases to the Cancer Registry during the early years of the study period, by which time the Registry had already been operational for 15 years, such a possible source of bias can not be completely ruled out in a retrospective setting. A similar increase in the incidence of CM was soon reported from the SEER regional cancer surveillance data base in the United States, supporting the argument that the incidence of CM may be growing globally in predominantly white Caucasian populations. More population-based data are thus needed to confirm this finding.

Most primary CM grow on the limbal or bulbar part of the conjunctiva that is directly exposed to UV radiation. In my series, only 8 patients had a primary tumor that was classified as entirely palpebral. Non-limbal tumors tended to be more often associated with PAM with atypia and it is possible that in some of these 8 palpebral primary tumors the actual origin was limbal or bulbar PAM. The biological similarity of CM and CMM, the increase in the incidence of both, and the fact that most CM originate in locations exposed to UV radiation lend support to the role of UV radiation in the pathogenesis of CM. Future studies will be needed to establish the direct mutagenic effects of UV radiation in CM, however, before any causal relationship can be confirmed.

The proportion of CM thought to arise from a pre-existing nevus in my study was 30% (95% CI, 20%-41%), a relatively high number compared to most studies but still smaller than the 39 % reported from Germany. Moreover, six patients had a history of both nevus and PAM. Taking this and the other available population-based and other larger studies together, and considering confidence intervals, it can be assumed that roughly one out of four to five melanomas may originate from a pre-existing conjunctival nevus.

My estimate of 61% (95% CI, 49%-72%) of CM originating from PAM with atypia is in line with previous reports. In my study PAM was evaluated clinically which probably causes some underestimation in the proportion of CM originating from PAM with atypia. It is well known that PAM may be amelanotic and only detectable histopathologically. Likewise, several patients developed clinical PAM during the follow-up despite it not being present at diagnosis. Consequently, superficial epithelial spreading of atypical melanocytes in the form of PAM at some point is characteristic of the majority of CM.

Fifty-four (59%) of the 85 primary CM were classified as limbal. These tumors are diagnosed earlier than nonlimbal melanomas. The median thickness of limbal CM in my study was only 1.1 mm (0.3-3.9) compared to 3.3 mm (0.2-8.8) for nonlimbal CM (Table 1 in I). My study was consistent with most previous ones in that limbal CM are associated with a
The 5-year cumulative survival after limbal CM was 94%, compared with 67% for bulbar and 38% for palpebral and diffuse CM combined (Fig 3F in I). Palpebral location was also associated with a short time to local recurrence. Moreover, of those 9 patients who died of melanoma after limbal primary CM, all but two had from 1 to 6 recurrences, mostly in nonlimbal locations. Those two patients with a limbal CM who did not develop recurrences but died of metastatic CM had thick primary tumors, 2.2 mm and 3.9 mm.

My second paper concentrated on analyzing a subtype of limbal melanoma that I named “corneally displaced CM”. These tumors have formerly been referred to as “corneal melanomas”. Because no melanocytes exist in the cornea, however, the origin of corneally displaced melanomas is presumably the limbal conjunctiva from where the atypical melanocytes migrate to the cornea in the form of PAM or a true invasive melanoma. These tumors seem in most cases to be limited to the epithelium and are easily peeled off, unless the Bowman’s membrane is disrupted by surgery. It is known, however, that advanced CM spreading to cornea can also disrupt Bowman’s layer and become intrastromal.

According to the current TNM classification corneally displaced CM are classified as T4 primary tumors along with tumors involving the eyelid or the orbit. While involvement of the eyelid and orbit definitively are predictors of poor prognosis, the prognosis of primary CM involving the cornea, at least when the CM is limited to the epithelium, is probably the same or better than for any limbal melanoma and should therefore be classified as T1 or T2, depending on the extent of the tumor in conjunctival quadrants. These tumors are normally detected early, before they grow thick. None of the four patients with corneally displaced CM died of melanoma and the tumor was in all cases limited to the epithelium. Likewise, the cases described in the literature support the hypothesis that this is a relatively benign form of CM. It should be noted, however, that I had no access to the original clinical and histopathologic and later follow-up data of the cases described in the literature, which is an obvious caveat in regards to this conclusion. Further clinical and histopathologic analyses of these rare tumors are warranted to confirm my theory.

By the Kaplan-Meier analysis and the univariate Cox regression, the thickness of the primary CM was significantly associated with mortality. According to a multivariate analysis, however, it was not unequivocally an independent prognostic factor, unlike tumor location and local recurrence. As stated earlier, the non-limbal CM were significantly thicker than the limbal ones. Even though the multivariate Cox regression suggested that it is the nonlimbal location itself rather than the greater thickness of these tumors that makes them more likely to
metastasize, I do not think any definitive conclusion on which of the two factors, location or thickness, is more significant, can be drawn from my results because these two variables were interrelated. The tumors that have the worst prognosis are the palpebral and diffuse CM. It may be speculated that tumors on the palpebral conjunctiva grow more easily deeper into the conjunctival stroma and thus have more direct access to the local lymphatics and blood vessels. The possibility of vertical growth is more limited for limbal and bulbar tumors because the conjunctiva is thinner and the sclera acts as a natural barrier preventing deeper invasion.

My analysis on metastatic pattern lends support to the theory that 2 mm may be close to the “critical” thickness that indicates a clinically significantly higher risk of both locoregional and systemic metastases.46,120,178

Multiple local recurrences of CM were very common. The 5 and 10 year cumulative probabilities of the first local recurrence that were 0.34 (95%CI, 0.25-0.46) and 0.36 (95%CI, 0.27-0.49), fall inside the confidence intervals of the other population-based studies (Table 2).

By Kaplan-Meier analysis, the location of the primary CM was associated with local recurrence mainly because palpebral location predicted frequent and more rapid recurrence. When analyzed by the univariate Cox regression, nonlimbal as compared to limbal location tended to be associated with time to the first recurrence in analysis of clinical prognostic factors (I) but not in the second analysis with four years longer follow-up (III). In the first analysis the association strengthened when recurrence was analyzed as a repeated event. The results of the Kaplan-Meier analysis further demonstrated that not all nonlimbal melanomas, but mainly palpebral ones were associated with high risk of recurrences (Fig. 2E in I). Univariate Cox regression also associated time to the first local recurrence with absence of epithelioid cells, small MLN, and high mitotic count, mostly features of thick and nonlimbal melanomas. For the purposes of clinical work, the conclusion probably should be that all CM have a high tendency to recur, but this tendency is particularly high for palpebral melanomas.

Repeated recurrences often lead to loss of the involved eye.132,142,169,199 During the follow-up of my patients, altogether 16 patients (19%) lost the involved eye. Even more importantly, each recurrence was statistically associated with a shortened time to death from melanoma. We applied recently developed statistical methods to analyze the effect of repeated recurrences on prognosis.32,190 According to our data, the model that gave each recurrence equal weight fitted with the data best.

At least two possible explanations can account for the causal relationship between tumor recurrence and mortality. First, it can be hypothesized that those primary CM that recur
Discussion

are more malignant per se leading not only to local recurrence but to death from melanoma more often than other CM. According to the univariate and multivariate Cox regression, high mitotic count was significantly associated with local recurrence, supporting the theory that these tumors are associated with a more malignant phenotype and probably a high growth rate. The other theory is that each recurrence adds to the tumor mass and that the probability of systemic dissemination increases linearly along with cumulative tumor burden.

I did not systematically study clinical or histopathologic characteristics of recurrent CM but the impression was that recurrences tended to grow on non-limbal locations even though the primary tumor had been limbal, as earlier described, and they could be larger and associated with more malignant characteristics compared to the primary CM. Further studies on clinical and histopathologic characteristics of recurrent CM are warranted.

The aim of my third paper was to analyze the histopathologic characteristics of CM. This was largely inspired by the advances in the research of UM that has shown that histopathologic factors consistently overshadow clinical ones in predicting outcome. I wanted to know whether it was possible to evaluate these factors in CM, what are the similarities and differences between CM and UM in this respect, and whether these factors had prognostic value in predicting local recurrence and mortality from CM.

CM is diagnosed earlier than UM, making the average histopathologic specimen smaller. The median thickness of a UM in a series from our institution was 8 mm, whereas the median thickness of CM in my study was 1.3 mm. This may limit the applicability to CM of histopathologic methods developed for predicting prognosis of UM, such as extravascular matrix loops and networks, and MVD. This same problem has been noticed when biopsied metastases of UM have been studied.

Classification of CM according to Callender resulted in difficulty. According to my data, thick and nonlimbal tumors less often contained epithelioid cells. Tumors with epithelioid cells were associated with longer time to local recurrence but there was no difference in survival according to cell type. The apparent conclusion that epithelioid cell type could be a benign feature of limbal and thin CM must be regarded with caution, because about half of the tumors did not fall into the two main categories. Secondly, I noticed that thin CM often grows in nests surrounded by lymphocytes and other cells, but thick melanomas are often tightly packed and contain few cells other than tumor cells. Consequently, it is possible that tumors growing loosely in groups of a few cells may be classified as epithelioid even though they are not truly composed of such non-cohesive cells as are the classical epithelioid
cells of UM. Taken together with previous studies, my findings do not support a key role for cell type in predicting prognosis of CM.

The mitotic count per ten HPF in those 44 primary CM in which it could be evaluated was generally low, in line with a previous analysis from the United States. Only 30% of the 44 tumors contained two or more mitoses per 10 HPF. Not surprisingly, tumor thickness was strongly associated with mitotic count. No mitoses were found in CM less than 1 mm in thickness. According to the Cox regression, mitotic count analyzed as a continuous variable was associated with a short time to local recurrence. There was a small subgroup of three patients with very high mitotic counts from 9.5 to 19 mitoses per 10 HPF. These could be equivalent to fast growing CMM described by a recent study. In that study, a subgroup of CMM estimated to grow more than 0.5 mm in thickness per month was identified, and they were characterized by a high number of mitoses. High mitotic count was a feature of thick CM, and it tended to be associated with nonlimbal location. Overall it was not, however, associated with prognosis. Moreover, the fact that most CM contained none or only one mitosis per 10 HPF makes mitotic count not a very useful prognostic factor in clinical ophthalmic pathology.

MLN in CM was similar in median to that observed in UM in our laboratory (median 4.1 um vs. 4.3 um). MLN was smaller in CM that recurred, probably due to the observation that spindle cells that have smaller nucleoli were more common in thick and nonlimbal CM. MLN did not predict mortality, contrary to UM in which large MLN is associated with death from melanoma.

Gathering of lymphocytes inside and around a malignant tumor is a common feature in different cancers and represents a first line defense against the tumor. I observed a significantly greater number of tumor infiltrating lymphocytes in limbal and in thin CM compared to nonlimbal and thick tumors. This finding probably represents normal growth and initial tumor response of a malignant CM during which lymphocytes that are abundant when the tumor is small are gradually replaced by tumor cells. In CMM and in some studies on CM, a high number of lymphocytes, especially of CD8+ lymphocytes, has been a favorable prognostic factor. In UM a high number of tumor infiltrating lymphocytes is paradoxically an unfavorable prognostic factor. Because of sample-size limitations, my study is unable to exclude the possibility that a small number of lymphocytes is associated with a moderately shorter time to local recurrence and tumor death (HR 0.70 and 0.66, Tables 2 and 3 in III).
Characterization of different subtypes of lymphocytes and their role in the primary defense against CMM has led to research and development of several cancer vaccines that are currently under clinical trials.\textsuperscript{17,188}

By using standard photographs published for grading tumor-infiltrating macrophages in UM, we found that 87\% of CM contained moderate to high numbers of macrophages, as compared to 83\% of UM in our laboratory.\textsuperscript{124} The number of tumor-infiltrating macrophages analyzed semiquantitatively in three categories did not predict either time to first recurrence or death from CM.

At least one closed extravascular matrix loop was observed in 82\% of patients with primary CM, compared to 24\% to 60\% in UM.\textsuperscript{68,70,123,128} Networks were present in 42\%, compared to 19\% to 45\% in different studies on UM,\textsuperscript{68,70,123,171} and the figures were thus roughly comparable in spite of the generally smaller size of CM specimens. No association between presence of these two extravascular matrix patterns and local tumor recurrence or survival could be detected in CM, in contrast to consistent findings of several studies of UM.\textsuperscript{70,171 68,70,123}

The median MVD and its range in CM and UM were fairly comparable (median, 48 and range, 13 to 99 vessels/0.313 mm\textsuperscript{2} vs. median, 40 and range, 5 to 121 vessels/0.313 mm\textsuperscript{2}).\textsuperscript{122} In contrast to UM,\textsuperscript{28,71,122} increasing MVD was not associated with melanoma-related mortality, however.

While the results of my analysis on potential histopathologic prognostic markers do not support an important role for them in predicting outcome of patients with CM, these findings should be considered as preliminary because of the relatively small set of patients, and because many of the methods used have not been applied to CM before.

From a clinical point of view, the majority of CM are thin limbal melanomas that are associated with good prognosis unless the tumor recurs. Meticulous treatment of the primary CM and close follow-up thereafter are warranted. It is very important to have the tumor removed with sufficient tumor free margins assessed by histopathologic examination. Cryocoagulation of the conjunctival margins and topical MMC are probably useful adjuvant treatments. Some studies suggest that radiation therapy is also effective in preventing recurrences, but this form of treatment seems to be associated with greater ocular complications compared to topical MMC. Well planned studies are needed especially to assess the effectiveness of MMC in preventing recurrences.

According to my analysis, it is unlikely that a patient with a primary CM that is less than 2 mm in thickness, limbal, or both would have locoregional or systemic metastases at the
Discussion

time of the diagnosis. Thus, SLNB would be unlikely to materially improve the prognosis of these patients.

When the primary CM is nonlimbal or thicker than 2 mm, the prognosis is worse and it can be assumed that SLNB would be positive in 20% to 40% of these patients. We consider these patients, probably along with patients with recurring CM, the best candidates for SLNB, at least until the survival benefit from the procedure is shown by future studies.
ACKNOWLEDGEMENTS

This study was carried out at the Department of Ophthalmology, Helsinki University Central Hospital, Helsinki, Finland during the years 1999-2008.

I am sincerely grateful to Professor Leila Laatikainen, MD, head of the Department of Ophthalmology during the first years of this study, for providing good research facilities and for her encouragement both in scientific and clinical work.

I am very grateful to Professor Ahti Tarkkanen, predecessor of Professor Laatikainen, for his continuous interest and support of my work. Without the database of the Ophthalmic Pathology Laboratory of the Department, founded by Professor Tarkkanen in 1962, this study would have never been possible. I also sincerely want to thank Docent Lauri Merenmies, MD, for his friendly support and advice, based on his long experience working at the Department of Ophthalmology, and its pathology laboratory.

My deepest gratitude is owed to my excellent supervisor, Professor Tero Kivelä, MD. His knowledge of ophthalmology, and dedication to ophthalmic research is incomparable. Without his admirable commitment and inspiring guidance this work would have never been possible. His patience with my work, and especially with me, during the countless hours we worked together was incredible. You truly could not wish for a better supervisor in any terms. Professor Kivelä is also thanked, as the current head of the Department of Ophthalmology, for providing good research facilities for this study.

I am very grateful to Docent Paula Summanen, MD, for her kind support and interest in my study over the years, and for so kindly teaching me clinical ophthalmology at the Diabetes Service of the Department during the past one and a half years. Evelyn Lumiste, MD, and the whole staff of the Diabetes Service are likewise thanked for their support and cooperation in everyday work.

I wish to thank my friends and colleagues in the Ocular Oncology Group. I started my melanoma research greatly encouraged by Teemu Mäkitie, MD, who was finishing his excellent thesis on uveal melanoma while I was beginning my career. Sebastian Eskelin, MD, and Ilkka Puusaari, MD, are thanked, as senior members of the group, for sharing their experience and advice. Likewise, Päivi Toivonen, MD, Emma Kujala, MD, Ranaa Al Jamal, MD, Anna Korsbäck MD, and Mamunur Rashid, MD, are thanked for their support and for the good times shared in work and out of work. Päivi Toivonen, MD, and Ranaa Al Jamal MD are also warmly thanked for participating in the histopathologic analysis of this thesis. My good friend Ranaa also deserves a special mention for telling the funniest jokes at the lab.
Acknowledgements

I also want to thank the investigators from the other research groups at the Department, for creating such a scientifically inspiring atmosphere that has been a privilege to work in. Especially, Waldir Neira, MD, from the Cornea Group and Sanna Seitsonen, MD, from the Retina Group, are thanked for their valuable advice during the last months of writing the summary. I also want to thank Ms. Sirkka Elomaa, Ms. Riitta Heino, Ms. Marjatta Koikkalainen, and Ms. Seija Lehtonen for their assistance and technical expertise during this study. Shannon Kuismanen, Ph.D, is gratefully acknowledged for her thorough checking of the English language of this text.

I further wish to thank all the colleagues, past and present, at the Department of Ophthalmology for making this hospital such an outstanding place to work. Likewise, I want to thank all the colleagues and other members of the staff of the other hospitals I have worked at over the years.

I also want to warmly thank my friends outside the medical profession for their long lasting friendship, and the fun times we have had over the years, and will hopefully have in the future.

I am grateful to my in-laws, Leena Wilska, LLM, and Ikka Räisänen, MD, for their support and the kind help they have always offered when needed.

My two brothers, Tapani and Jouni, and sister Saila, and their families are thanked for their support and friendship.

My parents, Paavo and Ritva, deserve my deepest gratitude for their unquestionable support and encouragement throughout my life. My father Paavo Tuomaala, MD, is also thanked for introducing me first to the world of ophthalmology. Without his example I might, as was my original career plan after high school, now be working as an employee of a Finnish consulate on some warm Caribbean island. That, thinking it over carefully, would probably not have been such a bad choice either.

My mother, Ritva Tuomaala, MD, is thanked for having always been available for advice when needed, in good and not so good moments of my life.

I wish to thank my beloved wife, Rosi, for her love, and patience, especially during the last few months of writing. She has admirably taken care of all the practical matters at home while I have been absent too much. Without her understanding and support this thesis would have never been finished.

Finally, I thank my lovely daughter Alma, who will be celebrating her second birthday at the same day as I will be presenting this thesis, for bringing so much happiness to our lives. She really is the sunshine of my life.
This work was supported by the Helsinki University Central Hospital Research Fund (TYH 1217, TYH 3203, TYH 5210), The Friends of The Blind, The Finnish Cultural Foundation, The Sigrid Jusélius Foundation, The Eye Foundation, The Eye and Tissue Bank Foundation, The Orion Research Foundation, The Paulo Foundation, The Instrumentarium Research Foundation, The Evald and Hilda Nissi Foundation, The Jalmari and Rauha Ahokas Foundation, and The Finnish Medical Foundation (Duodecim), Finland

Helsinki, February, 2008


References


References


References


References


References


