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## **New developments in analysis of ocular surface diseases|Nieuwe ontwikkelingen in analyse van ziekten van het oogoppervlak**

Keijsers, S.

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## **PART II**

### **PIGMENTED CONJUNCTIVAL LESIONS**



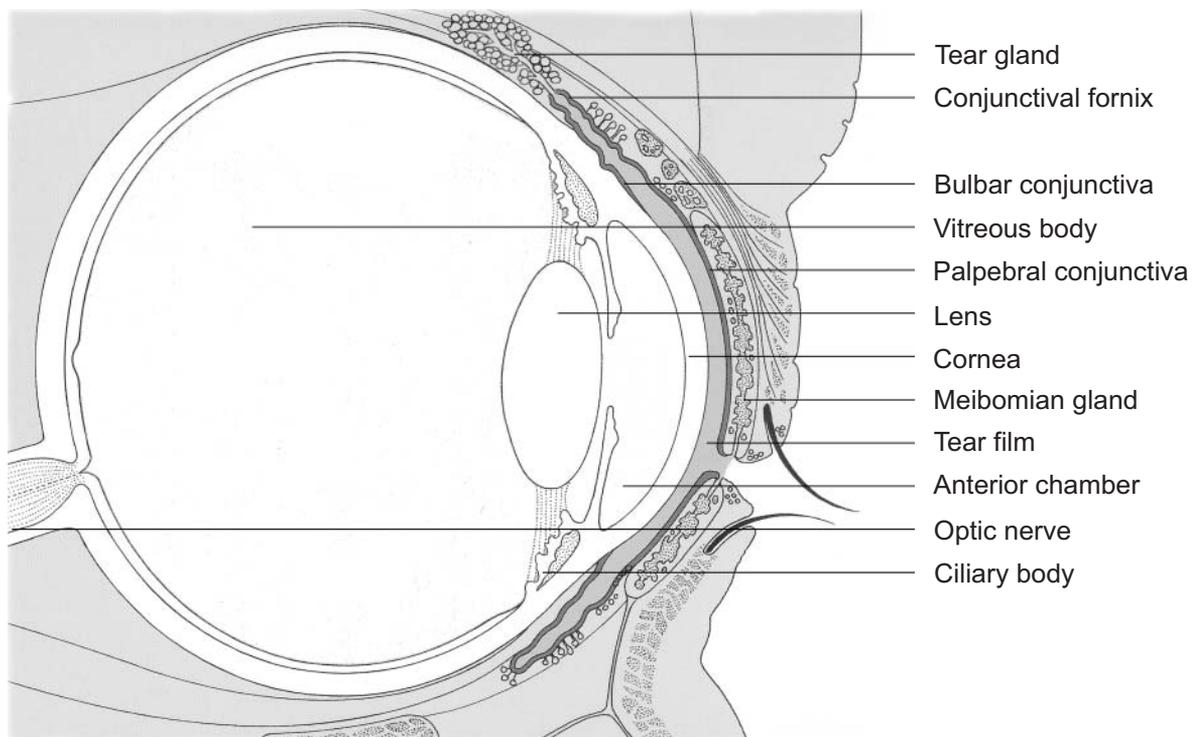
# INTRODUCTION

## INTRODUCTION

Conjunctival melanoma is the most potentially fatal pigmented lesion of the conjunctiva; it is a malignant lesion with a 30 % mortality in 10 years.<sup>1</sup> It is difficult but crucial to distinguish the malignant conjunctival melanoma from other benign melanocytic conjunctival lesions such as conjunctival nevi and conjunctival melanosis. Although it is a very rare disease, 0.2-0.8 cases per 1.000.000 inhabitants per year,<sup>1,2,3</sup> every ophthalmologist must be aware of this tumour. In the Netherlands, an average of about eight conjunctival melanomas can be diagnosed each year. The clinical presentation of conjunctival melanocytic lesions can help to distinguish between a nevus or primary acquired melanosis and a conjunctival melanoma, although it remains difficult to differentiate between them. Cytological and histological examinations are essential investigations to differentiate between these entities.

## ANATOMY OF THE CONJUNCTIVA

The most outer parts of the eye are the cornea and the conjunctiva. The cornea is a transparent avascular tissue, while the conjunctiva is opaque and vascularized. The bulbar conjunctiva is a free movable protective mucous membrane that covers the sclera and is continuous with the inner layer of the eyelids (palpebral conjunctiva). The conjunctival fornix is the junction of the bulbar and palpebral conjunctiva, thereby sealing off the orbital cavity from the outside environment (Figure 1). The circular zone where the conjunctiva



**Figure 1.** The anatomical structures of the human eye.

ends and cornea begins is the limbal region, where the corneal epithelial stem cells have their residence (see Part I). The conjunctiva consists of two or more layers of stratified columnar epithelial cells. Scattered among the basal cells on the basement membrane melanocytes are present, which stretch out their spidery offshoots between the epithelial cells. Also between the epithelial cells, goblet cells are present which produce mucous. Accessory tear glands are present in the subepithelial stroma of the conjunctiva and they continuously produce basal tears. In the tarsal plates of both the upper and lower eyelid meibomian glands produce lipids that prevent the evaporation of tears from the ocular surface. Lymphatic channels are present throughout the conjunctival stroma; the medial part of the conjunctival lymphatic vessels drain to the submandibular lymph nodes while the lateral part of the conjunctiva drains to the preauricular lymph nodes.<sup>4</sup>

Two special anatomical structures must also be mentioned, the plica semilunaris and the caruncle. The plica semilunaris is a conjunctival fold in the medial portion of the bulbar conjunctiva. The plica is thought to represent a remnant of the nictitating membrane that is found in many vertebrate species.<sup>5</sup> The caruncle is situated at the medial canthus, between the upper and lower tear ducts. It contains both characteristics of conjunctival and cutaneous tissue i.e. sebaceous glands and hair follicles.

During embryonic development, melanocytes migrate from the neural crest<sup>6</sup> to the basal layers of the conjunctival epithelium or to the area just underneath the basement membrane. Racial melanosis, primary acquired melanosis, conjunctival nevus, and conjunctival melanoma all find their origin in these melanocytes.

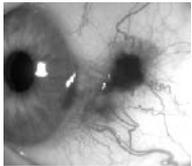
## NEVI

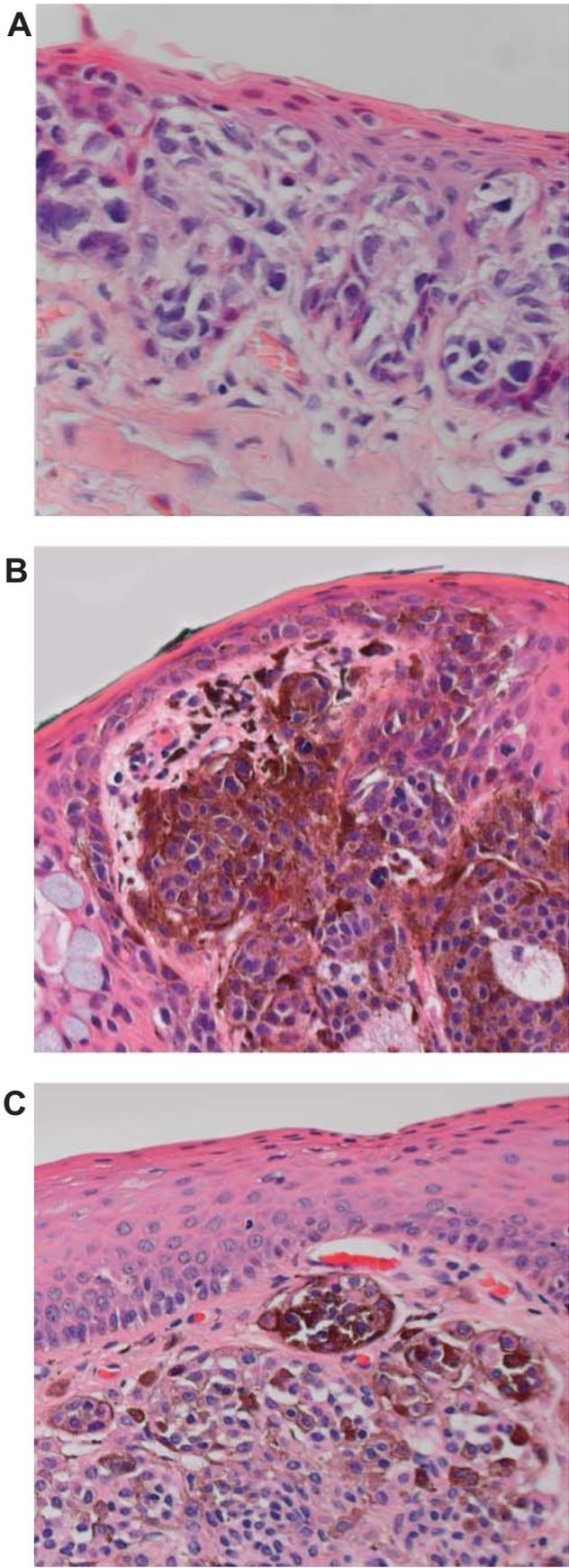
Conjunctival nevi can either be congenital or acquired. The acquired nevus is the most common conjunctival nevus and can be subdivided into junctional nevi, compound nevi, subepithelial nevi, and Spitz nevi. Where the congenital nevus presents itself during the first six months of life, the acquired nevi usually develop during the first or second decade. A nevus starts to develop as a benign proliferation of nevus cells (plump melanocytes without the spidery extensions) at the junction of epithelium and subepithelium (junctional nevus), and usually forms nests.<sup>7</sup> The junctional nevus is a clinically flat lesion since the nevus cells are confined to the epithelium, and are only seen in children.<sup>7</sup>

The nevus cells of a junctional nevus can descend to the subepithelial layers (superficial substantia propria) in a short period of time. The nevus cells frequently drag epithelial surface cells and goblet cells down to the subepithelial layer, where they can develop into inclusion cysts, which may enlarge gradually. The nevus is now a compound nevus, nevus cells are present at the epithelium and in the substantia propria.<sup>7</sup> Clinically, the lesion thickens and inclusion cysts can be seen with a slit lamp (Figure 3).

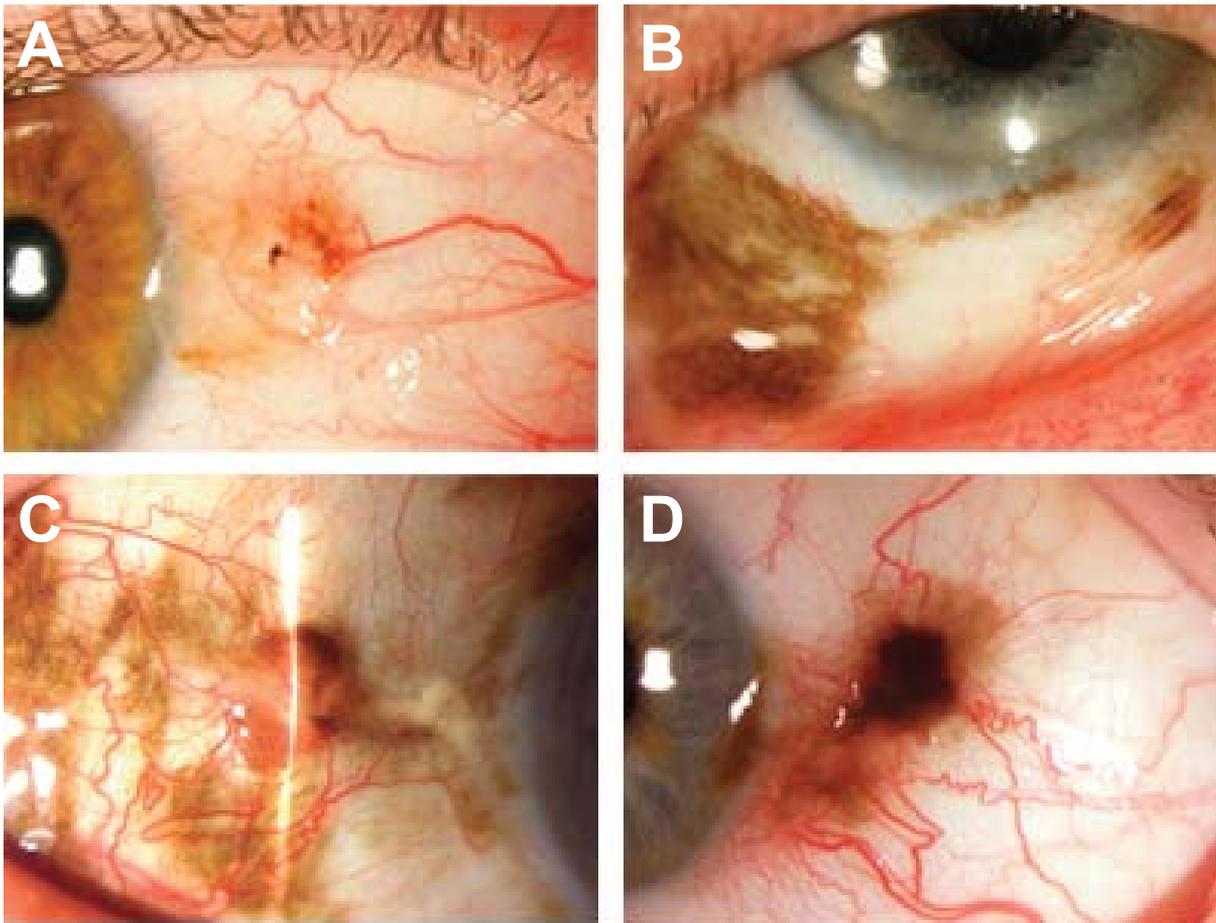
Over time the epithelial component of the junctional nevus may disappear, leaving only the subepithelial part of the nevus separated from the epithelium by a small band of fibrotic stroma.<sup>7</sup> Hence, the nevus is now called a subepithelial nevus, and is usually clinically elevated and may contain epithelial cysts.

Conjunctival Spitz nevi are rapidly growing nevi, appear solely during childhood, and are very rare.<sup>8</sup> The rapid growth makes these lesions very suspect for conjunctival melanoma.





**Figure 2.** Histological pictures of the three types of conjunctival nevi; **A**, junctional nevus; **B**, compound nevus; and **C**, subepithelial nevus.



**Figure 3.** Clinical examples of a conjunctival nevus (fig. 3A), PAM (fig. 3B), PAM with a conjunctival melanoma (fig. 3C), and conjunctival melanoma (fig. 3D).

Also histologically differentiation of a conjunctival Spitz nevus from a conjunctival melanoma is difficult, and even mitotic figures can be found.<sup>7</sup> However, the conjunctival Spitz nevus is strictly benign and used to be called “benign juvenile melanoma”, which is a confusing term since a melanoma cannot be benign.

The conjunctival nevus is a benign tumour that very rarely develops into a malignant conjunctival melanoma, i.e. less than one percent.<sup>9,10</sup> Epithelial cysts are an important clinical feature that usually indicates the benign character of a melanocytic lesion. Other important features are the free mobility of the lesion over the sclera and the localisation; nevi are usually situated at the interpalpebral bulbar conjunctiva near the limbus. Lesions in the fornix or the palpebral conjunctiva are suspicious of primary acquired melanosis (PAM) or conjunctival melanoma.

The management of an acquired conjunctival nevus consists of photographic documentation and regular follow up. Growth of a nevus can be a sign for malignant transformation, however, during puberty the lesions may acquire more pigment and can grow under the influence of hormones. The increase in pigmentation, enlargement of the intralesional cysts or inflammation of a nevus may simulate growth, increasing the suspicion of a melanoma, but is mostly restricted to childhood and adolescence. When additional information is required, cytological or histological biopsies can be performed to assess the risk of malignant transformation.

## MELANOSIS

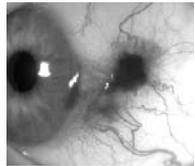
Conjunctival melanosis can be divided in two forms: racial melanosis and primary acquired melanosis (PAM) (Figure 3). Racial melanosis is very common in dark-skinned individuals, and generally affects the limbus of both eyes.<sup>7</sup> It is a benign condition that rarely develops into a conjunctival melanoma; histologically there is an increase in melanin production in spidery melanocytes without atypical characteristic. These rarely transform into melanocytes with atypia of the nucleus. Atypia of the melanocytes is characterized by abnormalities in nuclear size, nuclear shape, nuclear-cytoplasmic ratio, chromatin, nuclear membrane, nucleoli, or melanin.

PAM is an acquired melanosis, usually unilaterally in fair-skinned middle-aged individuals. Other terms for PAM used to be benign acquired melanosis, precancerous melanosis, idiopathic acquired melanosis, or intraepithelial atypical melanocytic hyperplasia. To promote a uniform nomenclature the World Health Organisation (WHO) introduced the term primary acquired melanosis. Clinically the pigmentation in PAM appears brown, diffuse, patchy, flat, and noncystic. PAM can wax and wane over time, i.e. extend and diminish in size within the epithelium, without any tumefaction.<sup>11</sup> They are mostly located at the limbal region, but may extend to the cornea and surrounding bulbar conjunctiva, fornix, and palpebral conjunctiva. Histologically, PAM is an intraepithelial proliferation of plump melanocytes (naevus cells) with or without atypia of the nucleus. The proliferation can be present in little nests in the basal layer of the conjunctival epithelium, or in a more linear increase in number of naevoid melanocytes. The atypia can be classified as mild, moderate or severe. This is important to estimate since PAM with atypia has a 50% chance to develop into a conjunctival melanoma.<sup>11,12</sup> Melanoma development in PAM without atypia or with mild atypia is very

rare.<sup>12</sup> PAM lesions that become elevated, or elevated areas within a larger patch of flat pigmentation, or increased vascularization are suspect of the development of a conjunctival melanoma, and must be treated accordingly. Frequently the tumour is unpigmented or of another hue than the original PAM. Unpigmented PAM exists, but is fortunately rare (PAM sine pigmento)

The management of PAM without atypia or with mild atypia consists of regular clinical and photographic follow up and, when indicated, cytological examination or biopsy to assess the atypia. However PAM can be a very extensive disease, and can require many biopsies over time, that can cause damage to the conjunctiva and sometimes limbus. Since cytology is a non-invasive method to assess the atypia, it is an alternative that has to be considered (Chapter 4 and 5). PAM with moderate to severe atypia can be either treated with mitomycin C, cryotherapy, or excision, depending on the localisation and extend of the lesion. PAM is notorious for its multiple recurrences despite adequate treatment; regular follow up is therefore a necessity.

Ocular melanocytosis can be confused with melanosis of the conjunctiva. However, ocular melanocytosis is part of the oculodermal melanocytosis (“nevus of Ota”), a congenital pigmentary skin disorder which may include the periocular skin, (epi-)sclera, uvea, orbit, meninges and soft palate. The pigmentation in PAM is located in the conjunctiva and directly subepithelial and is acquired. The pigmentation in ocular melanocytosis is located in the epi-sclera, and usually the conjunctiva is remarkable unpigmented; it is congenital, but may increase in pigmentation and extension with increasing age. Patients with ocular melanocytosis have an increased risk of uveal melanoma but not of conjunctival melanoma.<sup>13</sup>



## CONJUNCTIVAL MELANOMA

As has been mentioned in the introduction, conjunctival melanoma is a rare disease, which has the potential to metastasise and to cause metastatic death. Conjunctival melanoma usually arises from PAM, but it can also arise from conjunctival nevi or develop de novo.<sup>1,14,15,16,17,18,2,3</sup> Conjunctival melanoma with surrounding PAM with atypia is seen in approximately 70% of the conjunctival melanomas, indicating that, indeed, PAM with atypia is the most common precursor lesion of conjunctival melanoma.<sup>19</sup> It typically arises around the age of 60,<sup>20,2</sup> and can be located anywhere on the conjunctiva, but mostly in the bulbar conjunctiva near the limbus (Figure 3). It is a disease that primarily affects the Caucasian population, it is very uncommon in the African or Asian population, with a relative risk ratio of at least 8:1.<sup>21,22,23,24</sup> Clinically, conjunctival melanoma is characterized by horizontal and vertical growth with prominent feeder vessels. The pigmentation can vary from unpigmented to brown or black. Exfoliation and impression cytology shows severe atypical melanocytes.

## AETIOLOGY OF CONJUNCTIVAL MELANOMA

The aetiology of the conjunctival melanoma is not clear. Ultraviolet radiation has been mentioned to be involved in the development of conjunctival melanomas, since patients with xeroderma pigmentosum develop PAM with atypia and conjunctival melanoma more frequently.<sup>25,26,27</sup> Patients with xeroderma pigmentosum are prone to develop a variety of epithelial and melanocytic ultraviolet radiation-inducible tumours. In addition, many of the

conjunctival melanomas are found in the sun-exposed areas of the conjunctiva, although melanomas are also found in the fornix and the palpebral conjunctiva. In skin melanoma, the importance of ultraviolet radiation has been established; however, it is better to see conjunctival melanoma and skin melanoma as different entities, since the conjunctiva is a mucosa with considerably different anatomy and function. Although there was a major increase in skin melanoma in Denmark presumably due to increase in ultraviolet radiation, no increase in conjunctiva melanoma was seen.<sup>28</sup> Others claim an increase in conjunctival melanoma similar to the increase in skin melanoma.<sup>29,30</sup> So, there is no solid evidence that ultraviolet radiation is involved in the aetiology of conjunctival melanoma.

### **DIFFERENTIAL DIAGNOSIS OF CONJUNCTIVAL MELANOMA**

A conjunctival melanoma must be differentiated from other conjunctival melanocytic lesions such as a conjunctival nevi, racial melanosis, and PAM, but also from other melanocytic lesions that can be situated in deeper layers. Pigmented episcleral spots or Axenfeld's nerve loops are uveal melanocytes that have migrated to the episclera via either a perforating ciliary vessel or an intrascleral nerve loop. The spots may be painful and are usually located 3-4 mm behind the limbus. Ocular melanocytosis is also a melanocytic lesion and has been discussed previously. Tumour outgrowth of a ciliary body melanoma through the sclera can mimic a conjunctival melanoma,<sup>31</sup> but ultrasound biomicroscopy and ophthalmic examination can be helpful to differentiate between the two. Although it is very rare, a conjunctival metastasis of a skin melanoma must be included in the differential diagnosis.<sup>32</sup> Non-melanocytic lesions that can be mistaken for a conjunctival melanoma are pigmented squamous cell carcinoma, pterygium, pingueculum, Moll gland cystadenoma, apocrine adenocarcinoma, mascara, silver depositions, haemorrhage, and gunpowder.<sup>1,33</sup> For the clinical differentiation between melanin pigmentation or pigmentations of other origins, a UV-lamp can be used. For the differentiation of brown(ish) pigment in tissues, several histological stains are available. The lack of pigmentation in an amelanotic conjunctival melanoma makes the clinical diagnosis even harder.

### **CYTOLOGY**

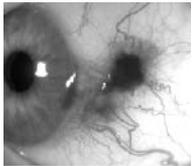
The Leiden University Medical Center has more than 25 years of experience with ocular cytology, especially with melanocytic lesions. It is one of our essential examinations for pigmented conjunctival lesions, however, there is not much literature on that subject. A grading system for melanocytic atypia in cytological samples is used in our clinic (Chapter 4). The primary objective for cytology in pigmented conjunctival lesions is the detection of conjunctival melanoma and the follow up of PAM and nevoid lesions. Exfoliative cytology and impression cytology are two different techniques to harvest cells for cytological examination. Exfoliative cytology can be performed through conjunctival scrapings<sup>34,35</sup> or by use of cotton wool swabs.<sup>36</sup> Impression cytology is either done with cellulose acetate filters<sup>37</sup> or with the Biopore membrane.<sup>38</sup> Advantages and disadvantages of both exfoliative and Biopore techniques are further considered in Chapter 5. Cytology can be helpful in differentiating PAM and nevoid lesions from non-melanocytic lesions and it can help to estimate the risk for melanoma development. For PAM lesions it is particularly helpful to know whether and

when a lesion without atypia turns to a lesion with atypia, or when the atypia further increases from mild to moderate or severe. Especially severe atypia is highly associated with the presence of a conjunctival melanoma (Chapter 4). However, cytology only assesses the superficial layers and the atypia of the sampled cells, and cannot differentiate between PAM with moderate/severe atypia and frank invasive melanoma.

An alternative for the cytological examination is a biopsy. A biopsy can reliably diagnose lesions as nevus, PAM, or melanoma, it can also further classify the grade of atypia within the melanocytic proliferation. However, a biopsy is an invasive method that cannot be performed endlessly on an eye with recurrent PAM lesions, while cytology is non-invasive, patient friendly and can be repeated many times. Both histological and cytological methods are essential tools for ocular oncology centres.

## HISTOLOGY

Histological material from melanocytic conjunctival lesions can be obtained by incisional or excisional biopsies. Incisional biopsies are primarily performed on large lesions that are difficult to remove in toto. However, an excisional biopsy is preferred since the lesion can be removed together with the acquired margins, thereby minimalizing the chance of recurrence of the lesion.<sup>16</sup> According to Shields, suspect bulbar lesions that occupy less than 4 clock hours or are less than 15 mm in diameter are suitable for excision.<sup>33</sup> Extreme care in handling the excised lesions will guarantee better material for the (ophthalmic) pathologist to estimate useful details such as margins of excision.



## CONJUNCTIVAL MELANOMA TREATMENT

The treatment of conjunctival melanoma has changed over time, from radical orbital exenteration to local irradiation or excision. Nowadays, the primary treatment for a conjunctival melanoma is local excision with a 3-5 mm free conjunctival margin,<sup>39</sup> thereby avoiding manipulation of the tumour, and with adjuvant therapy as brachytherapy or cryocoagulation.<sup>39,40,41,42,43</sup> Because conjunctival melanoma cells easily seed when manipulated, local recurrences may be reduced by applying absolute alcohol or Dakin's solution to devitalize any residual atypical melanocytes.<sup>44,45</sup> Excision of larger conjunctival melanomas can leave conjunctival defects that are too large for primary closure, mucosal grafts from the mouth or contralateral eye or amnion membranes can be used. When the tumour is not suitable for local excision, i.e. unfavourable location, very large tumours, or multifocal tumours, proton beam irradiation may be considered.<sup>47</sup> For very large conjunctival melanomas especially with extension into the orbit, orbital exenteration is often the only solution.<sup>48</sup> In large series, the number of exenterations is still uncomfortably high in these advanced cases.<sup>20</sup> Exenteration should only be used as a palliative treatment for no beneficial effect has been found of exenteration on survival.<sup>46</sup>

## RECURRENCES OF CONJUNCTIVAL MELANOMA

Despite complete microsurgical excision, local recurrences of conjunctival melanoma are

often seen, which can be either new primary tumour formation from remaining or recurrent PAM, from remaining melanoma due to incomplete removal or local spread due to surgery, or an in-transit metastasis in a lymphatic vessel. Five years after treatment, 26% - 46% of the patients have a local recurrence, increasing to 51% at 10 years.<sup>49,50,20</sup>

## **METASTASIS OF CONJUNCTIVAL MELANOMA**

Because of the existence of lymphatic vessels in the conjunctiva, conjunctival melanomas can spread to the regional lymph nodes (preauricular, submandibular, and cervical lymph nodes). In 65% of the patients with a distant metastasis also had regional lymph node metastases.<sup>51</sup> Haematogenous metastasis can be located in the lung, liver, brain, bone marrow, spleen, and gastrointestinal tract.<sup>17,52</sup> Because the lymph nodes can be the first step towards further spread of the conjunctival melanoma<sup>17,53,52</sup> and has a better prognosis than distant metastasis,<sup>54</sup> use of the sentinel node biopsy has recently been suggested. Good survival chances have been achieved with radical excision of the metastatic lymph nodes,<sup>53</sup> whether removal of the sentinel node also leads to longer survival is not known.

## **RISK FACTORS**

Patients with a conjunctival melanoma have a survival chance of 74% - 93% at 5 years, decreasing to 41% - 87% at 10 years.<sup>55,56,14,16,17,3,49</sup> There are several risk factors that decrease the chance of survival. First of all, tumour thickness is an important risk factor:<sup>57,43,49,58</sup> patients with a tumour thickness of 0.8 mm or more have a lower chance of survival.<sup>49</sup> A worse prognosis is also seen when the conjunctival melanoma is located in the fornix, palpebral conjunctiva, plica, or the caruncle.<sup>57,43,49,58</sup> Other risk factors can be multifocal tumours, orbital invasion, and recurrent disease. Histopathological risk factors are a high mitotic rate, mixed cell tumours, and a pagetoid growth pattern.<sup>49</sup> Whether the origin of the conjunctival melanoma (PAM, nevus, or de novo) is a risk factor for tumour related death is a topic of discussion and may vary with the number of cases of the study.<sup>57,43,16,49,59,53,41</sup>

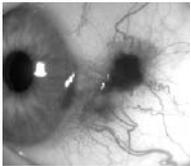
The local recurrence rate of conjunctival melanomas is influenced by incomplete removal of the lesion (i.e. positive margins), tumour thickness, and type of treatment.<sup>60,43,16</sup> Epithelioid cell type and mixed cell type conjunctival melanomas also have a higher rate of recurrence.<sup>43</sup> In Chapter 3 the risk factors for recurrences, metastasis and tumour related mortality for the Dutch population are further discussed.

## **MARKERS**

Histological markers for conjunctival melanomas could be helpful to differentiate a conjunctival nevus or PAM from a conjunctival melanoma. HMB 45, Melan A, and S100 have been used to identify conjunctival melanomas; however these markers only prove the melanocytic origin of the tumour and cannot differentiate between benign and malignant melanocytic lesions.<sup>57,60,61,55,62,63,64</sup>

S100 is a calcium-binding protein and involved in establishing the malignant and metastatic phenotype of various tumours.<sup>65,66,67</sup> The S100 protein family consists of over 20 members. The expression of S100A1, S100A2, S100A3, S100A4, S100A6, and S100B has been stud-

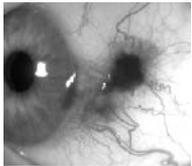
ied previously in cutaneous melanoma.<sup>68,69,70,71,72</sup> Serum S100B has proved its value as a marker for metastasis in skin melanoma, and is of prognostic importance.<sup>70,73</sup> Furthermore, in histological sections S100A6 seems of some use in the distinction between a Spitz-nevus and a cutaneous melanoma.<sup>72</sup> For conjunctival melanoma the research for markers has been limited by the rarity of the tumor, hence material is scarce. In Chapter 6 we will discuss the search for histological markers for conjunctival melanoma.



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