



Bone metastasis in a case of primary acquired melanosis with atypia resulting from conjunctiva melanoma

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ABSTRACT

A 66-year-old woman presented to a tertiary referral centre with a diagnosis of limbal stem cell deficiency secondary to multiple treatments for primary acquired melanosis (PAM) by her referring ophthalmologists. She was known with primary acquired melanosis with severe atypia of the right eye which had been treated with several excisions, cryotherapy and topical chemotherapy. She had PAM over 360° of alternating intensity. Throughout the follow-up period, she developed a cataract and subsequent a retinal detachment for which she underwent a phacovitrectomy on the right eye. There was no nodule present nor did the routine lymph nodes inspection show any enlargement. No abnormalities were observed on intranasal examination. Seven years after the last excision, a metastasis to the spine (Th3) was diagnosed. A complete systemic work-up and a pathological examination did not show any alternative primary melanoma in the body. The original histological material was reviewed again by an ophthalmopathologist. After a new round of stains and immunohistochemistry, the presence of atypical melanocytes in both the epithelium and stroma were found in a scar region leading to a renewed conclusion that the PAM in question was actually the primary melanoma.

1. Introduction

Primary acquired melanosis (PAM) is a benign but premalignant pigmented conjunctival condition that can occur in middle-aged individuals. It presents as a painless, flat, non-cystic pigmented lesion that can involve the conjunctiva, cornea, and eyelids and occurs mostly in the Caucasian population¹ In general, PAM accounts for 11% of all conjunctival lesions^{1–3}

In current practice, there is still much debate on the preferred treatment of this condition. Most centres base the treatment on the extent of pigmentation and its evolution.¹ In smaller lesions, observation, excision with cryotherapy, or topical treatment with mitomycin C are all viable options. In larger lesions, i.e. more than 3 clock hours, wide incisional biopsy and cryotherapy is advised.^{1,4,5} While some centres advise a radical approach to these pigmented lesions, others suggest a conservative approach as the size and pigmentation of PAM may 'wax

and wane'.⁵ Multiple surgeries and mitomycin C, however, can lead to excess conjunctival and corneal damage, scarring, discomfort, restricted eye movement and reduced vision.⁵

It is important to procure histological data from the pigmented lesions. In some centres, impression cytology diagnostic procedures are preferred as this can prevent the radical biopsies and the aforementioned ocular complications.⁶ However, impression cytology cannot distinguish between PAM with atypia and melanoma. Therefore, mapping biopsies should be performed when an atypical or malignant appearance is found in the cytological evaluation. A missed diagnosis of an invasive melanoma is associated with increased morbidity and mortality which can be devastating to the patient. PAM can be classified, based on histopathology, as Conjunctival Intraepithelial Neoplasia (C-MIN), formerly known as PAM with or without atypia. The degree of atypia can be graded on a scale of 1–10 according to a system described in the WHO classification of tumors of the eye.⁷ This is particularly

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importance as PAM with atypia (C-MIN score 2 or higher) can progress to a conjunctival melanoma. Up to 75% of all conjunctival melanomas arise from PAM with atypia. The chance of progression to melanoma from PAM with mild to no atypia is considered 0%, while it is nearly 50% in PAM with severe atypia.⁴ C-MIN scores of 5 and higher are viewed by some as melanoma in situ⁸ which in 50% of the cases may develop invasion in the stroma and thus to invasive melanoma.⁸

2. Case report

A 66-year-old female presented to her own ophthalmologist in 2007 with a red right eye (RE) together with bilateral blepharitis and was referred to a tertiary ophthalmic centre in the Netherlands for dry eye treatment. Treatment with lubricants for the dry eyes were prescribed and a bandage contact lens was used to help ease pain. At that time, the temporal cornea of the RE was found to be thinned.

At this visit, small mild limbal pigmentation of the right eye was noted. In 2008, the pigmentation was slightly progressive, and an excision of the melanosis RE from 3 to 9 o'clock which was complicated by corneal erosion. Histopathology demonstrated PAM with mild to moderate atypia associated with pterygium. Postoperatively, she was treated with several sessions of topical MMC 0.02% (5 times a day for one week and two sessions and three months later again two sessions of MMC 0.02%). Thereafter, for the pain associated with recurrent erosions and dry eye, she wore soft bandage contact lenses.

Two years later, progression with a secondary pterygium was seen and two excisional biopsies were performed. She was treated by resection of the melanocytic tissue and the pterygium with a conjunctivoplasty. During the surgery, cryotherapy was applied to the margins of the. The histopathology of one biopsy demonstrated recurrence of the PAM with mild to moderate atypia. The second biopsy showed severe atypia without invasive growth. She received further treatment with topical mitomycin C 0.02% (three separate sessions) together with steroid drops. At this stage, the best visual acuity of her right eye was between 0.6 and 0.8 decimal Snellen.

Unrelated to the PAM she underwent a correction (levator plication) of the right ptotic upper eyelid in another institute. An external approach was used without manipulation of the conjunctiva.

A third recurrence occurred in 2012 and she was again treated with 2 sessions of topical mitomycin C 0.04% of 2 weeks each. She also underwent a new biopsy, without total resection, that was initially interpreted as PAM with severe atypia on top of scar tissue but no signs of an underlying melanoma. The ptosis recurred and was treated with again a levator plication.

In 2014, she was referred to the University Hospital Antwerp because of limbal stem cell deficiency, persistent epithelial erosions and pain that occurred after the multiple treatments with the topical chemotherapy. The VA RE was 0.4 decimal Snellen. She underwent had two limbal stem cell transplantations in 2015 and 2016 respectively while her PAM was quiet using the technique previously described.⁹ On her first visit, there she had PAM of the limbal conjunctiva, spanning 3 clock hours (12h–3h) of light pigmentation without any clinical evidence of an underlying melanoma. Between the two stem cell transplantations, the pigmentation displayed a “wax and wane” appearance, with some consultations showing a bit more pigmentation than other times. Just after her second transplantation the pigmentation seemed more diffuse over 360° reaching the inferior nasal fornix to the eyelid margin and on the limbal cornea. Complete slit lamp examination did not show any irregularities in the pigmentation nor did it show any manifestations of a conjunctival melanoma (shown in Fig. 2). An ultrasound sonography of the eye was negative for any intraocular malignancy. Because of her ocular history of multiple treatments and a diffuse pigmentation over the conjunctiva, observation of the PAM was chosen as the course of action.

Over the next 3 years she had mild PAM over 360° of alternating intensity. The PAM did not show any clinical progression nor did it show

any signs of a conjunctival melanoma. Throughout the follow up she developed cataract and a retinal detachment for which she underwent a phacovitrectomy with silicon oil on the right eye. The PAM was only lightly present on the right eye then. There was no nodule present nor did the routine clinical check-up of the lymph nodes or nose show any atypia. The evaluation of the lymph nodes is done by means of palpation with every visit and an annual ultrasound in cases of severe PAM or conjunctival melanoma. Inspection of the lacrimal ducts at the conjunctiva and intranasal were normal. No abnormality or pigmentary lesions were observed in the left eye for the entire duration of the follow up.

In 2018, she presented to the emergency department with a pathological fracture of the spine. She underwent surgery for the fracture and a biopsy was taken. On pathological examination, a melanoma to the spine (Th3) was diagnosed. Further investigation with abdominal sonography and PET scan showed metastasis of the melanoma to both adrenal glands and liver, which were all confirmed as melanomas on pathological examination. Genetic analysis of the spinal melanoma and on the biopsies of the abdominal metastases were negative for mutations in the BRAF, KIT, and NRAS genes. No primary tumor was found on initial inspection. Further dermatological investigation showed no primary melanoma on the skin or in the oral or nasal cavities.

At this time, the PAM remained stable with all previous examinations and even showed a lighter pigmentation without any lesions or nodules. The PAM was suspected as the primary site as no other location for a melanoma was found. An expert pathologist in the field, familiar with the original histological material, performed a re-examination of the last biopsies of 2010. He concluded after new investigations and with new cuts and immunohistochemical stains (both H&E and HMB45 stain) of the tissue, especially in the “scar tissue region” (Fig. 1) the presence of atypical melanocytes in both the epithelium and stroma (picture B,C,D). This meant that there was invasive melanoma, initially misinterpreted as scar tissue which could explain the late metastasis.

A treatment with immunotherapy, nivolumab, and radiation therapy to spine were initiated. A year later chemotherapy with paclitaxel and carboplatin was added. More metastases to the abdominal lymph nodes were found and the patient was placed in a palliative care treatment. She passed away in the beginning of 2020.

3. Discussion

Although classified as a premalignant condition, PAM with atypia (C-MIN) is potentially serious with its ability to progress to a conjunctival melanoma.^{4,10,11} This potential risk of metastatic disease is an important aspect of this condition physicians should be aware about. Ackerman et al. believed that PAM with atypia should be called melanoma-in-situ, similar to lentigo maligna of the skin, as the other nomenclature can undermine the risk of progression.¹² According to the more recently proposed C-MIN scoring system a C-MIN score of 5 and higher is also considered equal to melanoma in situ.⁸ Risk factors for progression were noted to be the severity of atypia as well as the extent, in clock hours, of the lesion. Shields et al. noted that the risk of progression to melanoma in mild to no atypia was 0%, while in severe atypia this reaches nearly 50%.¹ This was confirmed in a multicenter validation study of the C-MIN scoring system.¹³ Furthermore, involvement of the palpebral conjunctiva, fornix, caruncle or plica location, and corneal involvement were also found to have an increased risk in progression towards melanoma.^{2,3}

Patients with a conjunctival melanoma develop metastatic disease in 20–30% of cases.^{14,15} Regional metastases to the lymph nodes are the most prominent mode of metastatic disease in about 40–60%. These include the pre-auricular lymph nodes as the most common and the deeper cervical nodes as the second most common involvement. Further studies show that in 38% of the cases distant hematogenic organ metastases without detectable regional lymph node disease is possible. Although seen in clinical practice, bone metastasis as a primary

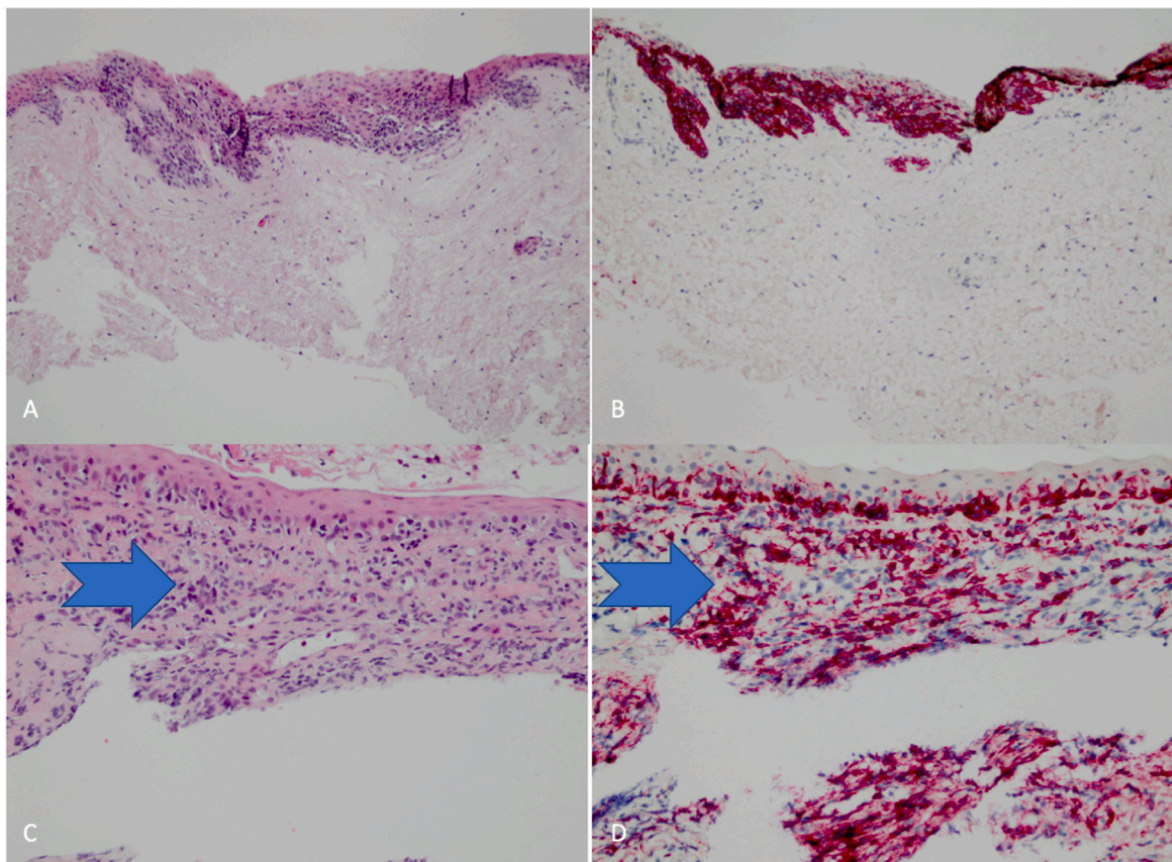


Fig. 1. Last biopsy of PAM with atypia after histopathological revision with new tissue analysis: A, a region of PAM with severe atypia showing intraepithelial nests and lentiginous spread of atypical melanocytes (Hematoxylin and eosin stain 100× amplification). B, immunohistochemistry accentuates intraepithelial ascension of melanocytes (HMB45 stain 100× amplification (B&D)). C, upon revision another region of PAM with atypia was identified showing atypical cells in the underlying stroma initially misinterpreted as scar tissue. (Hematoxylin and eosin stain 200× amplification). D, additional immunohistochemical staining proved these cells to be infiltrating melanoma (HMB45 stain 200× amplification).

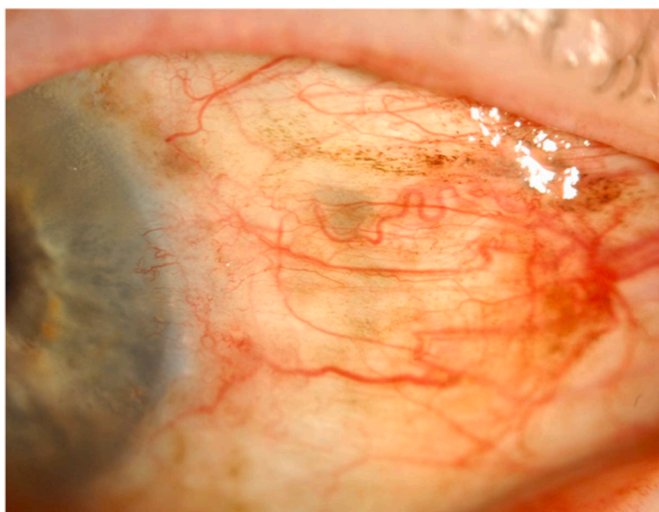


Fig. 2. Slit lamp examination of the right eye.

metastatic involvement is much rarer than liver, brain or pulmonary disease.¹⁶

The reason for distant metastasis in this case with PAM without clinical evidence of a melanoma can implicate three theories. *Firstly*, there is a possibility that even without clinical evidence, PAM can have small foci of melanoma present which can be misinterpreted on

histopathological examination. For this reason, in the upcoming WHO 5th edition immunohistochemistry with melanocytic markers is included as a desirable diagnostic criterium. The melanomas are only detectable on a cellular level so that on a slit lamp examination no abnormalities can be seen but may histopathologically be seen as micro-invasion. These small foci can easily be missed on different examinations and warrant an extremely close inspection of the specimen, with additional immunohistochemistry, to rule out any signs of melanoma. In our case, two experienced ophthalmic pathologists, experts in the field, had investigated the material. This was the case in our patient, where only signs of invasive melanoma were found after a revision was performed with step sections and immunohistochemistry by an experienced ophthalmopathologist. It is therefore that we strongly advised to maintain a low threshold for additional immunohistochemical investigations of the tissue for invasive melanoma by pathologists, especially in cases of repeat operations and scar tissue. Molecular and epigenetic investigations using recently developed microRNA also have an added benefit in distinguishing benign from malignant lesions in clinically suspected cases without a conclusive pathological investigation.¹⁷ BRAF, NRAS, NF1 and c-Kit mutations have been described as recurrent driver mutations in conjunctival nevi as well as in melanoma.¹⁸ Additional mutations in the TERT promoter and ATRX may be indicate progression to melanoma.^{19,20} TERT promoter mutations have been associated with metastatic disease in conjunctival melanoma.¹⁹ Moreover, TERT promoter mutations have been described in PAM with moderate and severe atypia, underlining their potential as melanoma in situ lesions.²¹ In the current case no mutations have been detected in the metastatic tumor, offering no targeted therapeutic options.

Secondly, there has always been a strong argument for a careful surgical approach for conjunctival lesions and neoplasia, more so for pigmented tumors. A no touch technique of resection is important as to not inadvertently seed tumor cells to adjacent healthy tissue.⁴ As an additional note there is a third possibility, that large surgeries such as a limbal stem cell transplantations significantly manipulate the conjunctiva such that malignant cells can be inadvertently disseminated hematologically in an iatrogenic matter. It has also been reported that an incisional technique in melanocytic tumors can increase the risk for numerous tumor recurrences.⁴ It may very well be possible in this case that because of the extent of the pigmentation and numerous surgeries this patient underwent, such as the stem cell transplantations and phacovitrectomy, an iatrogenic seeding with distant metastases occurred. For this reason, we would also advise caution for multiple surgeries in large pigmented conjunctival areas.

On the other hand, should multiple surgeries be necessary, appropriate measurements should be taken to minimize the aforementioned risks. Although the risk of local and hematogenic dissemination is very rare, as presented in this case report, one should consider - for the future - to perform preventive application of alcohol during surgery in severe PAM/melanoma in situ as is done during surgery of conjunctival melanoma.⁴

It is also important to understand the danger of wax and wanes in PAM as this may indicate the loss of pigmentation but not the vanishing of the PAM. This can also be the case when MMC sessions were done. So, in principle after the MMC treatment one should consider mapping biopsies to demonstrate the eradication of the PAM with atypia.²² This is important to as non-pigmented PAM can also give rise to amelanotic melanoma. These lightly-pigmented primary conjunctival melanoma more often give rise to metastases and are associated with a worse prognosis.²³

We would like to recommend a thorough work-up of all patients with PAM, and especially those with severe atypia. A close follow up, even in those with complete resection of the pigmented lesion, is paramount for the detection of recurrence and progression or metastasis. Our center performs an annual cervical lymph node ultrasound examination, as well as a liver sonography liver blood tests and X Thorax. Although there is still uncertainty on the role of positron emission tomography (CT scan PET), because of its high cost, false negative results and nonspecificity. It has been reported as a superior imaging modalities in detecting metastatic disease, specifically in case of bone involvement.⁶ Although more studies on this should be done to investigate the benefit of PET for screening purposes, we would recommend this for detecting metastatic disease, especially in bone pain without any other evidence of metastasis.

4. Conclusion

In conclusion, metastasis, even rare metastases such as bone, from conjunctival melanoma associated to PAM with severe atypia remains a possibility lifelong and ophthalmologists need to be alert for this. It is possible for these metastases occur due to operations on the affected eye, or by means of a pathologic changes not detected minimal invasive conjunctival melanoma. Revision of histopathology proved essential in answering the possibility of metastases and the change in histopathological diagnosis and therapy for PAM with severe atypia.

Statement of ethics

The subject has given her written informed consent to publish her case (including publication of images). All research was conducted ethically in accordance with the Declaration of Helsinki.

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Authorship

All authors attest that they meet the current ICMJE criteria for Authorship.

Author contributions

Jordy Goemaere: Design of the work, drafting the work, final approval of the version to be published, agreement for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Noémie Lauwers: Design of the work, critically revising the work, final approval of the version to be published, agreement for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Rob J W de Keizer: Design of the work, critically revising the work, final approval of the version to be published, agreement for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ronald de Keizer: Representative of Rotterdam Eye Hospital ocular oncology group providing additional clinical data, critically revising the work, final approval of the version to be published, agreement for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Rob Verdijk: Ophthalmic pathologist providing revision with new tissue analysis, critically revising the work, final approval of the version to be published, agreement for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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