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

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CHAPTER 4

PREDICTIVE VALUE OF EXFOLIATIVE CYTOLOGY IN PIGMENTED CONJUNCTIVAL LESIONS

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ABSTRACT

Purpose. Pigmented lesions of the conjunctiva are often difficult to classify clinically. Exfoliative cytology may be helpful, but reliable data regarding the sensitivity and specificity of this test are currently lacking. We determined the value of exfoliative cytology with regard to pigmented conjunctival lesions.

Methods. 294 smears from 182 patients were screened for malignancy within 6 months of exfoliative cytology. Smears were classified according to the following categories: grade 0, insufficient material for diagnosis; grade 1, normal conjunctival cells; grade 2, melanocytes with mild atypia; grade 3, melanocytes with moderate atypia; and grade 4, melanocytes with severe atypia.

Results. The sensitivity, specificity, positive predictive value, and negative predictive value of exfoliative cytology were 85 %, 78 %, 59 %, and 93 %, respectively.

Conclusion. Exfoliative cytology is a fast, easy, and non-invasive technique that may be used in the evaluation of patients with a pigmented conjunctival lesion.

INTRODUCTION

It is often difficult to correctly diagnose pigmented lesions of the conjunctiva on clinical grounds alone.[1] Yet it is important to differentiate between a benign conjunctival nevus, a potentially premalignant primary acquired melanosis (PAM), a malignant conjunctival melanoma, and pigmented squamous cell carcinoma, because the latter two are potentially lethal.

Conjunctival biopsies are usually taken to verify the clinical diagnosis of PAM or melanoma. However, a non-invasive and infrequently used alternative to conjunctival biopsy is the conjunctival smear. Whereas repeated biopsies can cause complications and discomfort to the patient, conjunctival smears can be obtained with minimal damage and discomfort by means of cotton swabs wiped across the conjunctiva. Lopez Cardozo, who first described exfoliative cytology, used a cotton wool tip to collect cells from pigmented conjunctival lesions.[2] In earlier studies, conjunctival scraping was used instead of cotton wool swabs, which is slightly more invasive.[3,4] Egbert et al. first described impression cytology, by which samples were obtained by touching the eye with a cellulose acetate filter or by Biopore membrane impression.[5,6] In our hospital, exfoliative cytology of conjunctival smears has been used for more than 25 years to assess pigmented conjunctival lesions. However, the exact sensitivity and specificity of exfoliative cytology have not yet been determined. Previous studies have reported predictive values from 73% to 100% for exfoliative or impression cytology on pigmented conjunctival lesions, but all examined only 30 patients or less.[7] In this study, we determined the predictive value, sensitivity, and specificity of exfoliative cytology in a large group of patients with a pigmented conjunctival lesion.
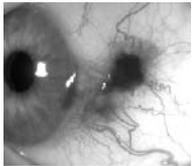
PATIENTS AND METHODS

We reviewed the records of all the patients who were diagnosed with a melanocytic conjunctival lesion (nevus, PAM, conjunctival melanoma), and for whom exfoliative cytology had been performed between 1975 and 2003 at the Leiden University Medical Centre. Exfoliative cytology was performed when the clinical diagnosis was uncertain and when a lesion was suspect for conjunctival melanoma or PAM with atypia. In this time period, 328 conjunctival smears were taken from 199 patients with a melanocytic conjunctival lesion. Of these samples, 34 were scored according to a grading system different from the currently used system and the slides were not available for re-evaluation. Slides of 294 smears from 182 patients were available for this study. For 138 patients, a single smear was performed. For 44 patients, multiple smears were taken (in total 156 smears), 101 of these smears were taken because of a primary tumour lesion or a recurrence; 46 smears were taken from the same lesion, because the lesions showed clinical changes. Nine exfoliations were performed after excision of the primary lesion to check complete removal of the lesion.

For all slides, the grade, number of cells, and possible problems with exfoliative cytology were recorded. It was also registered whether histological samples were taken from the same patient within six months after the cytological smear, such matched samples were available in 157 of the 294 cases. Conjunctival melanoma in situ was defined as a complete replacement of the epithelium with atypical melanocytes, without any signs of invasion into the deeper layers of the conjunctiva. PAM with atypia was defined as atypical melanocytes in

the basal layers of the epithelium, or nest formation of atypical melanocytes in the epithelium, or as individual atypical melanocytes in different layers of the epithelium (pagetoid form). No histological material was obtained when the lesion was considered benign; when in doubt the patients were followed regularly to confirm the benign character of the lesion. All cytological samples were reviewed by one cyto-pathologist (MV), all histological slides were reviewed by one ophthalmic pathologist (DW).

All smears were taken as described previously.[2] In brief, all suspected pigmented lesions were gently rubbed with a cotton wool tip, and the cotton wool tip was subsequently dabbed onto several glass slides. Glass slides were air dried, fixed with methanol, and subsequently stained with Giemsa. The exfoliative samples can contain conjunctival cells, goblet cells, melanocytic cells, inflammatory cells, and blood cells. The presence of melanocytic cells is important for the classification of pigmented conjunctival lesions. All samples were investigated for abnormalities in nuclear size, nuclear shape, nuclear-cytoplasmatic ratio, chromatin, nuclear membrane, nucleoli, and melanin. The samples were classified into five different categories (Figure 1): grade 0, insufficient material for diagnosis, grade 1, normal
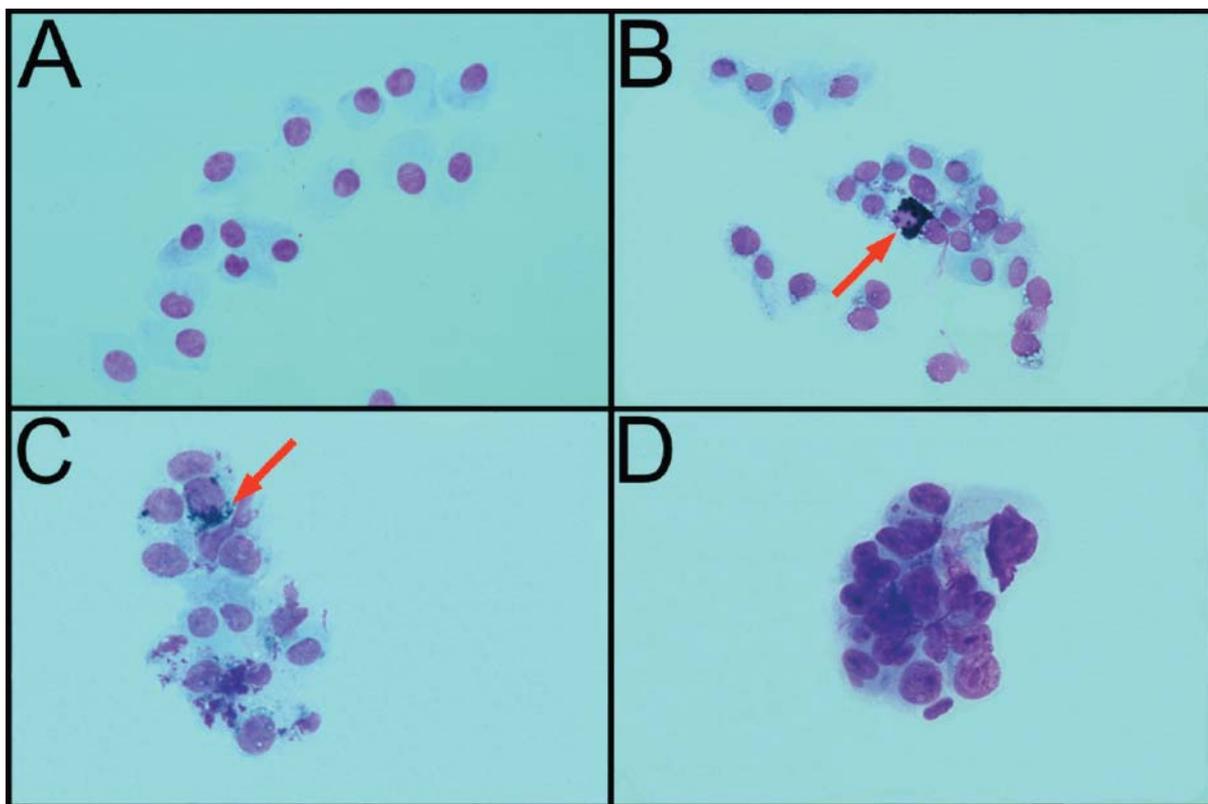

Figure 1. Different grades of atypia in exfoliative cytology samples.
Figure 1A: grade 1, normal conjunctival cells. Figure 1B: grade 2, melanocytes with mild atypia, characterized by slight increased nucleus-cytoplasmatic ratio, and few irregular nuclear membranes. Figure 1C: grade 3, melanocytes with moderate atypia, characterized by large nuclei, irregular nuclear membranes, anisokaryosis, and prominent nucleoli. Figure 1D: grade 4, melanocytes with severe atypia, characterized by very large nuclei and therefore severe increased nucleus-cytoplasmatic ratio, anisokaryosis, very irregular nuclear membranes, and large prominent macro-nucleoli. Red arrow indicates melanin pigment. Figures A-D are Giemsa stained and photographed with 400X magnification.

conjunctival cells with or without melanin pigment, or reactive conjunctival cells typical of inflammation; grade 2, melanocytic cells with mild atypia; grade 3, melanocytic cells with moderate atypia; and grade 4, melanocytic cells with severe atypia.

Statistics

Sensitivity (se), specificity (sp), positive predictive value (ppv), and negative predictive value (npv) were calculated with the following formulas:

se = TP/(TP +FN), sp = TN/(FP + TN), ppv = TP/(TP + FP), npv = TN/(FN +TN), where TP are the true positive results, TN are the true negative results, FP the false positive results, and FN the false negative results.

The 95% confidence intervals (CI) were calculated with the following formulas:

$SE(P) = \sqrt{(P(1-P)/n)}$, 95% CI = mean ± 2SE, where SE is the standard error, P is the percentage found, and n the total number of cases.

RESULTS

Samples were obtained from 83 men (45 %) and 99 women (54 %). The average age of the patients was 44 (± SD 22) years.

In 23 (8 %) smears (grade 0) the severity of atypia could not be estimated, primarily due to a low cell count and poor quality of the collected material. These cases were excluded from further analysis. Of the remaining 271 smears, 80 were classified as grade 1, 87 as grade 2, 26 as grade 3, and 78 as grade 4. Of the 157 histological samples, 35 were diagnosed as nevus, 16 as nevus with atypia, 6 as PAM without atypia, 18 as PAM with atypia, 17 as melanoma in situ, 63 as invasive melanoma, and two as a degenerative lesion. Table 1 shows the histological diagnosis versus the exfoliative grading.
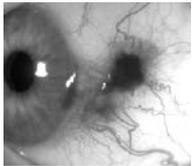
Table 1. Histological diagnosis and the corresponding exfoliative cytology category among 294 smears.

Category of atypia *	Nevus	PAM without atypia	PAM with atypia	Melanoma insitu	Invasive melanoma	Other[†]	No histo-logy	Total
Grade 0	4	0	0	2	6	0	11	23
Grade 1	17	3	1	1	4	0	54	80
Grade 2	16	1	1	1	5	1	62	87
Grade 3	4	0	8	0	5	0	9	26
Grade 4	10	2	8	13	43	1	1	78
Total	51	6	18	17	63	2	137	294

* Grading system for atypia, grade 0: insufficient material, grade 1: normal cells, grade 2: melanocytes with mild atypia, grade 3 melanocytes with moderate atypia, and grade 4 melanocytes with severe atypia.

† Degenerative lesions

Histologically confirmed conjunctival melanoma (invasive or in situ) was detected in 72 % (95% CI, 62-82) of the smears with grade 4 atypia, whereas melanoma was detected in 6 % (95% CI, 1-12), 7 % (95% CI, 2-12), and 19 % (95% CI, 4-35) of the cases with grade 1, 2, or 3 atypia, respectively (Table 2).

The calculated sensitivity, specificity, positive predictive value, and negative predictive value of atypia grades 1, 2, 3, and 4 to detect a conjunctival melanoma (invasive or in situ) are listed in Table 3. When smears with grade 3 and 4 atypia are grouped together this would result in a sensitivity of 85 % (95% CI, 77-93), specificity of 78 % (95% CI, 73-84), positive predictive value of 59 % (95% CI, 49-68), and a negative predictive value of 93 % (95% CI, 90-97) (Table 3).

Table 2. Cytology grading and melanoma incidence.

Category of atypia	Conjunctival melanoma †		Total	
	No	Yes		
Grade 0	15 65% (45-85)	8 35% (15-55)	23	
Grade 1	75 94% (88-99)	5 6% (1-12)	80	Low chance of melanoma
Grade 2	81 93% (88-99)	6 7% (2-12)	87	
Grade 3	21 81% (65-96)	5 19% (4-35)	26	High chance of melanoma
Grade 4	22 28% (18-38)	56 72% (62-82)	78	
Total	214	80	294	

*   Grading system for atypia, grade 0: insufficient material, grade 1: normal cells, grade 2: melanocytes with mild atypia, grade 3 melanocytes with moderate atypia, and grade 4 melanocytes with severe atypia.

†   Occurrence of histological-confirmed conjunctival melanoma (invasive or in situ) within six months of exfoliative cytology. Data are numbers, percentages within that grade of atypia, and between brackets 95% confidence intervals.

Table 3. Predictive values for the different grades of atypia to detect a conjunctival melanoma.

	Sensitivity	Specificity	Positive predictive value	Negative predictive value
	% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)
Grade 1	7 (1-14)	62 (56-69)	6 (1-12)	65 (58-72)
Grade 2	8 (2-15)	59 (52-66)	7 (2-12)	64 (57-71)
Grade 3	7 (1-14)	89 (85-94)	19 (4-35)	73 (67-78)
Grade 4	78 (68-88)	89 (85-93	72 (62-82)	92 (88-96)
Grade 3 and 4 together	85 (77-93)	78 (73-84)	59 (49-68)	93 (90-97)

The sensitivity, specificity, positive predictive value, and negative predictive value are expressed as percentages with a 95% confidence interval.

Table 4. Number of cells in 248 exfoliative smears.

| Number of cells	Number of smears (percentage)
Low	57 (23)
Moderate	71 (28)
High	110 (44)
Very High	10 (4)
Total	248 (100)

In 248 of the 294 smears the number of cells could be estimated: low, moderate, high, and very high numbers of cells were present in 57, 71, 110, and 10 smears, respectively (Table 4).

In 8 (35 %) of the 23 smears in which the lesion could not be graded by exfoliative cytology (grade 0), a conjunctival melanoma (invasive or in situ) was histologically confirmed within six months after cytological examination. In all of these cases, atypical cells were seen but the cell count was too low or the cell quality was too poor to grade the lesion.

DISCUSSION

Atypical melanocytic cells that ascend to the epithelial surface of the conjunctiva are indicative of melanoma and PAM with atypia. Benign lesions, such as conjunctival nevus or PAM without atypia, are not associated with superficial atypical melanocytes, although superficial atypical cells can be found in growing nevi in childhood and adolescence [3]. Thus benign lesions in adult patients usually have a normal cytology. PAM without atypia does not progress to melanoma [8]. Our results for the exfoliative cytology of pigmented conjunctival lesions (Table 2) indicate that lesions with melanocytes showing a low grade of atypia (grade 2) at their epithelial surface are rarely associated with a histologically confirmed conjunctival melanoma within 6 months. The likelihood of the presence of a conjunctival melanoma was higher when samples showed higher grades of melanocyte atypia (grades 3 and 4), increasing to 72 % (95% CI, 62-82) for grade 4 smears.

Therefore pigmented lesions with a low grade of atypia (grade 2) do not require aggressive treatment since the risk of melanoma is equal to that of controls (= normal cytological results), within six months of exfoliative examination. Patients should be followed each 6 to 12 months to detect whether the lesion is growing and smears should be taken repeatedly. For grade 4 smears the sensitivity, specificity , positive predictive value, and negative predictive value for diagnosing a conjunctival melanoma are reasonably well (78 %, 89 %, 72 %, and 92 %, respectively), however, when both grades 3 and 4 atypia are considered as a positive marker for conjunctival melanoma the sensitivity increases from 78 % to 85 % (Table 3). Because conjunctival melanoma is a potentially lethal disease (30 % 10-year mortality) [9,10,11,7,12,13,14,15,16], the sensitivity should be as high as possible in order to detect the highest number of conjunctival melanomas. Thus both grade 3 and 4 atypia (moderate and severe atypia) should be considered as positive clinical markers for both in situ and invasive conjunctival melanoma, since cytology cannot differentiate between in situ and invasive
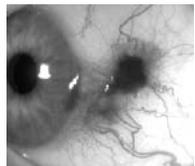
malignancies. If grade 3 or 4 atypia is found, patients should undergo excisional biopsy to confirm the findings. The sensitivity found in this study is comparable with previous studies [7].

Because the chance of conjunctival melanoma still exists in low-grade lesions, a biopsy is always needed when there is strong suspicion on clinical grounds that a pigmented lesion is a conjunctival melanoma. In these cases, atypical melanocytic cells have probably not yet reached the surface of the epithelium, and for this reason cytology reveals only normal conjunctival cells or only a very few atypical cells. It should be noted that four of the five melanomas found in patients with grade 1 cytology did have strongly reactive conjunctival epithelial cells but no melanocytic cells in their smears. Reactive cells can be found in inflamed conjunctivas, such as infected conjunctivas or after topical treatment with mitomycin C. Repeated smears for exfoliative cytology can probably decrease the numbers of missed melanomas in low-grade lesions.

To calculate the predictive value of exfoliative smears, we also used smears without a histological sample, this could be seen as a drawback of the study and a possible bias. However when only smears with a corresponding histological sample would be included, another bias exists since only lesions that are more suspect of conjunctival melanoma are excised. We wanted to present a more realistic picture, so all exfoliative samples were included in this study. For the samples without a histological sample, the clinical follow-up was used to confirm that these lesions were indeed benign.

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