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Original Article

Search for histopathological characteristics of inflammatory juvenile conjunctival nevus in conjunctival nevi related to age: Analysis of 33 cases

L. Nebot*; A. Sáez; P. Serret; C. Caral; R.B. García-Chamón; M.R. Bella

Abstract

Purpose: Conjunctival nevi in young individuals can correspond to the entity named Inflammatory Juvenile Conjunctival Nevus (IJCN), presenting clinically as a rapid growth lesion, and showing at the histopathological study an inflammatory infiltrate surrounding the lesion. All these findings can suggest a diagnosis of malignancy. Due to a case of IJCN diagnosed in our Pathology department, we realized that this entity is rarely reported in the literature and histopathological diagnostic criteria are not well defined. The aim of our study is to compare the histopathological characteristics of conjunctival nevi in patients aged thirty years or less to those in patients above 30 years, looking for the findings described in IJCN.
Methods: All the excisional specimens of resected conjunctival nevus in a tertiary hospital from 2000 to 2018 were retrieved from the Pathology department archives. Demographic data were recorded, and histopathological variables (histological type of nevus, lymphocytic infiltration, eosinophilic infiltration, presence of lymphoid follicles, stromal nevomelanocytic component, intraepithelial nevomelanocytic component, epithelial inclusions, quantity of goblet cells in epithelial inclusions, cellular atypia, mitoses and maturation of the lesion) were evaluated by three independent observers. Statistical analysis was performed comparing the two age groups.
Results: The study determined a significant predominance of the lymphocytic and eosinophilic infiltration in the group of patients aged thirty years or less respect to the elderly group. The percentage of stromal component of the lesion is larger in patients over thirty years compared to the younger group. There was no correlation between epithelial inclusions, maturation or cytological atypia and age groups.
Conclusion: We found some histopathological differences in conjunctival nevi related to young age, some of them coincident with the ones described in IJCN, which histopathologically could lead to a misleading diagnosis. However, we did not find significant differences related to age in many of the described histopathological findings described in IJCN. Larger series with a greater number of cases would be of interest to characterize more precisely this lesion.

Keywords: Conjunctival nevus, Inflammatory juvenile conjunctival nevus, Melanocytic conjunctival lesion, Lymphocytic infiltration

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Department of Pathology, Parc Taulí Hospital Universitari, Institut d'Investigació i Innovació Parc Taulí I3PT, Universitat Autònoma de Barcelona, Sabadell, Spain

* Corresponding author.
e-mail address: lnebot@tauli.cat (L. Nebot).

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Introduction

The most frequent tumours of the conjunctiva (50%) are melanocytic proliferations, 28% of these melanocytic lesions are conjunctival nevus [1]. This neoplasm appears most frequently in the bulbar conjunctiva (72%) [2]. Progression to melanoma has only been described in 1% of cases [3].

Inflammatory juvenile conjunctival nevus (IJCN) is a benign lesion located most often on the juxtalimbal conjunctiva, that occurs in children and adolescents (average age of surgery 11–12 years). In children is usually a lightly pigmented or amelanotic lesion that become pigmented at puberty or pregnancy [4]. Clinically, it may grow rapidly or increase its pigmentation, becoming suspicious of malignancy. In these cases, excision of the lesion is recommended. It is suggested that the growth of the lesions may be due to inflammatory infiltration and cystic degeneration. IJCN are associated with systemic allergy, allergic conjunctivitis and vernal conjunctivitis [5,6]. Levi-Schaffer *et al.* studied this issue and suggested an association between IJCN and allergic inflammation. They described an increased presence of nerve growth factor (NGF), eosinophils and mast cells in IJCN and demonstrated higher production of NGF by fibroblasts related to the lesion respect normal fibroblasts, which modulate eosinophil properties through NGF [6,7].

Histologically, most IJCN are described as compound nevi (97%) with intraepithelial and subepithelial melanocytic nests and solid or cystic epithelial inclusions, the latter being more frequently cystic with PAS stain–positive goblet cells. They show a remarkable stromal inflammatory infiltrate with lymphocytes, plasma cells and eosinophils [4]. Some authors describe a ''reverse'' subepithelial maturation in this entity, with melanocytes displaying nuclear and cytoplasmic size greater in depth than in the junctional component, a pattern of confluent growth in the junctional component, and certain degree of atypia [8]. Sometimes the prominent inflammatory component produces a distortion of the architecture and an apparent cytological atypia. It is described as a benign entity with some ''atypical'' features that can be associated with melanoma in cutaneous melanocytic lesions [4].

Classically, it corresponds to an enlarging lesion in the bulbar conjunctiva. Young age and cystic component are indicators of a benign lesion. The differential diagnosis of IJCN includes malignant conjunctival melanoma, lymphoma and primary acquired melanosis. The most important clinical and histological differential diagnosis is conjunctival malignant melanoma, whose clinical presentation can be similar to IJCN. However, cysts are rarely seen in melanomas, and unlike malignant melanoma, in IJCN there is no marked cytological atypia or mitotic activity in the stromal component [3].

The lymphocytic inflammatory infiltrate may suggest the diagnosis of a conjunctival lymphoma, but it is rare in young population, and the lymphocytic population in IJCN corresponds to an admixture of B and T cells. [4]

We received an excisional biopsy of a conjunctival melanocytic lesion in a 28 year old man, with a diagnosis compatible with IJCN. It is an uncommon lesion and there are few case series reported in the literature. The aim of this study is to describe the features of conjunctival nevi received in our Pathology department (Corporació Sanitaria Parc Taulí Hospital, Sabadell, Spain) in the last eighteen years, and compare the histopathological characteristics of young patients (aged thirty or less) to patients aged over thirty.

Materials and Methods

This is a retrospective observational study that analyses the histopathological characteristics of conjunctival nevus. Two age groups of patients were compared: children/young adults up to 30 years old and adults over thirty years old. All patients with diagnosis of conjunctival nevus in the database of Corporació Sanitaria Parc Taulí, Hospital, Sabadell (Spain), who were excised between years 2000 and 2018 were included in the study. The specimens fixed with formaldehyde and paraffin embedded were sectioned at 3 μm thick. Slides stained with Hematoxylin-Eosin were reviewed by three independent observers (two pathologists and a pathologist trainee).

The histopathological features evaluated were: histological type (compound, junctional or subepithelial), lymphocytic infiltration, eosinophilic infiltration, presence of lymphoid follicles, subepithelial nevomelanocytic component, intraepithelial nevomelanocytic component, epithelial inclusions (solid or cystic), amount of goblet cells in epithelial inclusions, cellular atypia, mitoses and maturation of the lesion. The features were evaluated semiquantitatively (0 to 3) or dicotomically (Yes/No) (Table 1).

The definite value for each item was the one determined by most of the observers. Statistical analysis was carried out using Statistical Package for the Social Sciences (SPSS) software. Descriptive statistics were presented as percentages and frequencies. Chi-square test (χ^2). was applied to compare the categorical variables of each histopathological

Table 1. Histopathological features.

Histological type of nevus	Compound Junctional Subepithelial
Lymphocytic infiltration	Grade 0: Absent Grade 1: Isolated inflammatory cells Grade 2: Groups of few cells Grade 3: Prominent groups of cells
Eosinophilic infiltration	Grade 0: Absent Grade 1: Isolated inflammatory cells Grade 2: Groups of few cells Grade 3: Prominent groups of cells
Lymphoid follicles	Yes/No
Stromal/intraepithelial nevomelanocytic component	0%/100% 25%/75% 50%/50% 75%/25% 100%/0%
Solid epithelial inclusions	Grade 0–3
Cystic epithelial inclusions	Grade 0–3
Maturation of nevus cells deeply	Yes/No
Goblet cells in epithelial inclusions	Grade 0–3
Cellular atypia	Yes/No
Atypical or deep mitoses	Yes/No

feature between the two age groups (≤30 or >30 years old). The significance level was fixed as 5% for each analysis. A p-value less than 0.05 was considered statistically significant.

Results

Thirty-three cases of conjunctival nevus were identified, 32 of them being evaluable histologically, while a remaining case was considered insufficient for its valuation. The series included 17 cases of patients aged thirty years or less (range: 5–30 years old), and 15 cases of patients over thirty years old (range: 31–71 years old). The histopathological features of each age group are summarized in Table 2.

There are 17 cases in the group of patients aged thirty years or less with histopathological characteristics of conjunctival nevus. Of these, 15 cases corresponded to compound nevi, 1 to a junctional nevus and 1 to subepithelial nevus. Of the 15 cases over thirty years old, 11 were compound nevi and 4 corresponded to subepithelial nevi. There were no significant differences in the distribution of the type of nevus according to the age group (p = 0.191).

Lymphocytic infiltration in the group of younger patients was considered grade 3 in 9 cases (52.9%), grade 2 in 3 cases (17.6%), grade 1 in 4 cases (23.5%) and grade 0 in 1 case (5.9%). In the group of older patients, the lymphocytic infiltration was grade 2 in 3 cases (20%), grade 1 in 9 cases (60%) and grade 0 in 3 cases (20%), with any case of grade 3, with statistically significant differences in lymphocytic infiltration between the two age groups (p = 0.008).

Eosinophilic infiltration was found only in the group of younger patients, in 8 of 17 cases (47.1%), with statistically significant difference (p = 0.024); it was considered grade 3 in 2 cases, grade 2 in 1 case, and grade 1 in 5 cases. In our series, only two cases showed lymphoid follicles and both were patients under 30 years of age.

Five cases showed no epithelial inclusions, solid or cystic. Four of these belong to the group until thirty years and one case belong to the oldest group. Solid and cystic epithe-

Table 2. Histopathological features.

		Group age*		Significance p-value
		≤30 y (n = 17) No. (%)	>30 y (n = 15) No. (%)	
Histological type of nevus	Compound	15 (88.2%)	11 (73.3%)	NS
	Junctional	1 (5.9%)	0 (0%)	
	Subepithelial	1 (5.9%)	4 (26.7%)	
Lymphocytic infiltration	0	1 (5.9%)	3 (20%)	p = 0.008
	1	4 (23.5%)	9 (60%)	
	2	3 (17.6%)	3 (20%)	
	3	9 (52.9%)	0 (0%)	
Eosinophilic infiltration	0	9 (52.9%)	15 (100%)	p = 0.024
	1	5 (29.4%)	0 (0%)	
	2	1 (5.9%)	0 (0%)	
	3	2 (11.8%)	0 (0%)	
Lymphoid follicles	Yes	2 (11.8%)	0 (0%)	NS
	No	15 (88.2%)	15 (100%)	
Stromal/intraepithelial nevomelanocytic component	0%/100%	1 (5.9%)	0 (0%)	
	25%/75%	2 (11.8%)	0 (0%)	
	50%/50%	6 (35.3%)	1 (6.7%)	NS
	75%/25%	7 (41.2%)	10 (66.7)	
	100%/0%	1 (5.9%)	4 (26.7)	
Stromal nevomelanocytic component	≤50%	9 (52.9%)	1 (6.7%)	p = 0.006
	>50%	8 (47.1%)	14 (93.3%)	
Solid epithelial inclusions	0	7 (41.2%)	8 (53.3%)	NS
	1	7 (41.2%)	5 (33.3%)	
	2	1 (5.9%)	2 (13.3%)	
	3	2 (11.8%)	0 (0%)	
Cystic epithelial inclusions	0	5 (294%)	1 (6.7%)	NS
	1	4 (23.5%)	6 (40%)	
	2	5 (29.4%)	3 (20%)	
	3	3 (17.6%)	5 (33.3%)	
Maturation of nevus cells deeply	Yes	13 (75.5%)	15 (100%)	NS
	No	4 (23.5%)	0 (0%)	
Goblet cells in epithelial inclusions	0	8 (47.1%)	3 (20%)	NS
	1	6 (35.3%)	8 (53.3%)	
	2	2 (11.8%)	2 (13.3%)	
	3	1 (5.9%)	2 (13.3%)	
Cellular atypia	Yes	2 (11.8%)	1 (6.7%)	NS
	No	15 (88.2%)	14 (93.3%)	
Atypical or deep mitoses	Yes	0 (0%)	0 (0%)	NS
	No	17 (100%)	15 (100%)	

*Age in years at time of surgery.
NS: No significance.

lial inclusions were present in 10 and 12 cases respectively in the group until thirty years, and in 7 and 14 cases respectively in group of patients older than thirty years.

In the group until thirty years, grade 3 solid and cystic epithelial inclusions were present in 2 (11.8%) and 3 cases (17.6%) respectively, while in the older group there wasn't any case of solid epithelial inclusion grade 3, although there were 5 cases (33.3%) of cystic inclusions grade 3.

In our series, older patients had a greater stromal nevomelanocytic component with statistically significant difference ($p = 0.006$). The stromal component constitutes more than 50% of the lesion in 93.3% of cases in the oldest group.

Cellular atypia was evaluated considering the usual atypia in nevus of young patients. Only 3 cases presented greater grade of atypia than expected, two of them in the group of patients until thirty years and the other in the group older than thirty.

Deep maturation of the lesion was absent in 4 cases (23.5%) in the youngest group and in zero cases (0%) in the oldest group of patients.

The statistical analysis showed no differences between age and the presence of cystic inclusions, solid inclusions, goblet cells, maturation or cytological atypia. (Figs. 1 and 2)

Discussion

Inflammatory juvenile conjunctival nevi are described as compound nevus with intraepithelial and stromal nevomelanocytic component [4,8]. In our series, in the group of younger patients ($\leq$30 years old), compound nevus represented 88.2% of cases, junctional nevus 5.9%, and subepithelial nevus 5.9%, while in the group of patients over thirty years old, compound nevus represented 73.3%, junctional nevus 0% and subepithelial nevus 26.7% of cases. The group of patients under thirty years had mainly compound nevus, while in the group of patients over thirty years there was a higher percentage of nevus of the subepithelial type. However, analyzing statistically these results we did not find statistically significant differences in the distribution of the types of nevus between the two age groups.

It has been described that conjunctival nevi appear in the first and second decades of life with the nevomelanocytic cell nests located in the epidermal-stromal junction, and during the second and third decade the nests migrate to the stroma forming the compound nevus. In the third and fourth decade the lesion usually is totally located in the subepithelial stroma [1,3]. This fact could explain why in our series the older group presented higher percentage of stromal component in the lesion.

Comparing the two age groups, there are significant differences related to inflammatory infiltrate between them. Nearly fifty-three percent (52.9%) of patients aged thirty years or less had severe lymphocytic infiltrate with prominent cell aggregates (grade 3), and two cases (11.8%) showed lymphoid follicles, while any patient of the group of older patients had severe lymphocytic infiltrate. Furthermore, patients aged over thirty years old had more frequently only isolated lymphocytes (60%) than the group of patients ages until thirty years old (23.5%). So the cases of the youngest group showed more prominent lymphocytic infiltrates compared to the group over thirty years. Patients under 30 years of age presented eosinophilic infiltration in eight cases

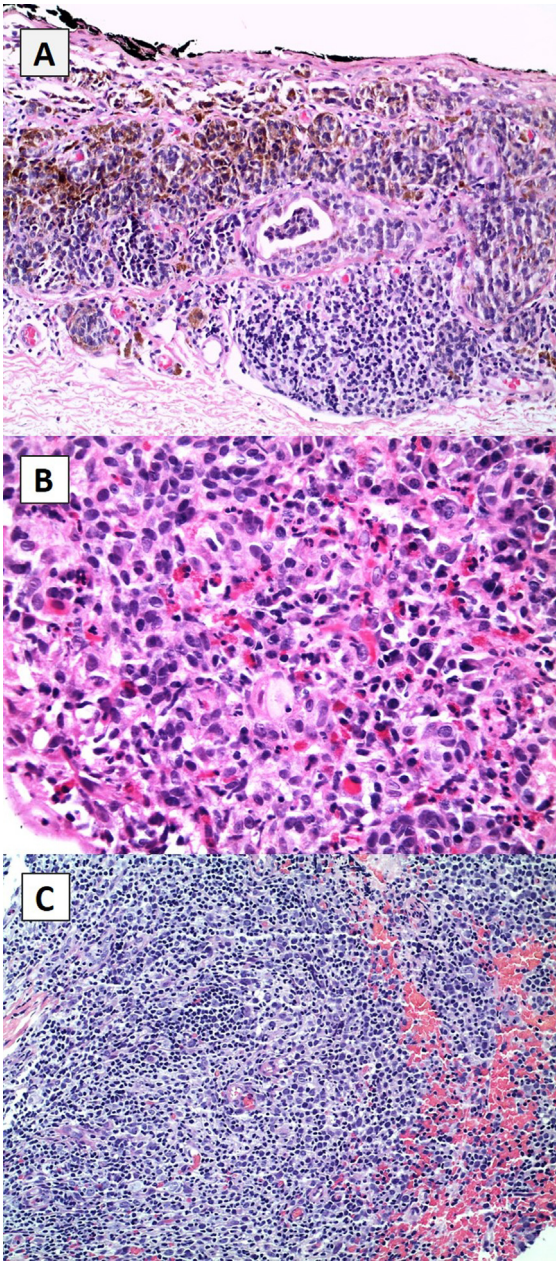

Fig. 1. A, Dense lymphocytic infiltrate grade 3 (Original magnification $\times$ 200 Hematoxylin and Eosin). B, Presence of a lymphoid follicle formation (Original magnification $\times$ 200 Hematoxylin and Eosin). C, Eosinophilic infiltrate grade 3 (Original magnification $\times$ 400 Hematoxylin and Eosin).

(47.1%). No eosinophilic infiltrate was found in patients older than 30 years age. There is a statistically significant high level of eosinophilic infiltration among the age groups. Zamir et al. found that 75% of conjunctival nevus of a serie of 63 patients younger than 20 years were compound nevus with a prominent inflammatory infiltrate constituted by lymphocytes, eosinophils and plasma cells with features of inflamed conjunctival nevus. Of these cases, 30% presented germinal centres and 77 % eosinophilic infiltrate [5].

Cystic inclusions are considered a sign of chronicity and are caused by the migration of subepidermal melanocytes [3]. Of the five cases that do not show intraepithelial inclusions (solid or cystic), four correspond to the group of

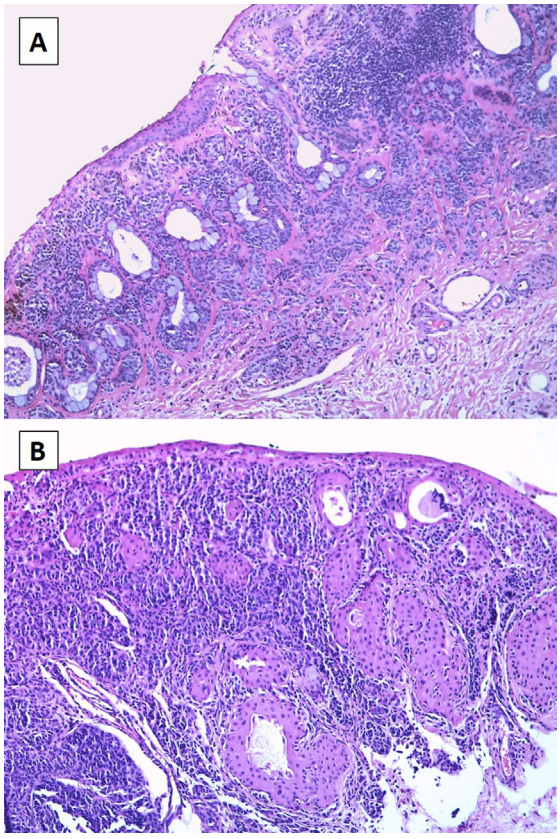

Fig. 2. A, Predominant component of goblet cells in epithelial inclusions (Original magnification × 100 Hematoxylin and Eosin). B, Prominent epithelial inclusions (Original magnification × 100 Hematoxylin and Eosin).

patients of thirty or less years old. However, we did not find a statistically significant association between age and epithelial inclusions grade.

Cellular atypia higher than the atypia expected in a conventional nevus was found in 2 cases in the youngest group and in one case of the oldest group. In addition, the two lesions belonging to the group of patients until thirty years old presented absent deep maturation of nevomelanocytic cells. Maturation of nevomelanocytes was the evaluated trait with the most discordance among the three different observers.

Colarossi *et al.* described a case of atypical juvenile conjunctival compound nevus with marked atypia and inflammation. It corresponded to a compound nevus with atypical cells in deep nests, with focal pagetoid spread but without mitotic activity. The final diagnosis was juvenile conjunctival atypical nevus [2]. Costea *et al.* also made the final diagnostic of inflammatory juvenile atypical compound nevus in a lesion with chronic inflammation, lack of maturation in the deep of the lesion, pagetoid spread and atypical nevomelanocytes without mitotic activity [1]. We consider that our three cases with certain degree of atypia did not have enough cytological atypia to make the diagnosis of atypical compound nevus of the conjunctiva. None of the three observers detected mitotic activity, as the cases of inflammatory juvenile atypical nevus reported in the literature [1,2]. Finally, no significant correlation was obtained between the age group and the atypia or maturation of the lesion.

To summarize, in an attempt to contribute to the characterization of IJCN, we described the histopathological characteristics of all conjunctival nevi received in our Pathology department from 2000 to 2018, looking for differences between two age groups. The younger group aged thirty or less, most frequently presented lymphocytic and eosinophilic infiltrates. We noticed also greater stromal component of nevus cells in the oldest age group. After reviewing the scant studies and reports about IJCN, we realized that there are not clearly defined diagnostic criteria for this entity. Most of the authors define this entity as a lesion with ''reverse'' maturation, confluent growth pattern in the junctional component, chronic inflammation and variable but low grade of cytologic atypia [3,8,9]. After the review of our cases and considering the lack of unification of diagnostic criteria reported in the literature, it is unclear if any of our cases could finally be diagnosed as IJCN. Our study is limited due to the small sample size. Larger series with a greater number of cases would contribute to a best characterization of this tricky and scarcely described lesion.

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