Correlations Between Clinical and Histological Aspects in Nasal Polyposis

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Introduction: The histological study of nasal polyps does not reveal any specific lesions but eosinophilic infiltration nasal mucosae seems to be characteristic of nasal polyposis. The aim of this work is to study possible links between certain histological and clinical aspects in nasal polyposis. Furthermore, we attempt to compare the quantification of tissue eosinophilia according to the number of eosinophils per field with the percentage figure obtained with respect to the total of inflammatory cells.
Material and method: We have studied 40 patients with idiopathic bilateral nasal polyposis, assessing the correlations between various clinical aspects such as their endoscopic and radiological status, association with asthma and intolerance to NSAIDs, against histological aspects of nasal polyps such as the frequency of metaplasia, fibrosis, and the degree of eosinophilic infiltration. A group of 12 healthy subjects allowed comparison of our results with healthy nasal mucosa.
Results: Tissue eosinophilia correlates statistically with clinical staging and tends to be higher in patients with ASA triad. The quantitative measurement of tissue eosinophilia (number of eosinophils per field) correlates with the percentage figure obtained (with respect to the total number of inflammatory cells in the infiltrate).
Conclusions: Eosinophil infiltration of the nasal mucosa is, together with oedema, the most constant histological characteristic of nasal polyposis and seems to be an important factor in the clinical behaviour of sinonasal polyposis. Quantitative measurement of tissue eosinophilia is easier and quicker to perform and equivalent to percentage evaluation.

Key words: Chronic rhinosinusitis. Inflammation. Respiratory mucosa.

Correlaciones entre aspectos clínicos e histológicos en la poliposis nasal

Introducción: El estudio histológico de los pólipos nasales no muestra lesiones específicas, pero la infiltración de la mucosa por eosinófilos parece ser una característica de la poliposis nasal. El objetivo del trabajo es estudiar las posibles relaciones entre algunos aspectos histológicos y clínicos en la poliposis nasal. Asimismo pretendemos comparar la cuantificación de la eosinofilia tisular según el número de eosinófilos por campo con la obtenida porcentualmente respecto al total de células inflamatorias.
Material y método: Hemos estudiado a 40 pacientes con poliposis nasal bilateral idiopática y valoramos las correlaciones existentes entre diversos aspectos clínicos, como el estadio endoscópico y el radiológico, la asociación con asma e intolerancia a antiinflamatorios no esteroideos, con algunos aspectos histológicos de los pólipos nasales, como la frecuencia de metaplasia, fibrosis y grado de infiltración eosinofílica. Disponemos de un grupo control de 12 sujetos con mucosa nasal sana para la comparación de nuestros resultados.
Resultados: La eosinofilia tisular se correlaciona estadísticamente con el estadio clínico y presenta una tendencia a ser más alta en el síndrome de ASA tríada. La medida de la eosinofilia tisular de forma cuantitativa (número de eosinófilos por campo) se correlaciona con la obtenida porcentualmente (respecto al total de células inflamatorias del infiltrado).
Conclusiones: La infiltración por eosinófilos de la mucosa nasal es, junto con el edema, la característica histológica más constante de la poliposis nasal y parece ser un factor importante en el comportamiento clínico de la poliposis nasosinusal. La medición cuantitativa de la eosinofilia tisular es más rápida y sencilla y con la misma significación que la porcentual.

Palabras clave: Rinosinusitis crónica. Inflamación. Mucosa respiratoria.

INTRODUCTION

Nasal polyposis is a chronic inflammatory disease of the mucous membrane of the nasal cavity and paranasal sinuses, of unknown aetiology, not mediated by immunoglobulin E

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(IgE). Eosinophils seem to play an important role and the condition leads to the formation of oedematous benign polyps from the sinuses to the nasal cavity.[1] The European Position Paper on Rhinosinusitis and Nasal Polyps[2] considered nasal polyposis as a type of chronic rhinosinusitis, but recommended excluding from the classification such other conditions as cystic fibrosis, primary ciliary dyskinesia syndrome, and the various forms of autoimmune vasculitis, such as Churg-Strauss syndrome.

The histological study of nasal polyps found no special features; the epithelium is generally ciliate, although it can sometimes present squamous metaplasia. The layer itself is thickened by oedema and shows infiltration of inflammatory cells, mainly eosinophils.[3-5]

Nasal polyposis is associated with relative frequency with intrinsic asthma and intolerance to non-steroid anti-inflammatory drugs (NSAIDs). When the 3 entities co-exist in the same patient, it is known as the ASA triad syndrome.[6] In the clinical assessment of nasal polyps it is considered important to ascertain the extent of the disease, for which endoscopic[7] and radiology[8] stagings are used.

The aim of this paper is to study possible correlations between clinical and histological aspects in 40 patients with idiopathic bilateral nasal polyps. We also aim to determine whether there are significant differences between the quantification of tissue eosinophilia in absolute terms (number of eosinophils per field) and as a percentage (percentage of eosinophils per field) with respect to the total number of inflammatory cells.

MATERIAL AND METHOD

Patients recruited for this study came from the otolaryngology out-patients' clinic of the Hospital General Universitario in Valencia. All patients were given full information and voluntarily consented to participate in this study.

The control group comprised 12 adult patients diagnosed with dysmorphia of the nasal septum, with surgical indication of septoplasty, with no allergy or clinical signs of bronchitis or rhinitis.

The treatment group consisted of 40 patients with nasal polyposis. It included patients with nasal allergy, asthma and intolerance to NSAIDs, as well as patients following treatment with topical corticosteroids. In the case of patients with seasonal allergies, biopsies were performed outwith the active season. The exclusion criteria were autoimmune disorders, treatment with systemic corticosteroids in the previous 2 months, unilateral polyposis, and children with polyposis.

The study noted age and gender in all patients. In the group with nasal polyposis, the following data were also collected: treatment with topical corticosteroids, allergy, asthma, and intolerance to NSAIDs. The allergy was diagnosed by identification with specific IgE and confirmed through skin tests. The diagnosis of asthma was established by the clinical signs and respiratory function tests through assessment by a pneumology specialist.

Patients were classified into 3 clinical groups:

– Group 1: isolated nasal polyposis
– Group 2: nasal polyposis + asthma (without intolerance to NSAIDs)
– Group 3: ASA triad

The endoscopic stage was determined through previously established criteria[7]:

– Stage 1: polyps which do not reach the lower edge of the middle cornet
– Stage 2: polyps which reach the lower edge of the middle cornet, but do not surpass it
– Stage 3: polyps which reach the lower edge of the middle cornet, but do not reach the bottom of the nostril
– Stage 4: polyps which reach the bottom of the nostril

The determination of radiological stage was conducted by means of computerized tomography (CT), according to the staging proposed by Lund et al.[8] The occupation of the frontal, maxillary, anterior ethmoid, posterior ethmoid, and sphenoid sinuses was evaluated: 0, no occupation; 1, partial occupation; 2, total occupation. The osteomeatal complex was similarly scored between 0 and 2 (non-occupied to occupied).

In the group with nasal polyposis, biopsies were obtained from the polyp, either during out-patient consultation by nasal endoscopy with topical anaesthesia, or by polypectomy during endoscopic surgery under general anaesthesia. For the control group, all biopsies were taken from the mucosa of the head of the middle cornet, at the start of the septoplasty under general anaesthesia.

The specimen was fixed in formaldehyde at 10% in distilled water. After preparation, it was included in paraffin blocks and then cut with the microtome into 5 µm slices. After removing the paraffin and putting the preparation into several xylene baths, the sample was washed with alcohol and finally with water, after which it was stained with haematoxylin-eosin and trichrome. After mounting it on the slide it was observes under the optical microscope (Nikon Eclipse E 200). Photographs were taken (digital camera Nikon Coolpix 4500) of the selected fields for each patient 1 at ×100 and 4 at ×400 resolution, and the cells were counted with the help of a microgrid.

The histological variables studied were as follows:

Metaplasia. We classified the type of metaplasia observed as squamous or mixed mucoid. Mucoid metaplasia was deemed to exist when a predominance of goblet cells was noted over mast cells.

Tissue type. We classified the type of tissue observed into lax, mixed, or fibrous.

Eosinophilic infiltration. We quantified the average number of eosinophils per field at ×400 in absolute terms and as a percentage of the total number of inflammatory cells. Both results were classified into 4 grades:

– Grade 0: less than 5
– Grade 1: between 5 and 19

– Grade 2: between 20 and 50
– Grade 3: more than 50

The following statistical studies were performed with the data obtained. For each variable we calculated: frequency, average, variance, standard deviation, maximum value, minimum value, and range.

Comparisons have been made between the experimental group and control group, between clinical and radiological stages, between clinical groups, between allergic and non-allergic patients and between the quantification of eosinophils in absolute and percentage terms.

RESULTS

Clinical Data

The average age of patients was 48.93 years. The youngest patient was 27 years old and the eldest 72. The average age in the control group was 37.8 years, with values ranging between 25 and 65. The group with nasal polyposis consisted of 14 women and 26 men, and the control group, 2 women and 10 men. Ten patients (25%) were diagnosed as having allergy, compared to 30 (75%) non-allergic patients. At the beginning of the study 18 patients were under treatment with topical corticosteroids, compared to 22 who were not.

The distribution by endoscopic staging was: stage 2, 10 patients (25%); stage 3, 25 patients (65%); stage 4, 5 patients (10%).

In the staging by CT, patients had an average score (standard deviation) of 16 (3) (range, 10-20).

Histology Data

In the nasal polyp group, we found 20 cases (50%) with metaplasia, 13 (32.5%) without metaplasia and 7 (17.5%) of non-assessable epithelium. Therefore, among the cases where we have been able to study the epithelium, 60.6% presented metaplasia compared with 58.33% in the control group (Table 1).

Lax tissue is the only one found in controls and is the predominant one in nasal polyposis. In nasal polyposis cases, fibrous tissue was also seen (Table 1).

Tissue Eosinophilia

In the controls, the quantitative average was 0.7 (0.62) eosinophils/field, ×400 (0-1.75). As a percentage, the average in the controls was 2.57 (2.06%) eosinophils/field, ×400 (0-6) (Table 2).

In polyposis, the mean number of eosinophils per field at ×400 was 42.98 (57.48) (range, 1-311). The mean percentage of eosinophils over total inflammatory cells was 41.26 (92.35%) (range, 1%-89.25%) (Table 2).

Comparisons

In the histological study, the group with nasal polyposis showed more fibrosis (*P*=.019) and more tissue eosinophilia than the control group, both statistically significant differences. There were no significant differences in metaplasia.

Table 1. Distribution of the Types of Epithelial and Connective Tissue Found in the Number of Cases

		Control, No. (%)	*Polyposis, No. (%)*
Epithelial	Ciliate[a]	4 (33.3)	13 (32.5)
	Squamous	5 (41.6)	12 (30)
	Mucoid	2 (16.6)	5 (12.5)
	Mixed	0	3 (7.5)
Connective	Lax	12 (100)	26 (65)
	Mixed	0	7 (17.5)
	Fibrous	0	7 (17.5)
	Non assessable[b]	1 (8.3)	7 (17.5)

[a]Represents the cases in which metaplasia was not observed.
[b]Represents the cases in which, due to lack of epithelium, the type could not be determined.

Table 2. Eosinophils per Field ×400 in the Control Group and in the Group With Polyposis

		Control	*Polyposis*
Number of eosinophils/field ×400	<5	12	4 (10%)
	5-19	0	13 (32.5%)
	20-50	0	14 (35%)
	>50	0	9 (22.5%)
Percentage of eosinophils/field ×400	<5	12	3 (7.5%)
	5-19	0	5 (12.5%)
	20-50	0	17 (42.5%)
	>50	0	15 (37.5%)

There were no significant differences between the endoscopic stages according to the epidemiological or allergy variables. Endoscopic staging presents a directly proportional correlation to radiological staging (*P*=.006). Tissue eosinophilia in endoscopic stage 2 was significantly lower than in stages 3 and 4 (*P*=.048). There were no significant differences between endoscopic stages 3 and 4 (Figure 1).

Isolated nasal polyposis and polyposis associated with asthma without intolerance to NSAIDs do not show major differences between them in any variable analyzed. These groups differ significantly on several variables with the ASA triad group which presents higher endoscopic and radiological staging (*P*=.0477) (Figure 2).

There are no statistically significant differences between the clinical groups regarding values of eosinophilia in tissue, although ASA triad shows a tendency towards higher eosinophilia in tissue. No significant differences were found either between allergic and non-allergic patients in any of the variables studied. We found no significant differences

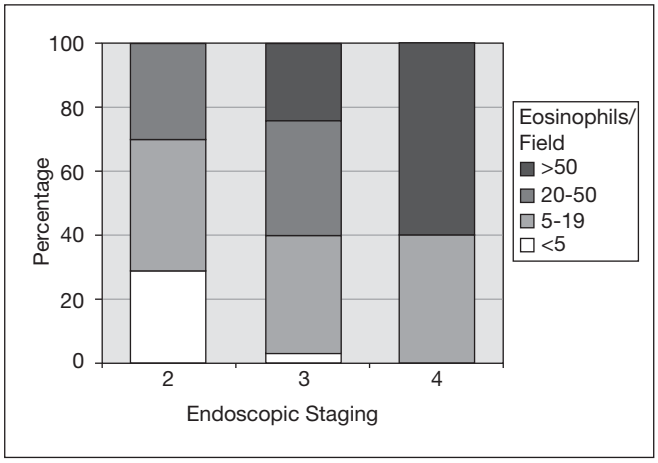

Figure 1. Distribution as percentage of cases (Y-axis) of the grades of tissue eosinophilia (number of eosinophils/field ×400) found in the clinical stages. The eosinophilia grade is directly proportional to the clinical stage.

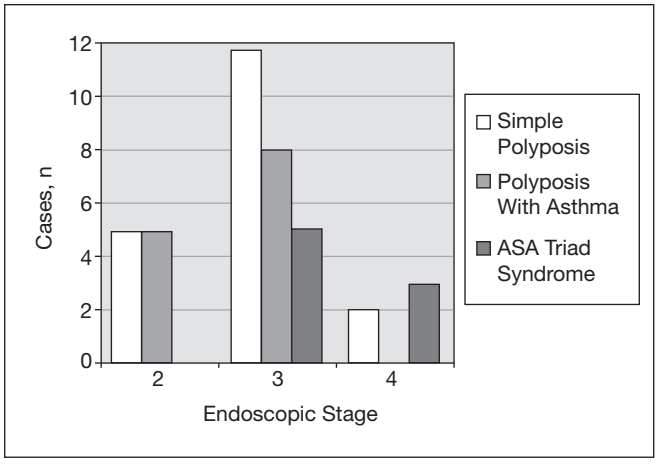

Figure 2. Distribution of groups by endoscopic staging. All cases of ASA triad were stages 3 or 4.

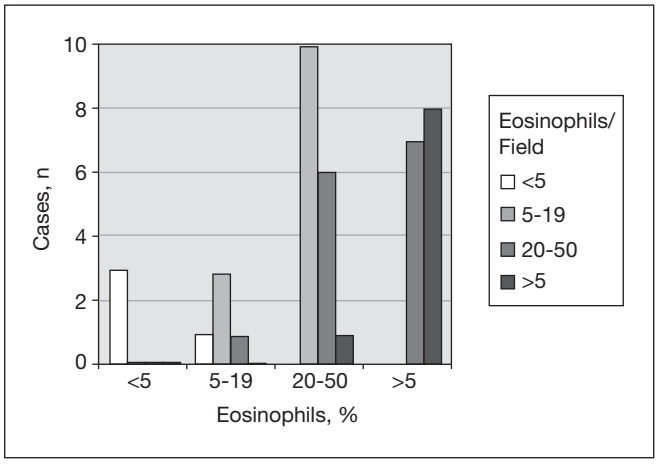

Figure 3. The bar chart shows the degree of quantitative eosinophilia (number of eosinophils/field ×400) according to the degree of percentage eosinophilia in the tissue (percentage of eosinophils/field ×400).

in the eosinophilia observed between patients undergoing treatment with topical corticosteroids and those who were not.

We found a correlation between the 2 methods of measuring eosinophilia in tissue (Figure 3). The correlation is statistically significant (P<.001) for both grades and continuous variables (polynomial regression analysis).

DISCUSSION

Squamous metaplasia has often been described in the anterior part of the polyp, ie, the part most exposed to the flow of inspired air.[9] In addition to squamous metaplasia, mucoid metaplasia can also be observed in the polyps,[10] where there is partial replacement of mast cells by goblet cells. In our study we found that 60% of the polyps showed metaplasia, mainly squamous. This percentage is not very different from that found in the controls, so we think that metaplasia is not a remarkable feature of polyps, and it probably depends on whether the portion of the polyp under study is exposed to inspired air.

Remodelling and fibrosis are considered features of intrinsic asthma, important for the course and prognosis of the disease. Given the co-morbidity of asthma with nasal polyposis, and because other authors[11] have emphasized the histological similarity of both diseases, we thought the study of this histological variable would be interesting. We found a clear dominance of lax connective tissue in the polyps, generally alone and sometimes associated with fibrous tissue (especially in areas near the pedicle). Fibrous connective tissue, also known as dense tissue, is characterized by consisting mainly of collagen fibres with few cells and fundamental substance.

The lamina inherent to almost all polyps presents intense oedema, resulting in abundant areas of pseudocystic appearance, thus diminishing the density of all elements, both fibres and tissue cells. The inflammatory cell infiltration is affected in the same way, thus concentrating in the sub-epithelium where the pseudocystic oedematous phenomenon is lesser or disappears altogether.

We noted that the proportion of fibrous tissue tends to be higher in early endoscopic stages. The explanation may be that the polyps in stage 2, presenting less oedema, exhibit a higher density of cells and fibres. Ultimately, from the cases studied, we can infer that the type of tissue in nasal polyposis did not present any significant fibrosis phenomenon because the predominant tissue is lax and, in general, there was a decrease of both fibres and cells in the tissue.

There is no unanimity among authors in the method of quantifying eosinophils, since some used the quantification of tissue eosinophilia in absolute values and others in relative values. In absolute terms, the results can be shown as the number of eosinophils per field or as the number of eosinophils per unit area. In relative terms, the results can be expressed as a percentage of eosinophils out of the total number of inflammatory cells.[5,12] In this paper, we have

reflected our results in both ways, allowing us to compare both methods.

In line with other authors,[13] we have encountered difficulties in percentile quantification, because the identification of some inflammatory cells can be very difficult without using immunohistochemical methods. Eosinophils stand out clearly due to their orange-stained acidophilic granular cytoplasm, but it can be very difficulty to identify them in other inflammatory cells.[13] Despite the difficulty in the exact identification of inflammatory cells in the absence of immunohistochemical techniques, quantification in percentile terms is possible as this task does not require perfect characterization of the cell type, since a general differentiation of structural cells and inflammatory cells is sufficient.

Although the assessment in quantitative terms (number of eosinophils/field ×400) is more accurate and faster, qualitative quantification (percentage of eosinophils/field ×400) is of interest because of the irregularity presented by global cell density in nasal polyposis. We found both methods for quantifying tissue eosinophilia to have statistical correlation and we believe that both help to understand the real status of eosinophilia.

In normal nasal mucosa, there are either no eosinophils or they are rare,[14] but there is no consensus on when to consider that they constitute tissue eosinophilia. The minimum figure we have found to consider eosinophilia (grade 0) is 5 eosinophils/field ×400 in absolute quantification and 5% as a percentage.[5,15] This figure is confirmed by published data and by comparison with our control group, which presented on average fewer than 2 eosinophils/field ×400.

Our data confirm that tissue eosinophilia is common in nasal polyposis, as 90% of our patients had eosinophilia in tissue. On the quantitative side, the majority tends to present between 20 and 50 eosinophils per field ×400. These results are comparable with those published by other authors.[4,5,15-17]

The quantitative results correlate statistically with the percentages, despite the latter tending to be higher. The largest difference is seen in grade 1 eosinophilia, which is quantitatively more common than in percentage terms. The explanation is that numerous patients with eosinophilia between 5 and 19 eosinophils/field ×400 showed percentages of eosinophilia over 20, meaning that the participation in the general infiltration tends to be higher with 5 to 19 eosinophils/field ×400.

We have seen the great utility of endoscopy and CT staging, since they offer valuable and concordant information on many aspects of nasal polyposis, with several statistically significant correlations with other variables. The scores obtained in the radiological staging are fairly homogeneous, as they were always between 10 and 20, with a possible range of 0-22. One possible explanation is that the involvement of the anterior and posterior ethmoid sinuses and the osteomeatal complex is almost constant in nasal polyposis, whereas, in contrast, the complete occupation of all the frontal and sphenoid sinuses is less frequent.

The fact that eosinophilia is no higher in allergic than in non-allergic patients helps to support the hypothesis of the scarcely relevant role of allergy in the pathogenesis of nasal polyposis.[18]

For ethical reasons, we have not considered it appropriate to recommend the withdrawal of treatment in patients already receiving topical corticosteroids[19] of proven efficacy. In taking this decision, we have taken into account other authors who have reported few changes in tissue eosinophilia in patients treated with topical corticosteroids.[5]

By way of conclusion, both endoscopic and radiological staging show significant correlation. Both offer valuable and consistent information on various aspects of nasal polyposis, since they present several statistically significant correlations with certain histological variables. Tissue eosinophilia correlates statistically with the endoscopic stage and tends to be higher in the ASA triad syndrome. The infiltration of eosinophils in the nasal mucosa, along with oedema, is the most constant histological feature of nasal polyposis. The measurement of tissue eosinophilia in quantitative terms (number of eosinophils per field) correlates with that obtained by percentages (with respect to the total number of inflammatory cells in the infiltrate). The quantitative measurement is faster and easier than calculating the percentage.

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