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Clinical factors influencing the eosinophil infiltration of nasal polyps*

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SUMMARY

Aims and methods: our study, based on a retrospective chart analysis, was aimed 1) to describe the varying degree of eosinophil infiltration in a series of 263 adult patients operated on diffuse and bilateral nasal polyposis (NPS) after failure of medical treatment, in 15 cystic fibrosis patients with bilateral nasal polyps, and in 31 patients with chronic sinusitis without polyps (18 bilateral, 13 unilateral) 2) to search for clinical factors that might influence the degree of eosinophil infiltration. Eosinophil infiltration was expressed semi-quantativily as a percentage of inflammatory cells.

Results: our study confirms that eosinophil infiltration is a striking feature of nasal polyposis. All patients with chronic sinusitis showed less than 10% eosinophils (mean ± SEM = 2 ± 2 %) whereas 88% of patients with NPS showed more than 10% eosinophils (50 ± 2 %). Cystic fibrosis lied in between with 40% of patients showing more than 10% eosinophils. In idiopathic bilateral NPS the number of eosinophils was increased in patients with asthma (58 ± 3%) and even more in Widal's triad (75 ± 4 %). Atopic patients did not have more eosinophils (52 ± 5%). Patients treated with systemic steroids within two months before surgery showed decreased eosinophil infiltration (22 ± 3 % vs 50 ± 2 for treated versus untreated) whereas patients treated with topical steroids did not (47 ± 2 %).

Conclusions: thus, a link might exist between clinical presentation and eosinophil infiltration. Chronic sinusitis and nasal polyps are probably not the same disease. Eosinophils appear as a link between nasal polyps, asthma and aspirin intolerance. Atopic status does not modify eosinophil infiltration of nasal polyps. Systemic steroids appear significantly more effective to reduce the eosinophil infiltrate than topical steroids in our selected group of operated patients.

Key words: nasal polyps, eosinophils, asthma, aspirin intolerance, cystic fibrosis, chronic sinusitis, steroids

INTRODUCTION

Nasal polyps originate from the respiratory mucosa covering the complex anatomy of the ethmoidal bones. Histologically, nasal polyps are characterised by stromal oedema and inflammatory cell infiltration, with a moderate - to - high infiltration of eosinophils (Jankowski et al., 1989; Stoop et al., 1989; Bachert et al., 2000). The aims of our study were 1) to evaluate the degree of eosinophil infiltration in a series of 263 adult patients with diffuse bilateral nasal polyposis, 15 cystic fibrosis patients with nasal polyps, and 31 patients with chronic sinusitis without polyps and 2) to search for clinical factors that might influence the degree of eosinophil infiltration.

In childhood nasal polyposis is a common manifestation of cystic fibrosis. In adulthood, nasal polyposis can occur either as an isolated disease or as a disease associated with asthma (Jankowski, 1997). Nasal polyposis, asthma, and aspirin sensitivity when present in one patient form the "aspirin triad" (Widal's triad in Europe, Samter's triad in USA). Despite the presence of eosinophils, the causal relationship between nasal polyposis and allergy remains doubtful (Keith et al., 1997). Nasal polyps have been described as neutrophilic in cystic fibrosis (Otsuka et al., 1987). In patients with the aspirin triad a marked infiltration with eosinophils seems always to be found (Ogino et al., 1986). The first question is whether or not the degree of eosinophil infiltration could be related to the clinical presentation of nasal polyposis.

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Systemic or topical steroids are efficient treatment of nasal polyposis (Lildholdt et al., 1997). Human eosinophils have glucocorticoid receptors (Peterson et al., 1981), and might be one of the main targets of corticosteroids. Their beneficial effect may be due either to an inhibition of mediator release or to a reduction in the number of eosinophils. Thus we looked at the consequences of topical and systemic steroid treatment on the density of eosinophils present in nasal polyps.

Our results, based on a retrospective chart analysis, indicate that clinical presentation and systemic steroids are factors influencing the degree of eosinophil infiltration of nasal polyp tissue. Our results may help to design future studies or trials based on histopathology, and to understand the pathophysiology of nasal polyposis as well as the mechanisms of action of corticosteroids.

PATIENTS AND METHODS

Patients

All patients' charts for this retrospective study were selected only if the histopathological report clearly described the eosinophil population in a semi quantitative way.

Two hundred and sixty three patients included in this study had idiopathic bilateral polyposis for which they were operated on. The diagnosis of nasal polyposis was based on rhinoscopic or endoscopic examination showing the presence of oedematous polyps in both nasal cavities. Idiopathic means that patients did not have cystic fibrosis, primary ciliary diskinesia, Young's syndrome, fungal sinusitis or other specific syndromes. Nasalisation was the surgical technique performed, i.e. an endonasal endoscopic complete ethmoidectomy with resection of the middle turbinate and large opening of the frontal, maxillary and sphenoid sinuses into the nose (Jankowski, 1995; Jankowski et al, 1997).

Fifteen patients with cystic fibrosis and operated on diffuse and bilateral nasal polyposis formed a group apart.

Thirty one patients operated on chronic sinusitis (functional ethmoidectomy) served as a reference group (18 bilateral, 13 unilateral). Chronic sinusitis was defined as chronic nasal or sinus discomfort that could clearly be related to sinus CT-scan opacities; surgery was only indicated after failure of medical treatment.

In addition to the histopathological report, each chart was checked in order to find the following data:

- did the patient report asthma?
- did the patient report aspirin intolerance?
- did the patient report atopy (based on skin tests or multi-RAST results)
- what was the treatment at admission at the hospital and during the two months before surgery? Systemic steroids? nasal topical steroids? treatment for asthma (inhaled topical steroids, ß2 mimetics, theophyllin,…)? other treatments? (The period of two months before surgery was chosen because this was the mean time between the patient's visit at the clinic and admission at the hospital for surgery; the patient's treatments were usually well recorded in the charts between these two points in time).

If and only if a clear answer to these questions was obtained, the chart was selected (one missing answer was however accepted in a few charts).

Methods

Our study is based on a retrospective chart analysis. However, the histopathological proceedings were performed in the same way for all patients of the study with constant criteria for a semi-quantitative evaluation of the eosinophil population.

Haematoxylin-eosin counterstained slides from formaldehyde fixed tissue specimens were scanned at low power to determine the areas having the highest content in inflammatory cells. In each specimen, more than 1.000 cells were counted in these areas, in random fields, using a x 40 objective magnification. The number of eosinophils divided by the total number of inflammatory cells counted provided the percentage of eosinophil content for each sample. The procedure was done by two independent pathologists without knowledge of the diagnosis or other clinical parameters. In case of disagreement (the two counts differed by $>$ 10 %) a consensus was reached by reviewing the case at a multihead microscope.

Statistics

Data are presented as the mean plus or minus the standard error of the mean (SEM). The parametric t-test was applied for comparison of means. Linear relationships between continuous parameters were measured by the correlation coefficient.

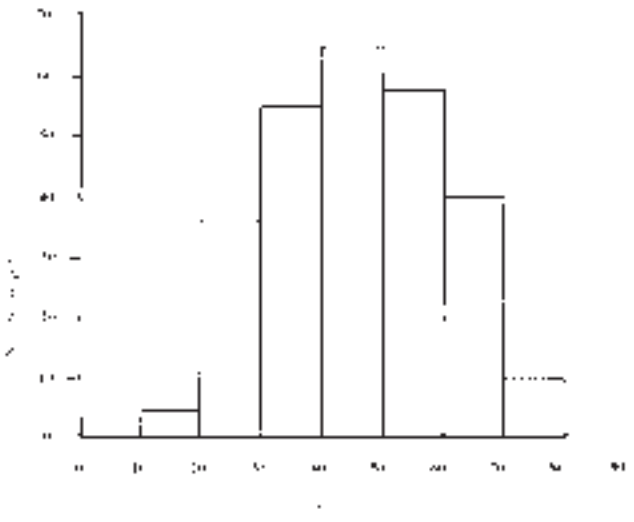

Figure 1. Age distribution in our series of 263 idopathic nasal polyposis patients.

RESULTS

1. Study population

1.1 Idiopathic bilateral nasal polyposis patients (n = 263)

Two hundred and sixty three cases were selected among 412 consecutive patients operated between 1993 and 1998. Males represented 60.5%. Mean age was 45 years (range: 8-77 years) (Figure 1). The polyp size score according to Rouvier's classification was at the time of surgery:

- stage 1 (small polyps reaching the lower edge of the middle turbinate) in 37 (14.1%) patients
- stage 2 (polyps extending between the lower edge of the middle and the upper edge of the inferior turbinates) in 63 (23.9%) patients
- stage 3 (large polyps extending below the upper edge of the inferior turbinate) in 154 (58.5%) patients; polyp size was not described in 9 (3.4%) patients.

Hundred and twenty nine (48.3%) patients reported asthma. Bronchial hyperresponsiveness to carbamylcholine was known in 7 (2.7%) patients without asthma attack but with suggesting symptoms. No history of asthma was reported by 127 (49.05%) patients.

Aspirin sensitivity with clinical manifestations was reported by 7 (2,7%) patients with isolated nasal polyposis and by 60 (22.8%) patients with nasal polyps and asthma (Widal's triad). No information was found in 31 (11.9%) charts.

Atopy (positive skin or multiRAST-tests) was found in 100 (38%) patients. No data were available in 21 (8%) patients.

Patients under systemic steroids at admission to the hospital were 83 (31.5%). There were only 57 (21.7%) patients treated with topical nasal steroids. Patients without systemic and/or topical nasal steroids counted 123 (46.8%). In the asthma population (n = 127), 72 patients (57%) had mild asthma without need for treatment at the time of surgery.

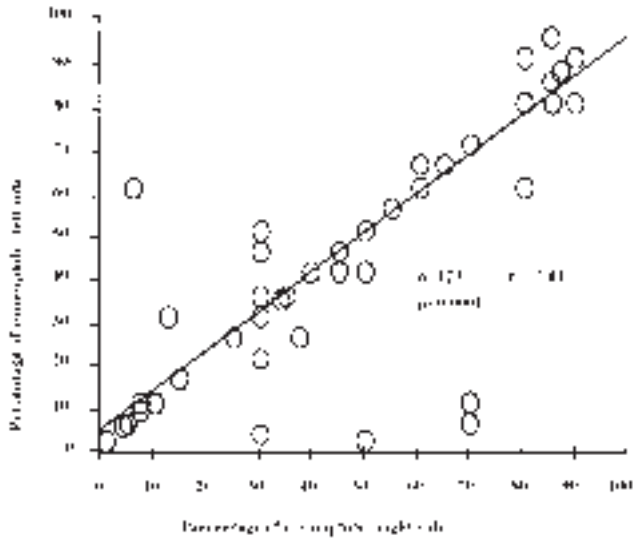

Figure 2. Correlation between right and left: eosinophil infiltration in nasal polyps, which clearly shows the symmetrical distribution of eosinophils in nasal polyps.

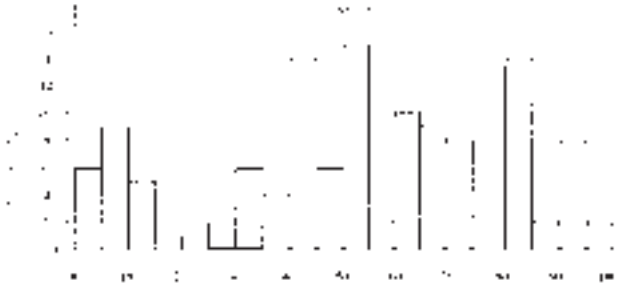

Figure 3. Histogram of the frequency distribution of eosinophils in untreated nasal polyps.

1.2 cystic fibrosis patients (n = 15)

The mean age was 14 years (range: 6-31 years). Males represented 57%. The polyp size score was stage 3 in all except one patient (stage 1). Only one patient out of 15 was atopic. Only two patients received topical nasal steroids at the time of surgery. None was under systemic steroid therapy.

1.3 chronic sinusitis patients (n = 25)

The mean age was 52 years (range: 15-76 years). Males represented 64.5 %. CT scan ethmopacities were found bilaterally in 18 patients, and unilaterally in 13. One patient reported mild asthma (no treatment). Five patients had bronchiectasies, and two others chronic bronchitis. One patient reported aspirin sensitivity. Atopy was found in 7 (28%) patients.

2. Eosinophil distribution in untreated nasal polyps (n = 123)

A good correlation was observed between the left and the right nostrils ($r = 0,43$, $p < 0.0001$) (Figure 2). As a consequence we report only the data of the left side in the following sections. The histogram of the frequency distribution of eosinophils in untreated nasal polyps is presented in Figure 3.

2.1 untreated nasal polyps: with (n = 50) versus without (n = 68) asthma

The mean number of eosinophils was 44 ± 3% in patients without asthma and 58 ± 3 % in nasal polyps of patients reporting asthma ($p < 0.002$).

2.2 untreated polyps with asthma: with aspirin sensitivity (n = 16) versus without aspirin sensitivity (n = 34)

In patients with nasal polyps reporting asthma (n = 50), those who also reported aspirin sensitivity (Widal's triad) showed significantly more eosinophils in their polyps (75 ± 4 %) than those without aspirin sensitivity (51 ± 4 %) ($p < 0.0002$).

2.3 untreated nasal polyps: in atopic (n = 33) versus non atopic patients (n =67)

No data about skin or multi-RAST tests were found in 23 charts of our 123 patients with untreated polyps. No difference in the number of eosinophils was observed between atopic (52 ± 5 %) and non atopic patients (51 ± 3 %).

3. Eosinophil distribution in nasal polyps of patients treated with systemic steroids (n = 83)

We found 83 charts in which the intake of systemic steroids during the last 2 months before surgery was recorded either as a short course or as a long-lasting treatment. The histogram of the frequency distribution of eosinophils in these patients is presented in Figure 4.

The mean number of eosinophils was 22 ± 3 % in these patients, i.e. significantly lower than in nasal polyps of untreated patients (50 ± 2 %, n = 123) ($p<0.0001$).

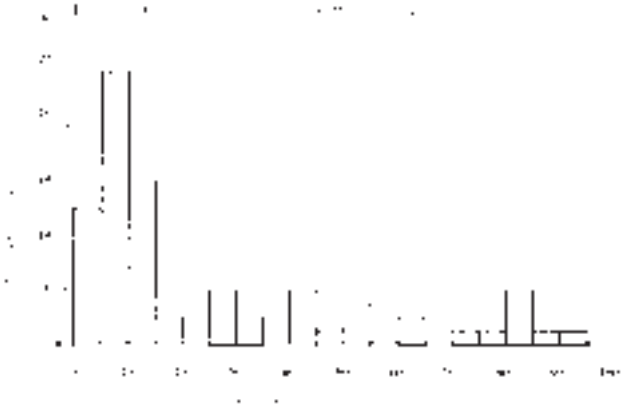

Figure 4. Histogram of the frequency distribution of eosinophils in patients having taken systemic steroids during the last two months before surgery.

3.1 isolated polyposis treated with systemic steroids (n = 28) versus untreated (n = 68)

The mean number of eosinophils was approximately four times lower in the treated (13 ± 3 %) than in the untreated group (44 ± 3 %) ($p < 0.0001$).

3.2 nasal polyposis + asthma patients treated with systemic steroids (n = 23) versus untreated (n = 34).

In patients with nasal polyps reporting asthma and no aspirin sensitivity, the mean number of eosinophils was more than one half lower in the systemic steroid group (19 ± 5 %) than in the untreated group (51 ± 4 %) ($p < 0.0001$).

3.3 widal's triad patients treated with systemic steroids (n = 25) versus untreated (n = 16)

The mean number of eosinophils is approximately one half lower in the treated group (34 ± 5% versus 75 ± 4%) ($p < 0.0001$).

4. Eosinophil distribution in nasal polyps of patients treated with topical steroids only (n = 55)

Among the 263 patients, 55 were only on topical steroids during the two months before surgery. The histogram of the frequency distribution of eosinophils in these patients is presented in Figure 5. The percentage of eosinophils was very similar in the treated (47 ± 2 %) and untreated groups (46 ± 2 %, n = 123). No difference in eosinophil infiltration due to topical steroids was found in the subgroups of patients with isolated polyposis (44 % versus 43 %) and of patients with polyposis and asthma (50 % versus 41 %). In the Widal's triad, patients treated with nasal steroids (n = 16) showed a slightly lower eosinophil content (61 ± 6 %) than untreated patients (75 ± 4 %, n = 16) ($p = 0.05$).

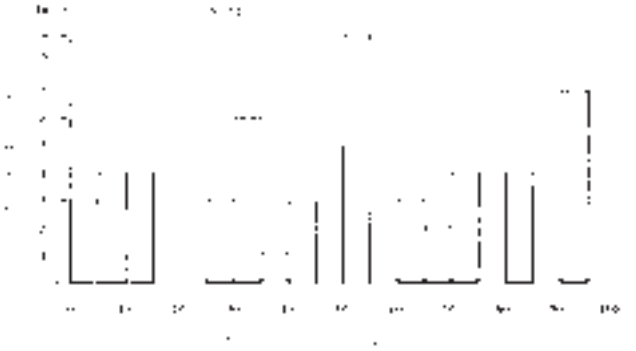

Figure. 5. Histogram of the frequency distribution of eosinophils in patients treated only with nasal steroids during the last two months before surgery.

5. Eosinophil distribution in nasal polyps of cystic fibrosis (n = 15)

None of these 15 patients reported systemic steroid therapy over the last two months before surgery; only two of them were on topical steroids. The histogram of the frequency distribution of eosinophils in these patients is presented in Figure 6. The correlation between left and right side was very high ($r2 = 0.98$, $p < 0.0001$). The mean number of eosinophils was 5 ± 1%.

6. Eosinophil distribution in a control group of chronic sinusitis (n = 25)

None of these patients reported systemic steroid therapy during the last two months before surgery. Eighteen patients were operated on bilateral sinusitis and the histopathologic study found a good correlation between the right and left sides (r2 =

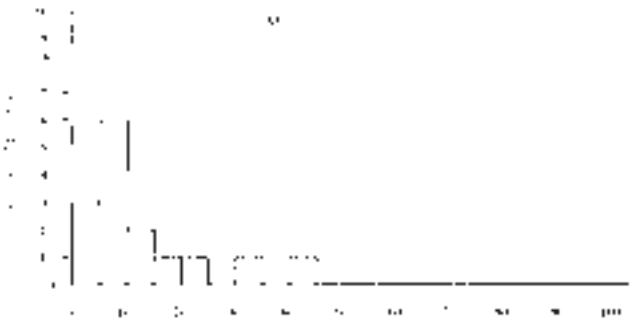

Figure. 6. Histogram of the frequency distribution of eosinophils in polyps from patients with cystic fibrosis.

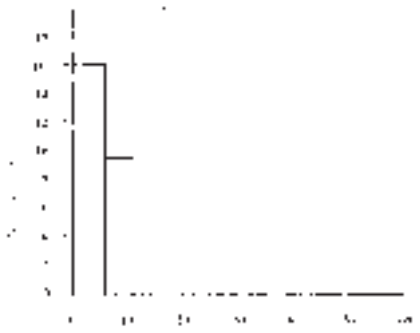

Figure. 7. Histogram of the frequency distribution of eosinophils in patients with chronic sinusitis.

0.49, $p = 0.0008$). The histogram of the frequency distribution of eosinophils in these patients is presented in Figure 7. All these patients showed less than 10% eosinophils. The mean percentage of eosinophils in chronic sinusitis was 2 ± 2 %.

COMMENTS

Our study confirms that eosinophil infiltration is a striking feature of nasal polyposis (Stopp et al., 1993; Bachert et al. 2000). In our control group of chronic sinusitis, all patients show less than 10% eosinophils. In our group of untreated idiopathic nasal polyposis, 83% (103/123) patients show more than 20% eosinophils. The eosinophil infiltration of polyps associated with cystic fibrosis seems to lie in between, with 40% of patients showing more than 10% eosinophils.

Polyps associated to cystic fibrosis have been described as neutrophilic (Sorensen et al., 1976). In the study by Rowe-Jones et al. (1997), polyps from patients without cystic fibrosis contain more eosinophils ($p < 0.001$) whilst polyps from patients with cystic fibrosis contain more neutrophils ($p < 0.001$) and plasma cells ($p < 0.03$). However, in our study eosinophils seem to be present in varying degrees in polyps from patients with and without cystic fibrosis. These data suggest that, both in non-cystic fibrosis and in cystic fibrosis patients, eosinophils could be key cells for understanding the pathophysiology of nasal polyposis (Jankowski, 1996; Bachert et al., 2000). The higher number of neutrophils frequently observed in cystic fibrosis could actually be the result of chronic bacterial infection, which is not usual in idiopathic nasal polyposis.

Approximately 20% (20/123) of our untreated idiopathic nasal polyps show less than 20% eosinophils. One major explanation of low eosinophilia could be associated bacterial infection, a factor we did not systematically record during surgery. Others explanations are however possible: depletion of eosinophils because nasal polyposis disease has come to a non-active phase of its evolution, heterogeneity in the tissue distribution of eosinophils, etc… It might also be that non-eosinophilic polyps represent a different species of nasal polyps with a different clinical behaviour, as suggested by Stammberger (1997).

Nasal polyposis has been regarded as an allergic condition as long as eosinophils were considered markers for allergy. Atopy is rather better defined today according to skin or multi-RAST tests. Our study confirms the results by Pawliczak et al. (1997), who used an advanced morphometric image analysis system: the density of eosinophils is similar in atopic and non atopic patients.

The reason why patients with isolated nasal polyposis have significantly less eosinophils in their polyps than patients with asthma or the Widal's triad is not clear. The number of eosinophils in polyps of the Widal's triad is almost twice the number found in isolated nasal polyposis. (Please remember that isolated nasal polyposis means diffuse bilateral polyposis and does not mean a single polyp). Could this number reflect the severity of the disease ? This would argue again for the key role of eosinophils.

Steroids are well known to be effective treatment for nasal polyposis. Intranasal steroids are, by far, the best documented type of treatment for nasal polyposis. Their mechanisms of action are not well understood. It has been shown mainly in biopsy studies, that topical steroids reduce the total number of eosinophils (Sorensen et al., 1977; Klemi et al., 1997; Tingsgaard et al., 1999). Our results, surprisingly, show no evidence of significant eosinophil reduction after topical steroid treatment. This might be explained in different ways: 1) our patients were operated on their polyps because the efficacy of topical steroids was not satisfactory (failure of topical steroids), 2) our specimens for histologic examination came from surgical removal of both superficial and deep polyps, the last ones being probably not reached by the limited intranasal distribution of a spray. It has also been suggested that, besides a small reduction in total number of eosinophils, topical steroids could mainly act by reducing eosinophils activation (Stopp et al., 1993; Kanai et al., 1994; Tingsgaard et al., 1999).

Systemic steroids are commonly used in the treatment of nasal polyposis because their efficacy is obvious. However, investigation of systemic steroids in polyposis has been remarkably insufficient. Oral steroids have, to our knowledge, not been compared with placebo in any polyposis study. Recently, Bachert et al. (2000) have suggested that oral corticoid treatment may lead to the shrinkage of nasal polyps by down regulation of the eosinophilic inflammation and reduction of the extravasation and deposition of albumin. Interestingly, our results indicate that systemic steroids appear remarkably efficacious to reduce the eosinophil content of polyp tissue. The content in eosinophils is lowered by approximately 3/4 in isolated polyposis, 2/3 in polyposis + asthma, and 1/2 in Widal's triad. A short course of systemic steroids has been shown to be as effective as polypectomy with a snare (Lildholdt, 1997). Thus the efficacy of both treatment could result from the same mechanism, i.e. a significant reduction in eosinophil content.

The reasons why eosinophils accumulate into nasal polyp tissue are unknown, but the hypothesis by Otsuka et al (Otsuka

et al, 1987) is very seducing: nasal polyposis is a self- perpetuating inflammatory reaction caused by tissue-derived growth factors and cytokines in an inductive microenvironment. In this theory, it is also suggested that the cytokines produced by eosinophils themselves may provide an autocrine pathway to maintain migration, viability, and effector functions. In this view, an effective treatment for nasal polyposis should be aimed at making the eosinophils disappear from the nasal mucosa.

In conclusion, we confirm once more that eosinophil infiltration is a characteristic feature of nasal polyposis. The eosinophil content seems related to the clinical presentation with higher counts in patients with asthma and even higher counts in the aspirin triad. On the contrary, atopy has no influence on eosinophil infiltration of the mucosa. Bacterial infection could explain the balance in favour of neutrophils in polyps associated with cystic fibrosis, and perhaps also in a few idiopathic polyposis. Systemic steroids remarkably reduce the eosinophil content, whereas topical steroids show no significant effect in our group of surgically selected patients. We believe that research for a curative treatment of nasal polyposis should be aimed towards eosinophils depletion and eradication.

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