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Short communication

Nasal biopsy is superior to nasal smear for finding eosinophils in nonallergic rhinitis

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The presence of eosinophils was compared in nasal biopsy and smear. Thirty-two nonallergic rhinitis patients, of whom six had nasal polyps, were included in the study. The specimens were studied light-microscopically after staining with hematoxylin-eosin. The association between the presence of polyps and the finding of eosinophils in the biopsy specimens proved to be significant. Ten normal subjects served as controls. It was far more simple to detect eosinophils in the biopsy samples than in the nasal smears. When we considered biopsies with at least four eosinophils in four fields as hypereosinophilic, our group of patients contained 25% nonallergic rhinitis with eosinophilia syndrome (NARES) patients.

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Identified by Paul Ehrlich in the late 19th century, the eosinophil is a bone-marrow-derived granulocyte (1). The numbers of this cell increase in worm infestation and during the course of certain types of allergic disease. There are still some aspects of the eosinophil that are poorly understood. This cell dampens the mast-cell-derived inflammatory mediators, but it also has a high potential for cytotoxicity. It contains proteins, such as major basic protein (MBP) and eosinophil cationic protein (ECP), that damage the epithelial cells in asthmatic patients (2).

Eosinophils are present in normal mucosa, but they appear in larger numbers in the nasal mucosa during the late phase of an atopic reaction (3). However, they are also found in nonallergic rhinitic noses. Jacobs et al. (4) pointed out the hyper-reactivity of the mucosa infiltrated by eosinophils, while Mullarkey et al. (5) described the development toward sinonasal polyps, intrinsic asthma, and acetylsalicylic acid (ASA) intolerance. The latter authors included patients with nasal polyps in their study, in contrast to the former. They called this disorder the nonallergic rhinitis with eosinophilia syndrome (NARES).

The triad of ASA intolerance, nasal polyps, and asthma is called the ASA-triad (6) or triad of Fernand-Widal in the French literature (7). It is believed that a link exists between NARES and the

ASA-triad. Some authors even think there is an evolutionary pathogenic mechanism beginning with NARES as a "slipping form" of vasomotor rhinitis caused by some kind of disturbance in the autonomic nervous system, leading to the blood eosinophilia with nonallergic rhinitis with secretory eosinophilia syndrome, and finally ending in the virtually complete ASA-triad (8).

Eosinophils can be demonstrated by nasal lavage tests, nasal smears, or biopsies. The problem with nasal smears and lavage tests is that eosinophils clump, and their number is extremely variable. They can be quantified by absolute numbers, or in a differential way. The finding of eosinophils in the nasal mucosa has clinical significance, since these patients more readily respond to steroids (9, 10). It was our experience that nasal smears and lavage tests are not very sensitive in detecting eosinophils. We wanted to investigate the suitability of nasal biopsies in nonallergic rhinitis patients. In order to be able to make our own diagnostic criteria, we compared the results with nasal smears and biopsies of normal subjects.

Material and methods

Thirty-two patients (mean age 40.9 ± 14.7 years) with a history of chronic nose obstruction, sneezing, and rhinorrhea were selected. Allergy was

excluded by standard allergen skin testing, the radioimmunosorbent test for total IgE, and the radioallergosorbent test. The patients were endoscopically investigated for the presence of polyps. With a cotton wool applicator, a nasal smear was made by rubbing vigorously the inferior turbinate of the most patent side of the nose. The smears were immediately applied to a glass slide and then inserted into 95% alcohol. Mucosal biopsies were taken with a 2-mm Gerritsma forceps (11) from that same inferior turbinate after local anesthesia with lidocaine 4%. The specimens were fixed in formaldehyde and 5- μ m sections were cut after routine paraffin embedding.

Ten patients who were to be operated for a deviated septum without mucosal disorder served as controls. Allergy was excluded in the same way as in nonallergic rhinitis patients. During septoplasty, a nasal smear and biopsy were taken and elaborated in the same way as described above.

The nasal smears and biopsies were stained with hematoxylin-eosin (HE). Two of the authors (J.-P. D., C. C.) used an Olympus BH2 light optic microscope, magnification $\times 400$, to read the specimens blindly and in an independent way, for the presence of eosinophils. The mean of their scores was used for statistical analysis. Because of the limited number of microscopic fields suitable for examination in nasal smears, it was only possible to take a common number of two fields into consideration. At least four fields could be examined in all nasal biopsy specimens.

Results

In only one of the 32 patients studied could eosinophils be demonstrated in the smear. Eosinophils were, however, found in at least one area of the nasal biopsy in 15 patients. The number of eosinophils per field varied from 1 to 20. Eight patients showed at least four eosinophils in four fields. These eosinophils were always extravascular in the submucosal tissue or intra-epithelial.

Nasal polyps were detected endoscopically in six patients. Five of those patients showed eosinophils in the mucosal biopsy, but none had positive nasal smears for eosinophils. The association of the presence (or absence) of eosinophils and polyps is shown in Table 1. This association between eosinophils and polyps was shown to have borderline significance ($P = 0.021$) by Fisher's exact test.

Eosinophils were not found in the nasal smears of 10 control patients. In the biopsy specimens of two normal subjects, an eosinophil was demonstrated only in a single microscopic field.

Table 1. Two-by-two table for presence and absence of eosinophils and polyps in 32 nasal biopsies

	Eosinophils		
	-	+	
Polyps	-	22	2
	+	4	4

Discussion

There are various methods to demonstrate eosinophils, some more sensitive than the others. In a light microscope, eosinophils are recognizable by their content of red granules with eosin staining. The May-Grünwald-Giemsa stain, Hansel stain, Wright stain (12), HE stain, and Luna stain (9) are all possible methods of light-microscopic investigation. In the electron microscope, the granules are characterized by a disc-shaped, crystalline structure (13). On activation, the granules release cytotoxic proteins such as MBP, eosinophil peroxidase, ECP, and eosinophil protein X. The presence of any of these four proteins in the human serum is often used as an index of eosinophil activation (1). Electron-microscopically, eosinophils exist in various densities from normodense to hypodense. Hypodense eosinophils are probably activated and more toxic than the normodense (14). Immunologic methods can demonstrate the presence of receptors for IgG, IgM, IgE (low-affinity receptors), and C3b on the cell surface (1). Anti-BMK13 is a paneosinophil marker and binds to MBP in resting and activated eosinophils.

Moneret-Vautrin et al. (8) have specified the criteria for diagnosis of NARES. Allergic skin tests with standard allergens have to be negative. The IgE titer may not surpass 50 IU/ml. Finally, more than 20% of eosinophils have to be present in at least 10 microscopic fields of a nasal smear. There is controversy about the last criterion, because Mygind (12) specifies 10% of eosinophils, and Mullarkey et al. (5) consider a smear "hyper-eosinophilic" when 25% or more of the inflammatory cells are eosinophils.

However, with our technique of taking nasal smears, we were not able to demonstrate eosinophils adequately. This is in contrast to the findings of Phillips et al. (10), who suggest that nasal smears accurately reflect the eosinophil content in the mucosa. We think this is not probable because most eosinophils are found in the subepithelial layer. Lans et al. (15) made nasal smears in 100 allergic and nonallergic patients, and found that in 57% of allergic patients the eosinophil count was more than 20%. No eosinophils were found in controls

nor in patients with nonallergic rhinitis. This led them to conclude that the nasal smear is an insensitive but specific test for allergic rhinitis. However, polyps and aspirin sensitivity were excluded in that study. Our inability to demonstrate eosinophils in nasal smears may be explained by the fact that we did not use a curet for sampling.

From our results, it was clear that, even with a simple HE-staining technique, eosinophils are much more easily found in mucosal biopsies than in nasal smears. Consequently, the following question arises: "When are biopsies hypereosinophilic?" Taking into account our results in normal subjects, one might consider biopsies hypereosinophilic when they contain more than one eosinophil in four fields. However, this criterion would introduce too many false positives. We propose sharpening the criterion and considering a biopsy specimen "positive" for eosinophils when at least four eosinophils are demonstrated in four microscopic fields. This means that in our group of nonallergic rhinitis patients, eight subjects (25%) had hypereosinophilic mucosa, and thus might be diagnosed as NARES patients.

Our findings seem in accordance with those of Moneret-Vautrin et al. (8), who consider 15% of nonallergic rhinitis patients to have the eosinophilic form. These patients complain of profuse watery rhinorrhea and sneezing, while the occurrence of hyposmia is striking. Probably there is a pathogenetic development toward the ASA-triad. The symptoms and polyps respond very well to corticosteroids (90%) and, to a lesser degree, to antihistamines (80%). In our group of eight patients with nonallergic rhinitis with eosinophilia, four had polyps in the middle meatus.

Our study suggests a strong relationship between the occurrence of nasal polyps and the presence of eosinophils in biopsies. As Stoop et al. (14) demonstrated, eosinophils play an important role in chronic inflammatory processes. Yamashita et al. (16) proposed a pathogenetic scheme for the formation of polyps. Eosinophilic mediators such as MBP and ECP, together with denervation of blood vessels and glands, cause an increased vascular permeability and edema that lead to the formation of polyps. Therefore, it seems reasonable that eosinophils at least betray a hyperreactivity state.

We are well aware that much more sensitive tests are available than HE staining with light microscopic investigation. There are also biopsy sites that are more representative of the presence of eosinophils and polyp formation than the inferior turbinate. However, the HE-staining technique is simple, cheap, and easy to perform, and the inferior turbinate as a biopsy site is easy to access. From our results, we can conclude that

- 1) biopsies are much more sensitive than nasal smears in detecting eosinophils
- 2) there is a correlation between the presence of polyps and the finding of eosinophils in the mucosa
- 3) in HE-stained normal nasal biopsies, very few eosinophils are demonstrated by light microscopic investigation.

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