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Eosinophil count in nasal secretions of subjects with and without nasal symptoms*

Roger Jankowski[1], Monique Persoons[1], Bernard Foliguet[2], Laurent Coffinet[1], Christine Thomas[2], Bettina Verient-Montaut[1]

[1] Department of Otorhinolaryngology, Head and Neck Surgery, Central Hospital, Henri Poincaré University, Nancy, France
[2] Laboratoire de Biologie Sexuelle, Maternité Régionale, Nancy, France

SUMMARY

The aim of this paper, based on a cross-sectional study of 129 patients with nonallergic chronic nasal symptoms and 40 healthy controls, was to examine the leucocyte differential count in nasal secretions as a diagnostic test. Nasal secretions were collected using preweighed suction glass canulas under controlled conditions (–100Pa, 30 sec). Leucocyte and differential counts were performed using a Thoma hemocytometer and on cytospin slides after May-Grünwald-Giemsa staining. The percentage of eosinophils (Eo) was significantly higher in patients (mean±SEM: 15.1±2.3%) than in controls (5±2.6%) ($p<0.04$). Comparison of the frequency distribution of the percentage of Eo in patients and controls clearly showed a subgroup of patients presenting with nasal secretion hypereosinophilia, and allowed us to set the positivity criterion at Eo=20%. Diurnal variations in Eo count in 11 controls and 8 patients confirmed the value of the cutoff point. In 28 patients with nasal polyposis who underwent surgery, a correlation was found between secretion and tissue eosinophelia ($r=0.58$, $p=0.001$). Patients with nasal secretion hypereosinophilia had no more leucocytes in their secretions than healthy controls, the increase in eosinophils being balanced by a decrease in neutrophils. In patients without hypereosinophilia, the number of leucocytes per milligram of secretion was four times higher (8672±2521) than in the controls (2020±823) ($p=0.06$) (cut-off point = 2500 leu/mg). These data show that the nasal cytogram can be modified either in qualitative or quantitative way, probably depending on the underlying inflammatory process.

Key words: nasal cytogram, nasal polyps, rhinitis, sinusitis

INTRODUCTION

Nasal secretions represent a first line defense medium, in which the leucocyte compartment (Jankowski et al, 1995) probably acts as an efficient part of the defense mechanism along with the mucociliary transport system and the biochemical properties of the mucus. If this assumption is true, inflammation of the nasal mucosa should quantitatively and/or qualitatively modify this leucocyte compartment.

The aim of this paper, based on a prospective cross-sectional study , was to describe the distribution of leucocytes and eosinophils in nasal secretions of a population of patients with a complete spectrum of chronic rhinosinopathy (from mild to severe disease), and to see if nasal cytology could help to differentiate healthy volunteers from patients.

The presence of eosinophils in nasal secretions is known since the first description by Eyermann in 1927. Eosinophil cells were initially regarded as indicative of allergic rhinitis, but have recently been associated with nonallergic rhinitis. Mullarkey et al (1980) and Jacobs et al (1981) described the NARES (Non Allergic Rhinitis with Eosinophelia Syndrome) and set empirically the cut-off point for hypereosinophilia at 20%. However, we do not know if significant eosinophelia may be found in healthy secretions, or in fact whether patients with chronic nasal symptoms have more eosinophils in their secretions than controls. If this is, indeed, the case what proportion of such patients have hypereosinophilia.

Chronic nasal complaints can be generated by structural abnormalities, inflammation of the mucosa, vasomotor or nerve dysfunctions, etc… There is no gold standard for nasal inflammation. Our secondary aim was to look at how qualitative or quantitative changes in the nasal leucocyte differential count may help to diagnose nasal inflammation.

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PATIENTS AND METHODS

Patients

Patients were identified from a ENT Clinic in a tertiary referral care center. Those patients included were those with chronic perennial nasal complaints lasting for longer than one year. Patients were primarily selected on a negative history of allergen-induced symptoms. Patients with a clear cut history of allergic rhinitis (rhinorhoea, sneezing, etc…, in response to known allergens) were excluded as an eosinophelia would be expected in this group (Pelikan, 1983).

Endoscopic findings could range from no detectable abnormality to diffuse and bilateral polyposis. Exclusion criteria included severe septal deviation (that would not enable collection of secretion with a suctioning canula), tumours (such as carcinoma or inverted papillomas), pseudotumours (e.g. antrochoanal polyps or mucocele), frank purulent secretions or crusts. CT-scan was not systematic in all patients, but CT-scan findings could range from no opacity to diffuse and bilateral opacification of the sinuses. Patients with specific diseases like cystic fibrosis, ciliary dyskinesia, immunoglobulin deficiency, Wegener's syndrome, etc… were also excluded.

The following criteria were used to select healthy volunteers: no past history of medical or surgical disease of the nose; no acute or chronic nasal symptoms; no allergic symptoms or past history of hay fever or periodic rhinitis, asthma, eczema, urticaria, food allergy; no use of topical drugs; no intake of steroids or anti-inflammatory drugs; smoking habits less than 10 cigarettes per day. Skin or RAST tests were not required. Forty healthy volunteers agreed to participate in the study. All subjects gave informed consent before entry, and the study was approved by the Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale (CCPPRB) de Nancy.

Table 1. Clinical and demographic data on the study populations.

	PATIENTS	CONTROLS
n	129	40
mean age	38 years	27 years
range	8-81 years	19-33 years
Sexe	71M/58F	17M/23F
chronic rhinosinopathy CRS	90 18 asthmatics 72 non asthmatics	0
Nasal polyposis NPS	39 21 asthmatics 18 non asthmatics	0
Skin or multi-RAST tests = not done	37 (29.1%)	
Skin or multi-RAST tests = negative	59 (45.7%)	
Skin or multi-RAST tests = positive	33 (25.5%)	

Study Design

1. Patients

1.1 Baseline-methacholine protocol

Two samples of nasal secretion were obtained with an eight-minute interval between both nostrils of each patient. The first samples were collected at baseline. Because nasal secretions can be scarce in some patients, 5 minutes later we induced nasal glandular secretion by spraying methacholine into the nose. The methacholine (Laboratoire Aldrich, St Quentin Falavier, France) at a concentration of 15 mg/ml was delivered by a metered-pump spray (VP3 pump, Laboratoire Valois Le Neubourg, France). Two doses of 0.1 ml each were sprayed into each nostril to deliver a total dose of 6 mg methacholine. Based on our previous data (Jankowski et al., 1995) the post-methacholine samples of nasal secretion were collected three minutes after the sprays.

1.2 Baseline-postbaseline protocol

In patients with asthma methacholine was not used on the recommendation of the CCPPRB, due to its potential bronchoconstrictor effect. The second samples were obtained 8 minutes later after saline was sprayed into the nose.

1.3 Diurnal variations in eosinophil count

Eight patients (7 CRS and 1 NPS) volunteered to stay in the department for 8 hours and had baseline secretion collections taken at 2 hour intervals.

1.4 Correlation between secretion and tissue eosinophelia in nasal polyposis

Twenty-eight NPS were operated on, and polyps tissue were systematically sent for histological examination. Tissue eosinophelia was expressed in a semi-quantitative way as a percentage of the total leucocyte count. Nasal secretions were collected the day before surgery.

2. Volunteers

Healthy volunteers were divided into three groups. Group 1 (n=10) was given the baseline-methacholine protocol. In group 2 (n=11), we designed a protocol to study the diurnal variations of the leucocyte compartment. Volunteers stayed in the lab for 8 hours. Secretions were collected on both sides at baseline every two hours. In group 3 (n=19), baseline secretions were collected only on the right side.

Nasal secretion collection

Subjects were first asked to blow their nose and to take a semi-seated position on an examination table. Four to 5 cm of a suction glass canula (Ets Descharmes, Nancy, France) were gently introduced under direct vision into the nose, along the nasal septum onto the floor, and then swept across the inferior and middle meatus as it was brought out. As blood contamination in the sample could change the leucocyte count, great care was taken to avoid undue trauma to the nasal mucosa. Due to the transparent nature of our canula we were able to confirm that, in fact, no blood contamination had occurred in any of our col-

Table 2. A- Baseline and post-methacholine secretion in non asthmatic patients (n=90)
B- Baseline and post-baseline secretion in asthmatic patients (n=39)
C- Baseline and post-methacholine secretion in healthy volunteers (n=10)

Table 2 A: non asthmatics	baseline secretion		post-methacholine secretion	
	right nostril	left nostril	right nostril	left nostril
Secretion weight(mg)	40.3±4.2	46.4±4.7	127.8±7.8	130.8±8.7
Total leucocyte number	294,246±139,052	222,194±85,755	300,193±123,956	359,774±144,049
Leucocytes/mg	6,514±2,160	3,776±946	1,760±495	2,495±1,014
Neutrophils(%)	80.1±3.2	73.7±3.7	84.4±2.6	85±2.5
Eosinophils (%)	14.3±2.7	15.4±2.82	13±2.4	12.4±2.3
Basophils (%)	0.07±0.05	0.1±0.1	0.07±0.06	0.1±0.09
Lymphocytes (%)	0.2±0.1	0.07±0.03	0.2±0.1	0.3±0.2
Monocytes (%)	0.8±0.2	0.6±0.1	1±0.3	1±0.3

Table 2 B: asthmatics	baseline secretion		post-baseline secretion	
	right nostril	left nostril	right nostril	left nostril
Secretion weight (mg)	34.9±5.9	51.6±7.8	85.6±12.5	98.9±14.6
Total leucocyte number	329,763±126,607	588,166±265,007	406,220±176,884	609,319±337,802
Leucocytes/mg	6,239±2,030	6,293±2,259	4,194±1,371	4,506±1,392
Neutrophils(%)	76.2±5.2	75.9±4.5	79.9±4.8	71±5.3
Eosinophils (%)	17±4.2	16.9±3.5	16.5±4.2	22.9±4.7
Basophils (%)	0.1±0.1	0.07±0.04	0.1±0.09	0.07±0.05
Lymphocytes (%)	0.3±0.2	0.21±0.1	0.1±0.06	0.07±0.04
Monocytes (%)	1±0.4	0.5±0.1	0.7±0.1	0.4±0.1

Table 2 C: controls	baseline secretion		post-methacholine secretion	
	right nostril	left nostril	right nostril	left nostril
Secretion weight (mg)	50.6±9.3	68.2±11.3	197.4±23.4	287.1±106.7
Total leucocyte number	135,662±81,648	18,210±7,013	111,115±98,845	17,264±4,209
Leucocytes/mg	3,866±2,660	399±161	439±363	99±35
Neutrophils(%)	98.1±1.7	71.1±18.3	93.9±3.2	93.7±1.8
Eosinophils (%)	1.7±1.7	0	0	0.3±0.3
Basophils (%)	0	0	0	0
Lymphocytes (%)	0.1±0.1	0.2±0.2	5.4±3.1	5.2±1.9
Monocytes (%)	0	0	0.7±0.4	0.6±0.3

lections. The suctioning depression was maintained at –100 Pa via a manometer. The suctioning time was 30 sec. Each canula was pre-weighed (Precisa 160M, Pag Oberlikon AG, Switzerland). The weight of secretion was calculated by subtracting the pre- from the post-collection weights. The canula was then sealed with a silicone plug at one end and filled with 2 ml of saline, in which a small amount of a mucolytic agent (Digesteur®, Eurobio Laboratoires, Les Ulis, France) was dissolved. After sealing, the canula was stored at +4° until processing within 1-24 hours of collection.

Cell processing

The sample was transferred to a tube, followed by a 2 ml saline rinse of the canula, and vigorously shaken to obtain a homogenized suspension. One millilitre of this suspension was transferred to a centrifuge (2.000*g* for 30 min). The supernatant (900 µl) was discarded. The cell pellet was resuspended in the remaining 100 µl. For the cell count, 10 µl of the final suspension was placed in a Thoma hemocytometer. After 5 min, the number of leucocytes was counted. This number, multiplied by the dilution factor, gave the total number of leucocytes in the starting suspension, i.e. in the amount of collected secretions. The instrumental reproducibility of this measure, tested on 11 samples, showed a coefficient of variation of 28%.

Differential counts were performed on cytospin slides. On the basis of total cell count, 200-500 µl of the starting suspension was centrifuged at 1.250*g* for 30 min. Slides were stained with May-Grünwald-Giemsa. We counted 100 leucocytes on each slide and categorized them as neutrophils, eosinophils, basophils, lymphocytes, and monocytes (a category that included true monocytes and mononuclear cells of unclear origin). The results were expressed as percentages. Epithelial cells were not counted.

Statistical analysis

Data are presented as mean±standard error of the mean (SEM). Parametric paired or unpaired *t*-tests were applied for comparison of means, and the chi-squared test for comparison of proportions. Linear relationships between continuous parameters were measured by the correlation coefficient.

RESULTS

The clinical and demographic data of the study populations are given in Table 1. A total of 139 consecutive patients participated in the study, but in 10 cases secretion collection or sample processing were defective. These 10 cases were excluded. The remaining 129 subjects were classified according to the following criteria: chronic rhinosinopathy without nasal polyposis (CRS) or with nasal polyposis (NPS), asthmatic (asth) or non-asthmatic (non-asth). The distinction between CRS and NPS was exclusively based on endoscopic findings. Patients with NPS showed bilateral benign edematous polyps protruding from the meatus into the nasal cavity (Lildholt, 1994). All other patients were considered as chronic rhinosinopathy without nasal polyposis (CRS). A patient was considered non asthmatic when he presented with no history or symptoms of asthma. If suspicious symptoms were found, the patients were referred to a pulmonary physician to clarify the diagnosis. All patients had a negative history of allergen-induced symptoms. However, many patients had previously undergone skin or multi-RAST tests at other institutions. We used these retrospective data and ended-up with a subclassification of our patients in three subgroups according to results of these tests (not done, positive, or negative skin or multi-RAST tests).

1. Baseline-methacholine protocol in non asthmatic patients (n=90)

The data is summarized in Table 2A. No significant difference was observed between right and left nostrils both at baseline and after methacholine stimulation. The comparison between baseline and post-methacholine samples showed that methacholine significantly increased the secretion weight ($p=0.0001$), but did not change the total number of leucocytes collected in 30 seconds. As a consequence, the number of leucocytes per milligram of secretion tended to be significantly lower in post-methacholine secretion ($p<0.10$). The percentage of neutrophils appeared significantly higher in post-methacholine secretions compared to baseline in both nostrils ($p<0.04$), whereas the percentage of eosinophils did not change.

In 10 right and 12 left nostrils, we could not get useful information at baseline, either because the number of leucocytes was too low (less than 100) or because cell identification was difficult. In these patients, the post-methacholine sample allowed a correct leucocyte and differential count in 6 right and 11 left nostrils (i.e. 17/180 samples ~ 10 %).

2. Baseline-post baseline protocol in asthmatic patients (n=39)

The data is summarized in Table 2B. No significant difference was observed between right and left nostrils both at baseline and in post-baseline secretions, except for the baseline secretion weight which was significantly higher in the left nostril ($p<0.008$). The comparison between baseline and post-baseline samples showed a significant difference only in the secretion weight ($p<0.0005$). No difference was observed in the eosinophil count. In 8 right and 4 left nostrils, we could not get useful information at baseline. In these patients, the post-baseline sample allowed a correct leucocyte and differential count in 6 right and 2 left nostrils (i.e. 8/78 samples ~ 10%).

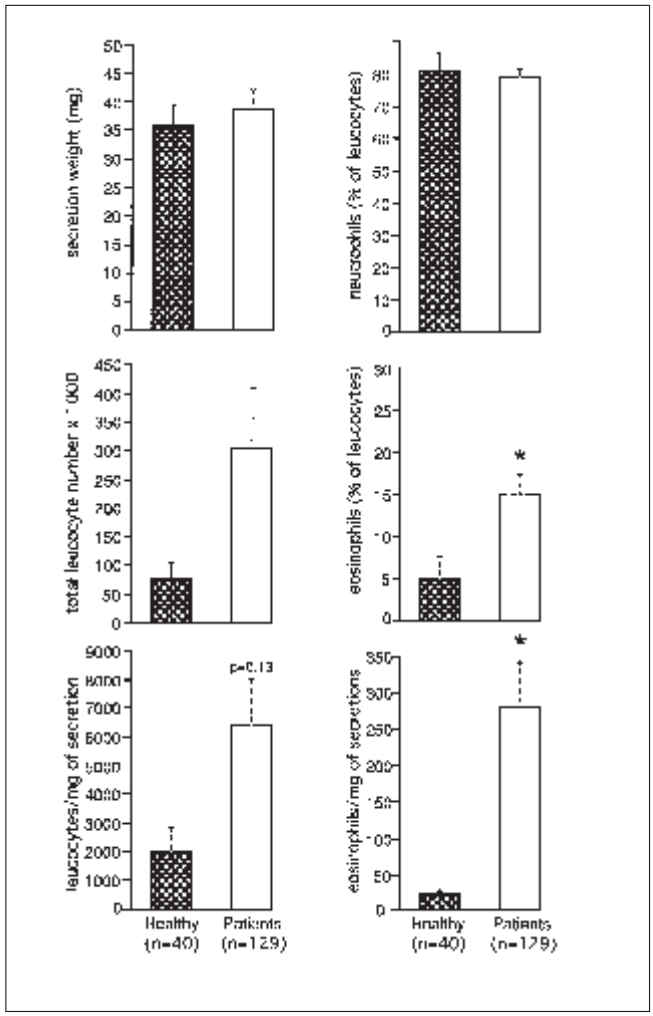

Figure 1. Comparison between baseline secretion of healthy volunteers and patients (right nostril) (*=$p<0.05$)

3. Baseline-methacholine protocol in healthy volunteers (group 1)

The data is summarized in Table 2C. At baseline, the amount of secretion collected on the left side was significantly higher than on the right side ($p=0.005$), and the number of leucocytes tended to be lower on the left side ($p=0.06$). Methacholine significantly increased the baseline secretion weight ($p<0.006$), but did not change the total number of leucocytes collected in 30 seconds. As a consequence, the number of leucocytes per milligram of secretion tended to be significantly lower in post-methacholine secretion. No difference was observed in the eosinophil count.

In 2 right and 3 left nostrils the leucocyte count was possible, but a satisfactory differential count could not be obtained at baseline. The post-baseline samples allowed a correct differential count in 2 right and 2 left nostrils (i.e; 4/20 samples ~ 20%).

4. Comparison between baseline secretion of healthy volunteers and patients

In order to simplify and clarify the presentation of our results, comparisons are only made with respect to the right nostril (Figure 1). The only significant difference was in the eosinophil count : 5±2.6 % in healthy volunteers versus 15.1±2.3 % in patients ($p<0.04$). The mean number of eosinophils per milli-

Table 3. Diurnal variations in the number of leucocytes and the percentage of eosinophils in healthy subjects (n=11) and patients (n=8)
Eosinophil count in nasal secretions

Healty Volunteers (n=11)		*#1*	*#2*	*#3*	*#4*	*#5*	*#6*	*#7*	*#8*	*#9*	*#10*	*#11*
Right nostril	8 o'clock	0	0	0	0	0.5	1	3	1	0	0	3
	10 o'clock	0	1	1	1	1	0	8	2	1	0	1
	12 o'clock	0	2	0	2	0.5	0	1	0	1	0	0
	14 o'clock	0	2	0	1	0.5	2	1	1	0	1	0
	16 o'clock	2.7	0	2	1	0.5	9.1	1	1	1	2	3.2
	Range	*0-2.7*	*0-2*	*0-2*	*0-2*	*0.5-1*	*0-9.1*	*1-8*	*0-2*	*0-1*	*0-2*	*0-3.2*
Left nostril	8 o'clock	0	0	0	0	0	1	2.0	0	0	0.1	0
	10 o'clock	0	1	5	2	0.5	1	16	0.5	0	0	6.6
	12 o'clock	0	2	0	1	1	1	14	1	0	4	0
	14 o'clock	5.3	3	2	3	0.5	2	7	1	4.8	1	5.6
	16 o'clock	0	1	1	2	0.5	1	10	1	0	0	0
	Range	*0-5.3*	*0-3*	*0-5*	*0-3*	*0-1*	*1-2*	*7-2 0*	*0-1*	*0-4.8*	*0-4*	*0-6.6*

Patients (n=8)		*#1*	*#2*	*#3*	*#4*	*#5*	*#6*	*#7*	*#8*
Right nostril	time 1	2	1	0	0	1	45	80	57
	time 2	0	1	11	0	0	60	84	26
	time 3	8	1	2	0	0	70	75	65
	time 4	2		0				96	
	Range	*0-8*	*1-1*	*0-11*	*0-0*	*0-1*	*45-70*	*75-96*	*26-65*
Left nostril	time 1	8	2	1	0	0	35	45	88
	time 2	8	2	0	0	1	55	78	77
	time 3	22	2	60	0	0	50	70	60
	time 4	7		0				80	
	Range	*7-22*	*2-2*	*0-60*	*0-0*	*0-1*	*35-55*	*45-80*	*60-88*

gram of secretion was 19±8 in healthy volunteers versus 274±67 in patients. The number of leucocytes per milligram also tended to be higher in patients than in healthy volunteers (6438±1613 vs 2020±823, p=0.13).

5. Frequency distributions of the percentage of eosinophils in healthy subjects and patients (Figure 2)

The highest eosinophil percentage was selected among the four samples from each patient. The large majority of selected samples came from baseline secretions. The post-methacholine sample was selected in only 8 out of 90 non asthmatic patients. The post-baseline sample was selected in 12 out of 39 asthmatic patients. The highest eosinophil percentage was also selected among all the samples of each healthy subject.

Figure 2 represents the eosinophil frequency distributions in healthy subjects and patients. In healthy secretions, only one subject out of 40 (2.5%) showed an eosinophil count higher than 20% (Eo = 87%). In patients, 46/129 (35.7%) showed an eosinophil count higher than 20%, whereas 83/129 patients showed an eosinophil distribution similar to healthy subjects. Accordingly, the cut-off point was set at Eo > 20%.

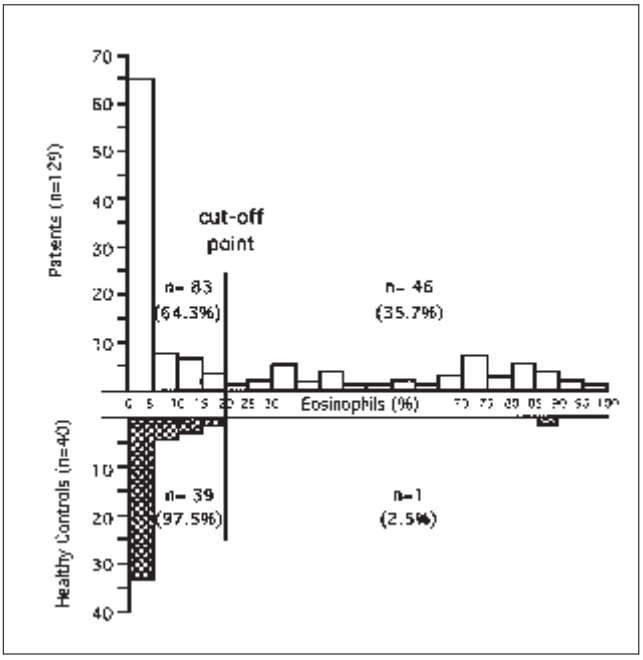

Figure 2. Frequency distribution of the percentage of eosinophils in healthy subjects (n=40) and patients (n=129).

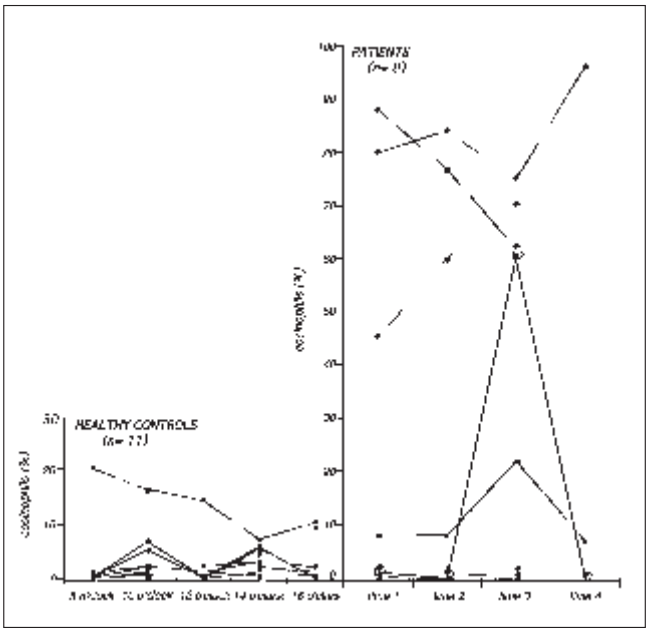

Figure 3. Diurnal variations of the percentage of eosinophils in one nares of 11 controls and 8 patients (The two nares have been investigated : See Table 3. To plot this graph the nares with the highest range was selected).

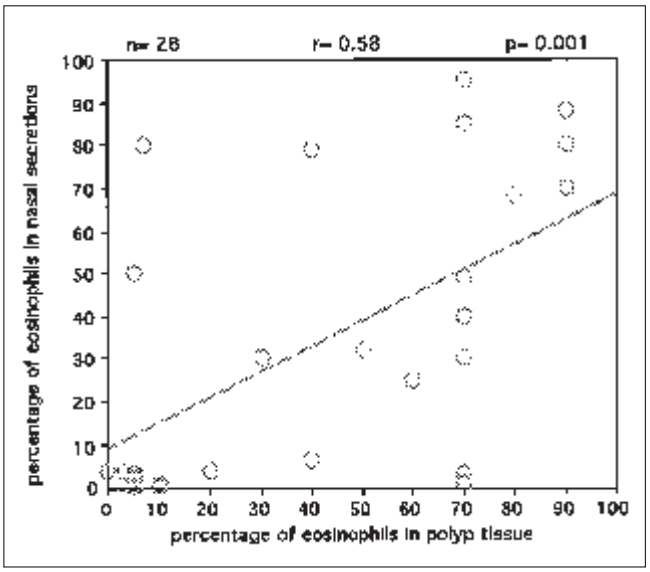

Figure 4. Correlation between secretion and tissue eosinophelia in nasal polyposis.

6. Diurnal variations in eosinophil count (Table 3)

6.1 Healthy volunteers (group 2, n=11)

Eosinophils were scarce in healthy secretions, but were observed in each subject at least in one out of 5 samples collected over an 8 hour period. The lowest diurnal range was 0-1%, the highest 7-20%. The scores were fairly similar in both nostrils.

6.2 Patients (n=8)

Four patients (#1, 2 ,4, 5) had a constantly low eosinophil percentage (range : 0-22%), and three patients (#6, 7, 8) a constantly high percentage (range 26-96%). Patient no.3 generally had a low percentage of eosinophils, but showed on one occasion a unilateral rise to 60%. Except for patient no.3, when the eosinophil count was low on one side, it was also low on the opposite side. The same was true for patients with high eosinophil counts.

Figure 3 represents the diurnal variations of the percentage of eosinophils in the nostril that showed the highest range in each patient and control.

7. Correlation between secretion and tissue eosinophelia in nasal polyposis (Figure 4)

In the group of NPS who underwent surgery (n=28), 12 patients had secretion eosinophelia lower than 20% and 16 were higher. Eleven patients had tissue eosinophelia lower than 20% and 17 were higher. A relatively poor correlation was found between tissue and secretion eosinophelia ($r=0.58$, $p=0.001$). Only two patients with low tissue eosinophelia (5 and 7%) had high secretion eosinophelia (50 and 80%, respectively). Inversely, three patients with low secretion eosinophelia (6, 3 and 1%) had high tissue eosinophelia (40, 70 and 70%, respectively)

8. Distribution of patients with and without nasal hypereosinophilia in the clinical subgroups

The data is summarized in Figure 5. The proportion of patients with Eo>20% was not significantly different in the three subgroups of skin (RAST) test>0, skin (RAST) test<0, and skin (RAST) test=ND ($\chi^2<5.99$). This was also the case when we considered only the two subgroups of skin (RAST) test>0 and skin (RAST) test<0 ($\chi^2<3.84$). Eo>20% was observed in approximately one half of patients (21/39) with nasal polyposis and one quarter (25/96) of patients with chronic rhinosinusitis ($\chi^2=7.83$, $p<0.01$). Eo>20% was also found significantly more frequently in patients with asthma ($\chi^2=7.83$, $p<0.01$) (in one half (21/39) of patients with asthma versus one quarter (25/90) of patients without asthma).

9. Nasal secretion eosinophelia and number of leucocytes

The data is summarized in Table 4. The total number of leucocytes in baseline secretions was fairly similar in both controls and patients with nasal hypereosinophilia (Eo>20%), but was approximately four times higher in patients without hypereosinophilia (Eo≤20%) ($p= 0.13$). Comparison of the number of leucocytes per milligram showed the same trend ($p=0.06$) (Figure 6). Patients without hypereosinophilia had mainly neutrophils in their secretions, and the percentage of eosinophils was significantly lower than in the controls ($p=0.03$).

The same conclusions could be drawn by comparing the subgroups of nasal polyposis and chronic rhinosinopathy with the controls. In each subgroup, patients without hypereosinophilia (Eo≤20 %) tended to have more leucocytes per milligram of secretion than controls (p-values were 0.06 and 0.05 in nasal polyposis and chronic rhinosinusitis, respectively) whereas no difference in leucocyte number was observed between controls and patients with nasal hypereosinophilia.

As patients without nasal hypereosinophilia tended to have significantly more leucocytes in their baseline secretions than controls, we compared the frequency distributions of the number of leucocytes in these two groups. Figure 7 shows that the cut-off point for the number of leucocytes per milligram could be set at

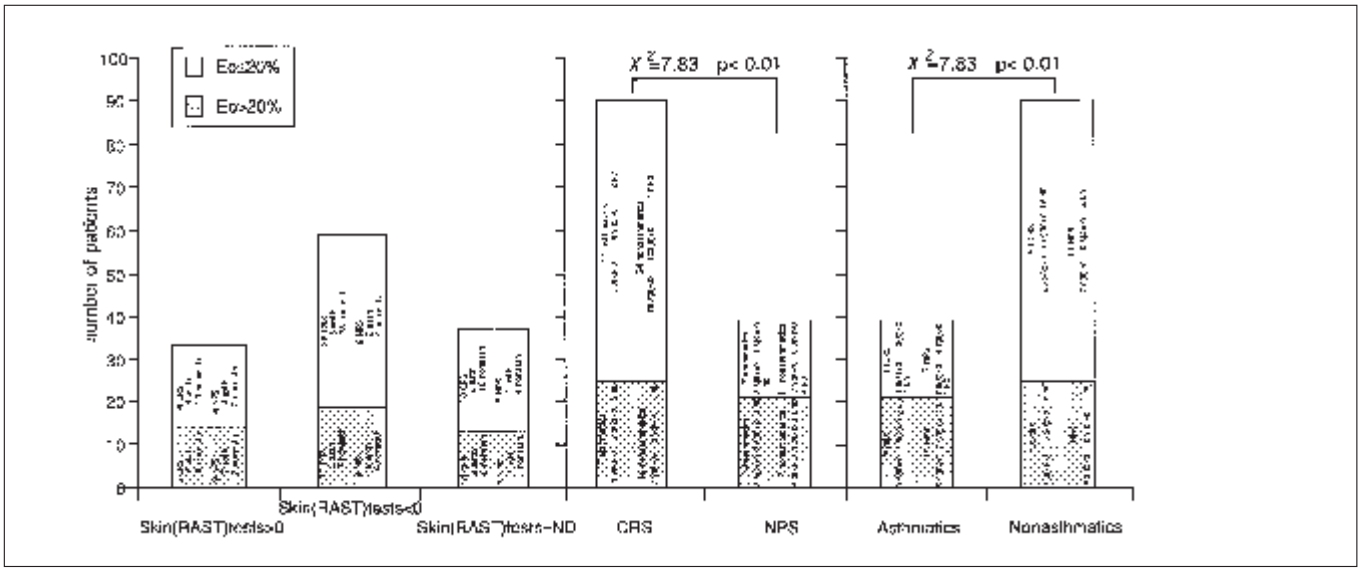

Figure 5. Distribution of patients with nasal hypereosinophilia (Eo>20%) and without nasal hypereosinophilia (Eo≤20%) in the clinical subgroups. Clinical classification of the 129 patients was based 1) on skin- or multi- RAST tests (positive, negative or not done) 2) absence (CRS = chronic rhinosinopathy) or presence of nasal polyps (NPS) 3) presence or absence of asthma.

Table 4. Nasal secretion eosinophelia and number of leucocytes. (Statistics compare patients to controls)

	controls (n=40)	patients (n=129)		nasal polyposis (n=39)		chronic rhinosinopathy (n=90)	
	Eo≤20%	Eo>20% (n=46)	Eo≤20% (n=83)	Eo>20% (n=21)	Eo≤20% (n=18)	Eo>20% (n=25)	Eo≤20% (n=65)
secretion weight (mg)	35.7±3.9	38.4±5.6	38.8±4.4	40.6±9.6	41.3±10.1	36.6±6.6	38.1±4.9
total number of leucocytes	76,381±29,623	101,740±326,965	417,625±158,463 (p=0.13)	176,773±103,043	289,883±115,869 (p=0.01)	38,713±14,926	452,999±200,012 (p=0.14)
leucocytes/mg	2,020±823	2,408±1,087	8,672±2,421 (p=0.06)	3,365±2,296	5,100±1,620 (p=0.06)	1,604±575	9,661±3,054 (p=0.05)
neutrophils (%)	80.6±6	58.4±4.6 (p=0.004)	90.2±2.7	53±7.1 (p=0.005)	91.5±5.5	63±6 (p=0.04)	89.9±3.2 (p=0.13)
eosinophils (%)	5±2.6	40.1±4.6 (p=0.0001)	1.3±0.2 (p=0.03)	45.6±7 (p=0.0001)	0.9±0.3	35.5±6.1 (p=0.0001)	1.4±0.3 (p=0.06)

2500, as less than 5% of controls had more than 2500 leucocytes/mg in their baseline secretions. According to this cut-off point, 46/83 patients without nasal hypereosinophilia showed values lower than 2500 leucocytes/mg, but 37 of these 46 patients were classified in the chronic rhinosinopathy group.

DISCUSSION

The cytologic investigation of nasal secretion is still not fully accepted as a part of the clinical diagnosis. Its real usefulness as a diagnostic tool remains controversial. A normal nasal cytogram has not yet been described. The presence of eosinophils in nasal secretions has been known since first described by Eyerman in 1927. Eosinophil cells were initially regarded as indicative of allergic rhinitis, but have recently also been associated to non-allergic rhinitis (Mullarkey et al., 1980; Jacobs et al., 1981). The aim of this work was to help to clarify the problem by describing the performance of the leucocyte count in nasal secretions of a large group of subjects.

No gold standard is available for the diagnosis of inflammation of the nasal mucosa. We made an assumption that symptom-free healthy volunteers had no nasal inflammation. It is more difficult to be certain that our patients all had on-going inflammation of the mucosa at the time of secretion collection. Inflammation is certainly a major factor in chronic nasal symptoms, but other non inflammatory factors such as dysfunction of the nasal blood vessels, anatomical abnormalities or nerve dysfunction can probably generate the same pattern of symptoms.

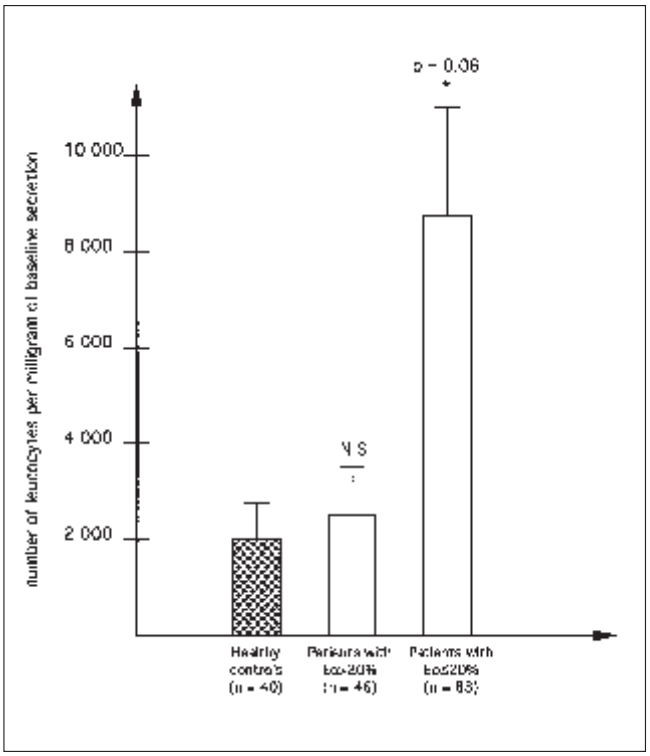

Figure 6. Comparison in the number of leucocytes per milligram of secretion between healthy controls, patients with nasal hypereosinophilia (Eo>20%), and patients without nasal hypereosinophilia (Eo≤20%).

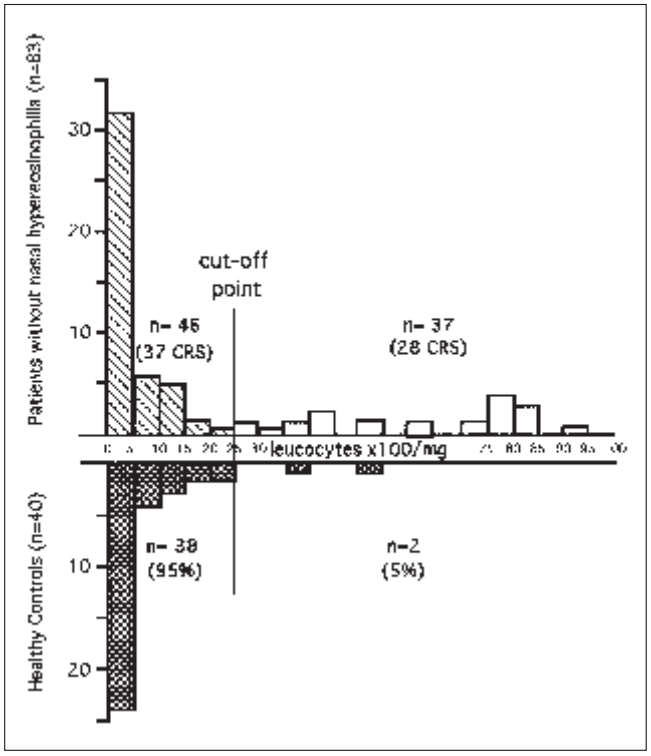

Figure 7. Frequency distribution of the number of leucocytes per milligram of secretion in healthy subjects (n=40) and patients without hypereosinophilia (n=83)
(Note: 17 values above 10.000 leucocytes/mg are not illustrated in the patient histogram).

Because baseline nasal secretion can be scarce in healthy volunteers and in some patients, we used methacholine to increase secretion and designed a time-series protocol. Methacholine is a glandular cholinoceptor agonist that increases nasal glandular, but not vascular, secretion (Raphael et al., 1988). Methacholine was sprayed into the nose but because of its bronchoconstrictor properties was considered a risk in asthmatic patients. The methods for collecting and analyzing nasal secretion have been discussed in a previous paper (Jankowski et al., 1995). Methacholine allowed us to obtain a reliable leucocyte differential count in 10% of patients, and 20% of volunteers, whose baseline samples showed no white cells. In asthmatic patients the post-baseline sample, without methacholine, also demonstrated no white cells in 10% of the cases. Methacholine significantly increased the secretion weight, but not the total number of leucocytes collected in 30 seconds, and therefore tended to decrease the number of leucocytes per milligram. The same situation was observed, but to a lesser extent, with the post-baseline sample in asthmatics. These results are not necessarily contradictory with our previous data (Jankowski et al., 1995) showing that methacholine increased the number of leucocytes because both the time-series design and patient selection were different. The use of methacholine seems unnecessary to get a reliable differential leucocyte count in clinical practice, but we would, however, recommend the collection of a second sample as it reduces the risk of getting blank samples from 15% to 5%. We also recommend taking specimens from both nostrils. Although we did not find statistical differences between right and left sides, the distribution of leucocytes between nasal cavities was found to differ in almost every patient and in a few patients one side was unreadable whereas the other was readable. This underlines how inhomogeneous the nasal secretion medium is, which is also reflected by the very large coefficient of variation we found in the leucocyte count.

The number of leucocytes in baseline secretions, expressed as the total number collected in 30 seconds or the number per milligram of secretion, does not statistically differ between healthy controls and patients. The mean is, however, more than three times higher in patients than in controls. The high variability in the leucocyte number, both in controls and patients, certainly explains the large overlap between the two distributions of leucocyte counts. Other explanations are that patients with chronic nasal symptoms do not all have nasal inflammation, or that many patients might have taken anti-inflammatory drugs a few days or weeks before, which is a major bias we did not care about during the study. Smoking habit is also a potential confounding variable we did not care about. At this point in time, the question as to whether or not the number of leucocytes could represent a diagnostic criterion of nasal inflammation remains to be solved.

More interesting was the differential leucocyte count in baseline secretions which showed a significantly higher percentage and number of eosinophils in patients than in controls. High percentages of eosinophils were found not only in patients with nasal polyposis, but also in patients with chronic rhinosinusitis. The role and significance of eosinophils in nasal inflammation

remains unclear. There is no doubt that eosinophils are associated with IgE mediated allergic rhinitis, but the association of non-allergic rhinitis with eosinophelia (Mullarkey et al., 1980 and Jacobs et al., 1981) also appears to be important in the understanding of diseases of the nose, especially nasal polyposis (Jankowski, 1996). Recent findings are consistent with the view that nasal polyposis is a chronic eosinophilic inflammatory disease, involving self-sustaining mechanisms, largely independent of allergen stimulation (Keith et al., 1997). With regard to allergy status, we have excluded allergic patients on the grounds of a strong allergic history. Whilst we would accept that one could have carried out more objective tests (such as skin tests), these are also subject to error and indeed positive results may not, in fact, be the primary cause of a patient symptoms (Keith et al., 1994).

The definitive criteria for nasal secretion hypereosinophilia are, however, not well established. Mullarkey et al. (1980) requires 25% or more eosinophils whereas Mygind et al. (1978) consider patients with 10% diffusely spread eosinophelia or 50% in a single part of a smear as being eosinophilic "positive". Moreover, the distribution of eosinophils in the nose has been found to vary within and between nares (Phillips et al., 1992). Although our technique of secretion collection by suctioning a large area of the nasal mucosa over a 30 second period seems more representative than nasal smears, we also found variations between nares, and between baseline and post-baseline (methacholine) secretions.

As a consequence the eosinophil status of each patient or control is best given by analyzing all the samples of each subject. We decided to select the highest eosinophil count as representative of a subject, because the presence of eosinophils appeared as a criteria that could distinguish healthy from non-healthy secretion, and because we made the hypothesis that the eosinophil count in diseased secretion could vary considerably from site to site and over time, the pathological feature being that the eosinophil count could reach high values never reached in healthy secretions. With this in mind, and in keeping with normal convention (Knapp et al., 1992), we took the cut-off point to be such that 97,5% of normal secretions was below this value, ie an eosinophil percentage reaching 20%.

We tried to confirm the aforementioned hypothesis by studying the diurnal variations in the eosinophil count. Eosinophils physiologically appear from time to time in healthy secretion, but their number never exceeds 20% and is usually below 5%. In patients, we observed three situations. Patients with a high eosinophil count stayed above 20% all day long, but the counts could vary from 40 to 90%. Patients with a low eosinophil count stayed below 20% all day long, with very mild variations. In one patient however, the eosinophil count raised once from 0 to 60%. This patient could have easily been classified in either the hypereosinophilic or in the noneosinophilic group. Using our criteria, this patient was included in the hypereosinophilic group. By choosing the highest eosinophil count, we have without doubt increased the sensitivity of the test. Further studies are, however, necessary to decide whether it is better to increase the sensitivity or the specificity of the test.

The number of eosinophils found both in nasal secretion and polyp tissue was slightly but significantly correlated. On the other hand, our group has previously shown that some patients with chronic rhinosinusitis without polyps can have very high number of eosinophils in their secretion but only very few in middle turbinate biopsies (Moneret-Vautrin et al., 1992). The kinetics of eosinophils in nasal tissue and secretion are, in fact, far from well understood. The link between non-allergic rhinosinusitis with hypereosinophilia and the further development of nasal polyps has been hypothesized by many authors (Mullarkey et al., 1980 and Moneret-Vautrin et al., 1990). In our study, approximately one half of patients with nasal polyps had less than 20% eosinophils in their nasal secretion whereas only one quarter showed both low secretion and tissue eosinophelia (Figure 4). The question is whether or not low tissue or secretion eosinophelia could signify that inflammation in such polyps is in a quiescent phase.

All our patients were considered non-allergic from a clinical point of view, but one quarter of them had positive skin or multi-RAST tests. The distribution of patients with secretion hypereosinophilia could not be predicted by the skin or multi-RAST tests. Interpretation of these results would actually have been enhanced if we had thought to include as a positive control a known allergic group in and out of season. Eosinophils are believed to play an important role in the inflammation associated with asthma. We found that half of the asthmatics had high nasal eosinophelia, supporting the hypothesis that in some patients with asthma sinus disease may be due to the same mechanisms that cause asthma (Adinoff et al., 1989). Figure 5 shows that there is no clear overlap between our clinical classifications of nasal diseases and nasal hypereosinophilia.

The more interesting observation in our study is, however, that the nasal cytogram can be modified in two ways, that could represent two different aspects of nasal inflammation. Inflammation can firstly modify the nasal cytogram in a qualitative way: patients with hypereosinophilia have no more leucocytes in their secretion than healthy controls, the increase in eosinophils being balanced by a decrease in neutrophils. On the other hand, inflammation can modify the nasal cytogram in a quantitative way. Patients without hypereosinophilia can actually be divided in two subgroups : one with and another without hyperleucocytosis, the cut-off point being set at 2500 leucocytes/milligram of secretion. The question remains, however, whether or not nasal secretion hyperleucocytosis can serve as a criterion to distinguish chronic nasal symptoms associated with nasal inflammation from chronic nasal symptoms of non-inflammatory origin. Our study cannot answer this question.

In conclusion, endoscopy and CT-scan imaging have dramatically improved knowledge and practice. Our study emphasizes the role of cytology in the investigation of nasal secretion. We believe that the leucocyte compartment found in nasal secretion is part of the first line of defense of the nose, and perhaps of the whole respiratory mucosa, and that knowledge of its pathophysiology could lead to a better understanding of respiratory diseases.

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R. Jankowski
Department of Otorhinolaryngology,
Head and Neck Surgery
Central Hospital-Henri Poincaré University
29 Avenue De Lattre de Tassigny
F-54035 Nancy Cedex
France
Tel.: +33-3-83851152
Fax: +33-3-83852258