RHINOLOGY

Exploring the role of nasal cytology in chronic rhinosinusitis

Il ruolo della citologia nasale nella rinosinusite cronica

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SUMMARY

Objective. Characterising the eosinophilic profile represents the main step in chronic rhinosinusitis (CRS) endotyping. The aim of the study is to verify the correlation between different methods for tissue eosinophilia quantification.
Methods. 33 CRS patients undergoing endoscopic sinus surgery and 30 controls undergoing non-CRS surgeries were enrolled. Blood venous sampling, nasal biopsy on uncinate process (UP), nasal cytology on inferior turbinate (IT) and middle meatus (MM) were performed.
Results. Differences in eosinophil count in blood (P=0.0001), UP (P<0.0001), IT (P = 0.01) and MM (P = 0.0006) were significant between CRS cases and controls. A weak correlation was found between UP and blood eosinophil count (r = 0.34, P = 0.006) and between UP and IT eosinophil count (r = 0.30, P = 0.017). Moderate correlation between UP and MM (r = 0.51, P < 0.0001) was shown. ROC analysis predicted eosinophilic CRS with an overall low sensitivity. Once allergic patients were excluded from the analysis, the sensitivity decreased for sampling on IT and increased for MM sampling.
Conclusions. This study suggests that MM cytology gives more accurate information on the degree of tissue eosinophilia. Replication in wide and unbiased cohorts is necessary to verify these results and define accurate thresholds.

KEY WORDS: rhinosinusitis, nasal polyps, rhinitis, eosinophilia, biomarker, cytology

RIASSUNTO

Obiettivi. L'identificazione del profilo eosinofilico è uno step fondamentale nell'endotipizzazione della rinosinusite cronica (RSC). Lo scopo dello studio è verificare il grado di correlazione tra le diverse metodiche di quantificazione dell'eosinofilia.
Metodi. Sono analizzati, per 33 pazienti RSC candidati a chirurgia endoscopica nasosinusale e 30 controlli sottoposti a chirurgia non RSC-correlata, un campione di sangue venoso periferico, una biopsia del processo uncinato (PU), un citologico sul turbinato inferiore (TI) e un citologico nel meato medio (MM).
Risultati. Differenze tra RSC e controlli negli eosinofili su sangue periferico (P = 0,0001), PU (P < 0,0001), TI (P = 0,01) e MM (P = 0,0006) sono risultate statisticamente significative. È stata dimostrata una correlazione debole tra sangue periferico e PU (r = 0,34, P = 0,006) e tra PU e IT (r = 0,30, P = 0,017), e una correlazione moderata tra PU e MM (r = 0,51, P < 0,0001). Le curve ROC hanno predetto forme di RSC eosinofila con una sensibilità globalmente bassa. Escludendo i pazienti allergici la sensibilità si riduce ulteriormente per TI mentre aumenta per MM.
Conclusioni. Il presente studio suggerisce di eseguire il prelievo citologico nel MM al fine di identificare le RSC eosinofile. Sono necessari studi più ampi per verificare i risultati e definire valori limite adeguati.

PAROLE CHIAVE: *rinosinusite, polipi nasali, rinite, eosinofilia, biomarker, citologia*

Introduction

Chronic rhinosinusitis (CRS) is a generic term for different disease entities, each representing the downstream consequence of a specific immune-mediated inflammatory mechanism. This is why a blanket treatment approach has been proven unsuccessful in some cases [1]. The phenotypic dichotomy of CRS with and without nasal polyps (CRSwNP and CRSsNP, respectively) is progressively being replaced by a more complex biomolecular classification of subtypes (or endotypes) [2].

Although the current therapeutic strategy is focused on immunomodulation (i.e., monoclonal antibodies), more scientific evidence is needed to find accurate predictive molecular markers of CRS endotypes to better tailor effective regimens.

To date, biological agents tested or in use for moderate/severe inflammatory disorders of the airways and skin target the T helper 2 (Th2) pathway. Conversely, few treatments are available for non-Th2 and non-eosinophilic cascades [3]. However, based on the rate of non-responders to biological therapies, clinical translation of the endotyping process is urgently needed [3]. Currently, the eligibility for these treatments depends on the demonstration of an eosinophilic inflammatory profile (i.e., blood eosinophil count and serum IgE). However, cut-off values are not clearly defined, with the only exception being thresholds applied in clinical trials [4].

All these premises are even more vague when applied to CRS. A basic attempt of CRS endotyping is based on the identification of the predominant immune cells in the inflamed sinonasal mucosa; in particular, it is key the distinction between eosinophilic and non-eosinophilic mediated CRS (ECRS and non-ECRS, respectively) [2]. The clinical interest in ECRS arises as it generally shows a poor response to medical and surgical therapies [5]. The therapeutic impact would be significant, as cases with intense eosinophilia would justify higher dosage of steroids and, theoretically, selected biological antagonists of type 2 inflammation [6].

Although no unanimous histopathological criteria exist for discriminating between ECRS and non-ECRS, it is current practice to define western ECRS when tissue eosinophil count > 5 cells/HPF [5]. Moreover, a tissue eosinophil count > 10 cells/HPF correlates with poorer outcomes [5]. Diagnosis requires obtaining tissue for histopathological analysis. As sinus mucosa needs to be collected, biopsies may not be straightforward or performed under local anaesthesia. This is why different, less invasive, surrogates have been tested to improve their reliability to predict tissue eosinophilia. It is worth mentioning the JESREC score, which defines ECRS in presence of differently matched clinical criteria [7]. Much less widespread among rhinologists is assessment of the degree of eosinophilia through nasal cytology. This technique has been reported as an efficient method to differentiate among various forms of non-allergic rhinitis [8]. However, it is still debated if it might be of interest in defining CRS inflammatory profiles. Controversies are related to both sampling site and method of analysis, and only few reports have examined the cellular inflammatory pattern of different endonasal subsites in CRS.

In light of these premises, we wished to verify in a sample population (including CRS patients and controls) the existence of a correlation among the degree of tissue, blood and cytological eosinophilia. Moreover, standard cytological data was integrated with analysis of a cytological sample obtained from the middle meatus region. Lastly, by sorting the study population into cases (patients with CRS) and controls, we investigated the existence of significant differences in the degree of eosinophilia and association with the most typical clinical features related to CRS.

Materials and methods

An observational prospective study was conducted according to the declaration of Helsinki and was previously approved by the Institutional Review Board of the hospital.

Study population

Clinical data were obtained from patients affected by CRS who underwent endoscopic sinus surgery (ESS) at the same tertiary care center in the period between January 2018 and July 2018.

CRS was diagnosed according to the latest European guidelines [9]. Each CRS case was assessed by SNOT-22 questionnaire for symptoms, Lund Kennedy (LK) and Lund Mackay (LM) scores to assess the endonasal status and the degree of opacification of the sinuses, respectively, and skin prick test to investigate allergic sensitisation to common inhalants. Data on asthma, aspirin sensitivity and smoking habits were self-reported by patients. Exclusion criteria were genetic syndromes, congenital or acquired immunodeficiency, malignancy or history of the head and neck cancers, systemic autoimmune diseases, and drug abuse.

Patients scheduled for other non-CRS surgeries (septoplasty and dacryocystorhinostomy) in the same time-lapse served as control group. Each control was assessed by SNOT-22 questionnaire as well as LK and LM scores to exclude CRS. Control patients affected by asthma and aspirin sensitivity were excluded a priori. Lastly, skin prick tests were performed to investigate allergic sensitisation to common inhalants.

All cases and controls were considered eligible for enrolment only after a washout period of 15 days from oral and topical steroids and 1 month from oral antibiotics.

All collected data were entered in a specific CRS database as previously reported [10].

Sampling steps

At the beginning of the surgical procedure under general anesthesia, the following sampling steps were taken.

A peripheral blood venous sample from antebrachial vein was collected for blood and leukocyte formula counts. White blood cells (WBC) were expressed both as absolute count (cell x 10^9/L) and percentage of the total WBC count.

A nasal cytological sampling was performed under endoscopy along the inferior turbinate (IT) and the middle meatus – lateral nasal wall (MM) mucosa. The procedure consisted in swiping gently a disposable plastic nasal curette (Rhinoprobe®), equipped with a small distal collection chamber, on the mucosal surface. Samples were swiped on the central area of a slide and smeared with May Grunwald-Giemsa as described by Gelardi et al. [11]. Slides were observed through an optical microscope (Nikon Eclipse 600®) at different magnifications (100x, 200x, and 400x). Observed cells included intact respiratory epithelial, flaking, and immune cells (i.e., eosinophils, neutrophils, mastcells, macrophages, and plasma cells) and were counted in 10 consecutive fields at 400x. Eosinophils were expressed both as mean of eosinophil cells per high-power field (HPF) 400x and percentage of eosinophils on total immune cells. This latter parameter was intended to incorporate also the effect of the neutrophilic degree of infiltration of the specimen.

A mucosal biopsy was collected on the uncinate process (UP) at the same side of the cytological sampling. All samples, sized > 0.4 mm, were fixed in 10% buffered formalin, dehydrated by alcohol passages with increasing concentrations of ethanol, clarified in BioClear® and embedded in paraffin. Histological sections, with a thickness of 3 mm, were stained with haematoxylin-eosin. A conventional morphological evaluation was carried out according to the 2017 WHO classification criteria. Additional histopathologic features were taken into consideration as reported by Snidvongs et al. [12]. Moreover, an immune cell count was performed in 5 HPF using a 400x objective corresponding to an area of 1 mm². Tissue eosinophil count was graded in three classes: < 5 cells/HPF, 5-10 cells/HPF and > 10 cells/HPF [12].

Statistical analysis

An ad hoc electronic database was created to collect all study variables. Qualitative data were summarised with absolute and relative frequencies. Mean and standard deviation (SD) or median and interquartile range (IQR) were used for quantitative variables with a parametric and non-parametric distribution, respectively. Chi-squared or Fisher's exact test were used to detect significant differences for qualitative variables. Student's t and Mann-Whitney tests were used for quantitative variables following their parametric or non-parametric distribution. Spearman's correlation was used to assess the relationship between the different measurements of eosinophils. P values < 0.05 was considered statistically significant. Stata 15 statistical software was used for each statistical computation**.**

Results

A total of 33 CRS patients and 30 controls were recruited. Baseline characteristics are shown in Table I. The CRS group included 21 (63.6%) CRSwNP and 12 (36.4%) CRSsNP. Allergic sensitisation was diagnosed in 13 cases (39.4%), asthma in 14 (42.4%), and aspirin intolerance in 3 (9.1%). Sixteen (48.5%) CRS patients had underwent previous surgery elsewhere. Median baseline SNOT-22 score was 30. Mean baseline LK and LM scores were 6.1 and 13.5, respectively.

Only 5 (16.7%) controls showed allergic sensitisation to inhalants.

The median blood eosinophil count was 0.3×10^9/L in CRS group (min 0.03, max 1.14) and 0.2×10^9/L in control group (min 0.01, max 0.36) [P = 0.0001]. The median percentage of blood eosinophils was 3.9% (min 0.4, max 13.3) and 2% (min 0.2, max 6) in CRS and control group, respectively [P = 0.0008].

Increased overall degree of inflammation was found in UP CRS samples [P = 0.003]. Eosinophil counts in UP samples were significantly different between cases and controls [P < 0.0001]. In detail, among CRS group, the eosinophil count was <5 cells/HPF in 18 (54.5%) cases, 5-10 cells/HPF in 3 (9.1%), and > 10 cells/HPF in 12 (36.4%); in control samples, the eosinophil count was < 5 cells/HPF in 29 (96.7%) cases and 5-10 cells/HPF in 1 (3.3%).

Similarly, cytological analysis showed higher overall inflammatory infiltration in CRS cases, confirmed at both IT (P = 0.01) and MM scraping (P = 0.0006). Median IT eosinophil count was 0.5 cells/HPF in CRS group and 0 cells/HPF in control group [P = 0.0002]. Median IT eosinophil percentage was 4.2% and 0% in CRS and control group, respectively [P = 0.002]. Median MM eosinophil count was 0.3 cells/HPF in CRS group and 0 cells/HPF in control group [P = 0.006]. Median MM eosinophil percentage on total immune cells was 1.9% and 0% in CRS and control group, respectively [P = 0.01]. On the whole, these data

Table I. Demographic data of control and CRS groups.

	Control group N = 30	CRS group N = 33	*P* value
Males, n (%)	17 (56.7)	8 (24.2)	0.009
Mean (SD) age, years	52.1 (16.8)	52.7 (15.5)	0.88
CRS with nasal polyps, n (%)	-	21 (63.6)	-
Previous surgery for CRS, n (%)	-	16 (48.5)	-
Allergy, n (%)	5 (16.7)	13 (39.4)	0.05
Asthma, n (%)	0 (0.0)	14 (42.4)	< 0.0001
Aspirin intolerance n (%)	0 (0.0)	3 (9.1)	0.24
Smoker, n (%)			
Nonsmoker	29 (96.7)	24 (72.7)	
Smoker	1 (3.3)	4 (12.1)	0.03
Former	0 (0.0)	5 (15.2)	
Median (IQR) SNOT-22 score	-	30 (25-42)	-
Mean (SD) LK score	-	6.1 (2.8)	-
Mean (SD) LM score	-	13.5 (5.7)	-

SD: standard deviation; CRS: chronic rhinosinusitis; IQR: inter-quartile range; LK: Lund-Kennedy; LM: Lund-Mackay; SNOT-22: Sino-nasal outcome test 22

showed a significant difference in terms of eosinophilic infiltrate between CRS cases and controls (Tab. II).

A weak correlation was seen between UP eosinophil count and blood eosinophil count (r = 0.34, P = 0.006), and between UP and IT eosinophil count (r = 0.30, P = 0.017), whereas a moderate correlation was observed between UP and MM eosinophil count (r = 0.51, P < 0.0001). Subgroup analysis showed that in the control group only the correlation between UP and IT cytology was confirmed, whereas the opposite was seen in the CRS group (loss of correlation between UP eosinophil count and IT cytology and confirmed correlation between UP eosinophil count and MM cytology) (Tab. III).

No significant differences were observed in terms of tissue eosinophilia (blood, UP, IT, MM eosinophil count) across different clinical parameters, including sex, age, presence of nasal polyps, previous surgery, allergy, asthma and smoking habit. Similarly, no significant differences were evident comparing UP, IT, MM eosinophil count and clinical staging scores (SNOT-22, LK score, LM score). Conversely, higher levels of blood eosinophilia were associated with an increase in endoscopic and radiological scores (LK score, P = 0.03; LM score, P = 0.01).

The CRS group was then classified in ECRS and non-ECRS on the basis of the histopathological threshold (ECRS, eosinophil count ≥ 5 cells/HPF; non-ECRS, eosinophil count < 5 cells/HPF). The analysis of different clinical and biological parameters showed only a significant difference between the two groups for MM eosino-

phil count (MM eosinophils/HPF 400x, P = 0.003; MM eosinophil percentage, P = 0.005) (Tab. IV). The absence of a significant difference for asthma, aspirin intolerance and polyp phenotype might be justified by the small size of the sample.

ROC curve analysis on IT eosinophil count predicted ERCS with a sensitivity of 51.5% and specificity of 90% [positive predictive value (PPV) 85%; negative predictive value (NPV) 62.8%; area under the curve (AUC) 0.76, range 0.65-0.87], on IT eosinophil percentage on total immune cells with a sensitivity of 48.5% and specificity of 80% (PPV 72.7%; NPV 58.5%; AUC 0.72, range 0.59-0.84), on MM eosinophil count with a sensitivity of 42.4% and specificity of 90% (PPV 82.4%; NPV 58.7%; AUC 0.69, range 0.57-0.81), on MM eosinophil percentage on total immune cells with a sensitivity of 42.4% and specificity of 87.7% (PPV 77.8%; NPV 57.8%; AUC 0.67, range 0.55-0.80). Once allergic patients were excluded from the CRS population, ROC curve analysis on IT eosinophil count predicted ERCS with a sensitivity of 11.1% and specificity of 90.9% (PPV 50%; NPV 55.6%; AUC 0.53, range 0.27-0.80), on IT eosinophil percentage on total immune cells with a sensitivity of 11.1% and specificity of 81.8% (PPV 33.3%; NPV 52.9%; AUC 0.51, range 0.24-0.77), on MM eosinophil count with a sensitivity of 33.3% and specificity of 90.9% (PPV 75%; NPV 62.5%; AUC 0.81, range 0.61-1), on MM eosinophil percentage on total immune cells with a sensitivity of 55.6% and specificity of 81.8% (PPV 71.4%; NPV 69.2%; AUC 0.85, range 0.67-1).

Table II. Blood, histological and cytological features of control and CRS groups. Statistical difference is expressed as p value; significant results (P < 0.05) are highlighted in bold.

		Control group	CRS group	*P* value
Peripheral blood eosinophilia				
Median (IQR) blood eosinophil count, 10^9/L		0.2 (0.1-0.2)	0.3 (0.2-0.4)	**0.0001**
Median (IQR) blood eosinophils, %		2.0 (1.1-3.2)	3.9 (2.4-5.8)	**0.0008**
Uncinate process (UP) histological features				
Overall degree of inflammation, n (%)	Absent	14 (46.7)	5 (15.2)	
	Mild	16 (53.3)	22 (66.7)	**0.003**
	Moderate	0 (0.0)	6 (18.2)	
Inflammatory predominance, n (%)	Lymphoplasmacytic	16 (53.3)	27 (81.8)	
	Absent	14 (46.7)	5 (15.2)	**0.01**
	Eosinophilic	0 (0.0)	1 (3.0)	
Neutrophilic infiltrate, n (%)		4 (13.3)	4 (12.1)	1.0
Eosinophil count, n (%)	< 5/HPF	29 (96.7)	18 (54.5)	
	5-10/HPF	1 (3.3)	3 (9.1)	**< 0.0001**
	10/HPF	0 (0.0)	12 (36.4)	
Inferior turbinate (IT) cytological features				
Median (IQR) eosinophils/HPF 400x		0 (0.0-0.2)	0.5 (0.0-1.3)	**0.0002**
Median (IQR) eosinophil percentage on total immune cells		0 (0.0-0.6)	4.2 (0.0-12.5)	**0.002**
Eosinophil grading, n (%)	< 5%,	24 (80.0)	20 (60.6)	
	5-19%	3 (10.0)	9 (27.3)	
	20-50%	3 (10.0)	1 (3.0)	**0.05**
	50%	0 (0.0)	3 (9.1)	
Median (IQR) mast cell count		0 (0-1)	3 (1-8)	**< 0.001**
Median (IQR) neutrophil count		8 (2-43)	46 (8-300)	0.06
Median (IQR) macrophage count		2 (1-3)	3 (1-4)	0.25
Median (IQR) plasma cell count		0 (0-0)	0 (0-0)	0.17
Median (IQR) total immune cells		12 (4-50)	80 (20-409)	**0.01**
Middle meatus (MM) cytological features				
Median (IQR) eosinophils/HPF 400x		0 (0.0-0.2)	0.3 (0.0-3.5)	**0.006**
Median (IQR) eosinophil percentage on total immune cells		0 (0-4)	1.9 (0-30)	**0.01**
Eosinophil grading, n (%)	< 5%,	24 (80.0)	18 (54.6)	
	5-19%	4 (13.3)	4 (12.1)	
	20-50%	0 (0.0)	6 (18.2)	**0.04**
	50%	2 (6.7)	5 (15.2)	
Median (IQR) mast cell count		1 (0-1)	3 (1-12)	**< 0.0001**
Median (IQR) neutrophil count		3 (2-13)	19 (4-200)	**0.04**
Median (IQR) macrophage count		1 (1-2)	3 (2-6)	**0.001**
Median (IQR) plasma cell count		0 (0-0)	0 (0-0)	0.33
Median (IQR) total immune cells		7 (4-18)	95 (13-253)	**0.0006**

IQR: interquartile range; UP: uncinate process; IT: inferior turbinate; HPF: high power field; MM: middle meatus

Table III. Spearman's rank-order correlation between histological samples, blood tests and cytology. Statistical difference is expressed as p value; significant results (P < 0.05) are highlighted in bold.

Spearman's rho	Uncinate process (UP) eosinophil count	
	rho	*P* value
Total population (n = 63)		
Blood eosinophil count, 109/L	0.34	**0.006**
Blood eosinophils, %	0.26	**0.038**
IT eosinophils/HPF 400x	0.30	**0.017**
IT eosinophil percentage on total immune cells	0.20	0.111
MM eosinophils/HPF 400x	0.51	**< 0.0001**
MM eosinophil percentage on total immune cells	0.48	**< 0.0001**
Control group (n = 30)		
Blood eosinophil count, 109/L	-0.17	0.36
Blood eosinophils, %	-0.07	0.69
IT eosinophils/HPF 400x	0.40	0.03
IT eosinophil percentage on total immune cells	0.26	0.16
MM eosinophils/HPF 400x	0.19	0.32
MM eosinophil percentage on total immune cells	0.11	0.56
CRS group (n = 33)		
Blood eosinophil count, 109/L	0.20	0.26
Blood eosinophils, %	0.11	0.56
IT eosinophils/HPF 400x	0.04	0.83
IT eosinophil percentage on total immune cells	-0.06	0.74
MM eosinophils/HPF 400x	0.53	**0.002**
MM eosinophil percentage on total immune cells	0.51	**0.002**

UP: uncinate process; IT: inferior turbinate; HPF: high power field; MM: middle meatus; CRS: chronic rhinosinusitis

Table IV. Blood, histological, cytological and clinical differences between non-ECRS and ECRS group. Statistical difference is expressed as P value; significant results (P < 0.005) are highlighted in bold.

CRS group	Non-ECRS N = 18	ECRS N = 15	*P* value
Median (IQR) blood eosinophil count, 10^9/L	0.3 (0.2-0.4)	0.4 (0.2-0.6)	0.19
Median (IQR) blood eosinophils, %	3.7 (2.3-5.4)	3.9 (2.4-8.0)	0.42
Median (IQR) IT eosinophils/HPF 400x	0.4 (0.1-0.8)	0.7 (0-1.8)	0.66
Median (IQR) IT eosinophil percentage on total immune cells	4.6 (3-9.1)	3.4 (0-15)	0.94
Median (IQR) MM eosinophils/HPF 400x	0.05 (0-0.2)	3.3 (0.4-10.3)	**0.003**
Median (IQR) MM eosinophil percentage on total immune cells	0.2 (0-7.7)	23.3 (1.9-68.8)	**0.005**
Asthma, n (%)	8 (44.4)	6 (40.0)	0.80
Allergy, n (%)	7 (38.9)	6 (40.0)	0.95
Aspirin intolerance, n (%)	2 (11.1)	1 (6.7)	1.0
CRSwNP, n (%)	12 (66.7)	9 (60.0)	0.69

IQR: interquartile range; IT: inferior turbinate; HPF: high power field; MM: middle meatus; CRS: chronic rhinosinusitis

A cut-off of ≥ 1.9% of MM eosinophil percentage provided the best sensitivity and specificity (88.9% and 81.8%, respectively) (Tab. V).

Discussion

The term ECRS was introduced to identify a subgroup of patients with CRS and eosinophilic infiltration of nasal polyps, likely to occur consequent to eosinophil dysregulation [13]. The aetiology of ECRS encompasses a wide variety of stimuli and overlapping pathogenic mechanisms [13]. There is evidence that ECRS is associated with greater symptom severity [14], extensive sinus disease and comorbidities (asthma), with intermittent acute exacerbation of secondary bacterial infections [5]. Moreover, ECRS patients seem to have a poorer response to medical and surgical treatments with high polyp recurrence rate and severely impaired quality of life [5]. Therefore, early detection of ECRS, preferably in outpatient settings, is key to guide overall long-term management and improve prognosis.

In daily practice, diagnostic criteria for ECRS are based on clinical features. Traditional traits include asthma (late-onset), nasal polyps, aspirin intolerance, high serum eosinophilia and IgE. Although the presence of polyps predicts high tissue eosinophilia, a remarkable number of CRSsNP show the same degree of eosinophilic inflammation (19%) [12]. For this reason, the most reliable way to diagnose ECRS remains histopathological assessment. However, relying on biopsy as the main diagnostic tool of ECRS opens several issues. 1) Unless adequately aware, the pathologist's report often concludes generically with "chronic inflammation" [12]. 2) Diagnosis requires obtaining sufficient tissue for histopathological analysis. As sinus mucosa needs to be collected, and not just nasal polyp samples, biopsies may not be straightforward or performed under local anaesthesia. Moreover, biopsy – due to its intrinsic invasiveness – is not an early step in the CRS diagnostic workup. 3) To date, the definition of eosinophilia in CRS has not reached consensus among researchers [5]. This controversy concerns both the method and interpretation of the results. Actually, it is accepted practice to define western ECRS when tissue eosinophil count is > 5 cells/HPF. Moreover, a tissue eosinophil count > 10 cells/HPF was demonstrated to correlate with poorer outcomes and overall prognosis [5].

To overcome the aforementioned disadvantages of biopsy, other types of biological samples have been considered as possible indirect assessments of tissue eosinophilia. A number of studies demonstrated that there is an association between peripheral eosinophilia and tissue eosinophilia in paranasal sinuses. Our study confirmed this correlation, albeit weak (r = 0.341). The cut-point of > 0.3 x 10⁹/L or 4.4% of WBC is that adopted for administration of biological agents in asthma, though still within the normal range. Other thresholds have been proposed to gain better diagnostic reliability. However, their broad variability prevents drawing firm conclusions [13]. Blood eosinophil count shows low specificity depending on other comorbidities (parasitic infections, allergy, autoimmune disorders, adverse drug events, etc.); moreover, local eosinophilic activation is often independent on blood eosinophils [14]. It is reasonable that on-site biomarkers might provide a more specific overview on cellular inflammatory pattern. In some studies, indeed, asthma subtypes are defined on induced sputum, a non-invasive well standardised procedure of bronchial cytological assessment, able to sort asthma into eosinophilic, neutrophilic, mixed-granulocytic or pauci-granulocytic subtypes [16]. Similarly, the degree of nasal eosinophilia, together with other inflammatory cells, can be measured by cytological analysis. Numerous techniques have been described to obtain nasal specimen for cytological assessment. Among them, nasal scraping, performed along the medial aspect of the inferior turbinate, has shown several advantages [8]. Although the technique has been validated as a semi-quantitative analysis for diagnosis of cellular rhinitis and correlations have been demonstrated between nasal and bronchial inflammatory cytological patterns, its role in CRS has not been clarified [17]. One controversial issue is linked to the sampling site. Some studies debate its usefulness when performed along the inferior turbinate. For example, De Corso et al. reported that inferior turbinate eosinophilic inflammation represents an early marker for severe CRSwNP [18]. Similarly, Gelardi et al. showed that the

Table V. Sensitivity, specificity, positive likelihood ratio and negative likelihood ratio of cytology performed on the inferior turbinate and middle meatus in the non-allergic CRS population.

N = 20	Best cut-off	Sensitivity, %	Specificity, %	LR+	LR-	Correctly classified, %
IT eosinophil count in 10 consecutive HPF 400x	≥ 7	66.7	54.6	1.5	0.6	60.0
IT eosinophil percentage on total immune cells	≥ 1.3	66.7	45.5	1.2	0.7	55.0
MM eosinophil count in 10 consecutive HPF 400x	≥ 5	88.9	72.7	3.3	0.2	80.0
MM eosinophil percentage on total immune cells	≥ 1.9	88.9	81.8	4.9	0.1	85.0

IT: inferior turbinate; HPF: high power field; MM: middle meatus; LR+: positive likelihood ratio; LR-: negative likelihood ratio

association of eosinophilic-mast cell inferior turbinate infiltration and the presence of asthma and aspirin sensitivity is correlated with an increased risk of polyp relapse [19]. Our analysis confirmed the existence of a significant degree of correlation between tissue eosinophilia and IT cytological eosinophilic count (r = 0.30, P = 0.017). To make matters complicated, She et al. demonstrated a lack of significant correlation between the total and individual inflammatory cell counts in inferior turbinate versus paranasal sinus mucosa, questioning the diagnostic value of nasal cytology for CRS [20]. If it is true that the term CRS has been coined precisely to express that every sinus inflammation translates contextually into an inflammation of the nasal mucosa (and therefore also of the inferior turbinate) and that transcriptomic studies showed a substantially overlapping gene expression profile of various nasal subsites [21,22], it is also true that clinical practice teaches that the phenotypic manifestations of CRS usually spare the mucosa of the inferior turbinates [23]. This aspect is the inspiring concept underlying the recent "reboot approach" [24]. Moreover, this region, in addition to a different embryological origin, shows a morpho-histological structure that is not identical to that of the middle meatus [22,25].

She et al. demonstrated that 66% of CRS patients with CRS show marked inflammation in the inferior turbinate, but that the inflammation is much more intense in maxillary sinus mucosa [20]. Furthermore, the inflammatory response in the ethmoid sinus seems even more severe than in maxillary sinus or inferior turbinate in other series of patients with chronic sinusitis [26]. Taken together, these findings suggest that the paranasal sinuses, especially ethmoid, possibly play a pivotal role in CRS. It follows that sampling a typical site of CRS manifestation might be more representative of CRS-related inflammatory profile; moreover, the cellular inflammatory pattern of the inferior turbinate can be clearly influenced by the coexistence of allergic and non-allergic rhinitis. Furthermore, as reported by Armengot et al., a significant correlation exists between ethmoid tissue eosinophilia and MM cytological eosinophilia [27]; the same moderate correlation emerged from our data (r = 0.51, P < 0.0001). However, the estimated accuracy of nasal cytology seems limited because overall sensitivity values are low. Interestingly, once allergic patients were excluded from the analysis, the sensitivity further decreased for cytological sampling on IT and slightly increased for cytological MM sampling. This fact suggests that the allergy comorbidity can act as a confounding factor and should be taken into account when interpreting nasal cytology findings. These results, moreover, lead to further reflection. Apparently, nasal cytology might not be the ideal screening test for ECRS due to its low sensitivity. However, when ap-

plied to patients clinically suspect for ECRS, this test might confirm diagnosis and drive treatment selection. Lastly, it is reasonable to think that the degree of neutrophilic infiltration also produces an effect in terms of CRS classification. Thus, a more comprehensive grouping should account for mixed-granulocytic and pauci-granulocytic CRS cases, apart from the classical ECRS and non-ECRS subtypes.

Of course, the study is somewhat limited. It represents a preliminary exploration of the role of nasal cytology in CRS in a relatively small population. The technical choice has fallen upon nasal scraping because it was the only available in our center. Nonetheless, the literature concerning this topic is limited and extremely variable in terms of sampling site and processing techniques, which makes it difficult to carry out comparisons and draw solid conclusions.

Conclusions

In summary, assuming that a re-classification of CRS is a pressing clinical need, as well as the identification of reliable biomarkers, nasal cytology conceptually represents an interesting tool. In the same way as bronchial cytology for asthma, nasal cytology can allow for cellular profiling of CRS which, albeit in its initial stages, is a step forward the endotyping process and the thoughtful application of innovative biological therapies. Additionally, it shows several practical advantages, such as good tolerability and compliance, limited costs and an easy-to use approach. It is reasonable to think that nasal cytology in the MM might provide more accurate information on the degree of tissue eosinophilia in CRS. The next steps would be to verify these results across other wide and unbiased cohorts (eventually comparing different sampling methods) and to define thresholds values with the best accuracy. However, at present, its semi-quantitative nature, the lack of standard cut-offs and the discrepancy of reported results limit its systematic use in CRS workup, while remaining undisputed its role in chronic rhinitis.

Acknowledgements

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