Nasal fluid cytology and cytokine profiles of eosinophilic and non-eosinophilic chronic rhinosinusitis with nasal polyps*

Zhenzhen Zhu[1,#], Weiqing Wang[1,#], Xinyao Zhang[2], Xiaowei Wang[1], Yang Zha[1], Yujie Chen[1], Liangrui Zhou[3], Wei Lv[1]

[1] Department of Otolaryngology-Head and Neck Surgery, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Peking Union Medical College, Beijing, China

[2] Department of Clinical Laboratory, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Peking Union Medical College, Beijing, China

[3] Department of Pathology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Peking Union Medical College, Beijing, China

Rhinology 58: 4, 314 - 322, 2020
https://doi.org/10.4193/Rhin19.275

***Received for publication:** August 12, 2019
Accepted: January 13, 2020

# these two authors contributed equally to the work

Abstract

Background: Chronic rhinosinusitis with nasal polyps (CRSwNP) is a heterogeneous disease with different clinical characteristics and different treatment responsiveness. The aims of this study were to compare the nasal fluid cytology and cytokines between eosinophilic CRSwNP (eCRSwNP) and non-eosinophilic CRSwNP (neCRSwNP) and establish a new multivariate model to predict eCRSwNP before surgery to improve personalized treatment for CRSwNP patients.

Methods: Eighty-six consecutive patients with CRSwNP and sixteen healthy controls were recruited in this study. Nasal fluid (NF) was collected from all subjects and nasal polyp tissue was collected during the surgery. The differential cell counts and concentrations of IL-6, IL-8, TNF-α and IL-10 in NF were measured. Univariate and multivariate logistic regression were used to identify predictors for eCRSwNP.

Results: There were more inflammatory cells in NF of CRSwNP than controls. The eosinophil percentage was significantly higher in eCRSwNP than neCRSwNP and controls. The level of IL-8 was significantly higher in neCRSwNP than in eCRSwNP and controls. Blood eosinophilia, nasal fluid eosinophilia, higher total ethmoid score / total maxillary score (E/M ratio) and higher visual analogue scale (VAS) score of CRS were associated with eCRSwNP, the area under receiver operating characteristic curve (AUC) was 0.800, 0.755, 0.703 and 0.648, respectively. Using the coefficients of multivariate regression, we set up a scoring system to predict eCRSwNP with three of the variates and the AUC was 0.883.

Conclusion: ECRSwNP, neCRSwNP and healthy controls demonstrated different cytology and cytokine profiles in NF. A new preoperational multivariate prediction model for eCRSwNP with NF eosinophilia, blood eosinophilia and higher E/M ratio was established.

Key words: chronic rhinosinusitis with nasal polyps, eosinophil, nasal fluid, multivariate prediction model

Introduction

Chronic rhinosinusitis (CRS), characterized by nasal discharge, nasal obstruction, facial pain, and reduction of smell [(1)], is one of the most common chronic diseases in China. Based on the presence or absence of nasal polyp, CRS is currently divided into CRS with nasal polyps (CRSwNP) and CRS without nasal polyps (CRSsNP). As a heterogeneous disease, CRSwNP is further categorized as eosinophilic CRSwNP (eCRSwNP) or non-eosinophilic CRSwNP (neCRSwNP) depending on tissue eosinophilia. Compared with neCRSwNP, eCRSwNP often shows higher disease severity and higher risk of comorbid asthma [(2)]. These two subtypes of CRSwNP also respond distinctly to different treatments.

For example, eCRSwNP usually responds well to steroid therapy [3] but is more likely to relapse after surgery [4], while neCRSwNP responds well to macrolide therapy [5]. Besides tissue eosinophil content, they also possess distinct histological and immunopathological patterns [6, 7]. The different sites of biopsy, such as nasal polyp or ethmoid mucosa, could also affect tissue eosinophil counts [8]. Previous studies showed that eCRSwNP accounted for most cases in western countries [9, 10] and approximately more than half of CRSwNP cases in Eastern Asia were non-eosinophilic [7, 11]. However, our recent study in Chinese CRSwNP patients [12] together with studies in Korea [13] and Thailand[14] demonstrated that the proportion of eCRSwNP has significantly increased over time in Eastern Asia. In the era of precision medicine, otolaryngologists should personalize the regimen of different subtypes of CRSwNP with the change of disease spectrum. But the golden standard to diagnose eCRSwNP or neCRSwNP is based on the infiltrating inflammatory cells in the histologic sections, which is unavailable for patients whose first choice is medical therapy but not operation. Therefore, it is crucial to establish a prediction model for eCRSwNP before initial treatment plan decided. Previously, it was reported that higher peripheral blood eosinophil percentage and higher total ethmoid score / total maxillary score (E/M ratio) of computed tomography (CT) could be used as indicators of eCRSwNP[15-17]. However, the effectiveness of the previous model using single parameter to discriminate eCRSwNP was not satisfactory. The inflammatory cells and cytokines of nasal fluid (NF), mostly secreted by tissue of nasal cavity and paranasal sinuses, could reflect the inflammatory patterns of sinus tissue. We could often see eosinophils and neutrophils migrating from the lamina propria of nasal polyps through the epithelium in the histologic sections (Figure S1 in the Online Supplement). However, the differences of cytology and cytokines in nasal fluid of eCRSwNP and neCRSwNP remained unclear. The purposes of this study were: 1) to analyze the cytological characteristics and levels of several cytokine in NF of eCRSwNP, neCRSwNP and healthy controls; 2) to analyze the correlation between the cytology and cytokine levels in NF and those in sinonasal tissue; 3) to create a new multivariate prediction model with better effectiveness to discriminate eCRSwNP from neCRSwNP before surgery.

Material and methods

Subjects

A total of 86 consecutive patients with CRSwNP and 16 healthy controls without nasal disease from 2017 to 2018 were enrolled prospectively in Peking Union Medical College Hospital (PUMCH). The study was permitted by ethics committee of PUMCH and written informed consents were obtained from all patients and healthy controls. The diagnosis of CRSwNP was made according to European position paper on rhinosinusitis and nasal polyps 2012 (EPOS 2012) guidelines[1]. Subjects with fungal sinusitis, cystic fibrosis, antrochoanal polyps, immunodeficiency or sinonasal tumor were excluded. Concomitant asthma was diagnosed by pneumologists previously based on the history and pulmonary function test. Overall subjective symptom including nasal obstruction, nasal discharge, reduction or loss of smell, facial pain or pressure and headache, were evaluated using visual analogue scale (VAS). The complete peripheral blood cell count and differential white blood cell counts were measured by an automatic hemocyte analyzer. The atopic status of the patients was assessed by Phadiatop test (Phadia, Uppsala, Sweden) for detection of IgE against various common inhalant allergens. Blood total IgE and specific IgE were also measured before the surgery. Lund-Mackay score system was used to evaluate preoperative CT scan. Oral and intranasal glucocorticoids were stopped 1 month before the surgery and there was no acute onset of asthma 1 month before the surgery.

Sample collection, processing and measurement

All patients with CRSwNP underwent endoscopic sinus surgery. Nasal fluid was collected the day before the operation using a nasal nebulization machine (Neb-nid, Flaem Nuova SPA, Italy) as previous study mentioned[18]. Briefly, approximately 6 millimeters isotonic (0.9%) saline at room temperature was sprayed into each nasal cavity with the subject seated, leaning forward slightly. The overall volume of nasal fluid returned was 8-10 ml. 5 ml fluid was centrifuged at 400g for 5 minutes, the supernatant was discarded and cell pellets were resuspended in the residual fluid (about 200 microliter). The cell suspension was centrifuged at 800 r/min for 4 min by cytospin (Thermo Scientific Cytospin 4, Shandon, UK) and the cytospin slide was stained using the Wright-Giemsa method by an automatic slide-pushing dyeing machine (Sysmex, Japan). All cytospin slides were evaluated by the same experienced cytologist who was blinded to clinical and histological characteristics. A total of 200 nucleated cells were counted and differentiated as eosinophils, neutrophils, lymphocytes, monocytes / macrophages and epithelial cells at high magnification (x1000) and results were presented as percentage of the total cells. Another 2 ml returned fluid was filtered with 40μm-nylon sieve for the detection of IL-6, IL-8, TNF-α and IL-10 by IMMULITE 1000 Automatic Chemiluminescence Immunoassay (Siemens Medical, USA). The detection limit was 2pg/ml for IL-6 and IL-8, 5pg/ml for TNF-α and IL-10. The processing and measurement were conducted within 2 hours.

Nasal polyp tissue was obtained during the operation, fixed in 10% formalin promptly, and embedded in paraffin subsequently. The 4μm-paraffin section was made by a microtome and stained with hematoxylin and eosin (H&E) method. The number of eosinophils, lymphocytes, neutrophils, and plasma cells from 5 randomly selected non-overlapping fields was counted at ×400 magnification. All sections were reviewed by 2 independent physicians who were blinded to the clinical data. In cases of dis-

Table 1. Clinical characteristics of eCRSwNP and neCRSwNP.

	No. of patients with known information, eCRSwNP vs neCRSwNP	eCRSwNP n (%) or median (IQR)	neCRSwNP n (%) or median (IQR)	P value
Age	65:21	46.0 (35.5-57.0)	47.0 (35.5-57.5)	0.984
M/F	65:21	46/19	16/5	0.630
Smoking	64:21	23 (35.9%)	9 (42.9%)	0.570
Atopy	62:17	15 (24.2%)	3 (17.6%)	0.749
Asthma	65:21	23 (35.4%)	2 (9.5%)	**0.023**
Recurrence	65:21	15 (23.1%)	7 (33.3%)	0.394
VAS	63:20	7.00 (5.00-8.00)	5.50 (4.25-7.00)	**0.045**
LM Score	64:18	19.50 (15.00-23.00)	17.50 (16.00-22.25)	0.982
E	64:18	7.00 (5.00-8.00)	6.00 (4.75-8.00)	0.419
M	64:18	2.00 (2.00-3.75)	3.00 (2.00-4.00)	**0.036**
E/M ratio	64:18	2.00 (2.00-3.38)	2.00 (1.94-2.00)	**0.006**
T-IgE	62:17	68.10 (34.20-169.25)	72.80 (36.85-178.50)	0.802
B-EOS%	64:21	5.60 (4.33-8.23)	2.30 (1.20-4.50)	**< 0.001**

M/F = male/female; Recurrence = Previous nasal polyp surgery; VAS = Overall discomfort evaluated by visual analogue scale; LM Score = Lund-Mackay CT score; E = Total ethmoid sinus score; M = Total maxillary sinus score; E/M ratio = Total ethmoid sinus score/total maxillary sinus score; T-IgE = Level of serum total immunoglobulin E; B-EOS% = blood eosinophil percentage; IQR = Interquartile ranges.

agreement (when 2 counts differed by >10%), a consensus was reached by our research team reviewing the specimen together. The eCRSwNP was diagnosed when the tissue eosinophil count exceeded 10% of the total inflammatory cells as proposed in previous studies[15, 19].

Statistical analysis

SPSS, version 23.0 (SPSS, Chicago, USA) and GraphPad Prism, version 7 (GraphPad Software Inc, USA) were used to perform data analysis. Continuous variables expressed as median and interquartile ranges (IQR) or mean±standard deviation (SD) were analyzed by Mann–Whitney U test and categorical variables expressed as number (percentage) were analyzed using chi-square test. Correlation analysis was done using the method of Spearman. Receiver operating characteristic (ROC) curves were used to evaluate the prediction ability for eCRSwNP and the best cutoff value was determined by the lowest value of $(1\text{-sensitivity})^2+(1\text{-specificity})^2$. Continuous variables associated with eCRSwNP were transformed into categorial binary data based on the optimal cutoff value. These variables were then tested through univariate logistic regression and variables with $P < 0.1$ were included in multivariate logistic regression using forward stepwise selection. Significant variables selected from the multivariate logistic regression were used in the multivariate prediction model of eCRSwNP using a score based method as Sullivan et al mentioned previously[20]. The Hosmer-Lemeshow test was preformed to assess the goodness of fit of the logistic model, the ability of multivariate score model to predict eosinophilic subtype of CRSwNP was evaluated through ROC curve. The additional predictive value of nasal fluid eosinophilia to the multivariate prediction model was determined by integrated discrimination improvement (IDI)[21]. Significance was accepted when $P < 0.05$.

Results

Subject characteristics

Among the 86 CRSwNP patients, 65 (75.6%) cases were eosinophilic and another 21 cases were non-eosinophilic CRSwNP. The demographic and clinical characteristics were demonstrated in

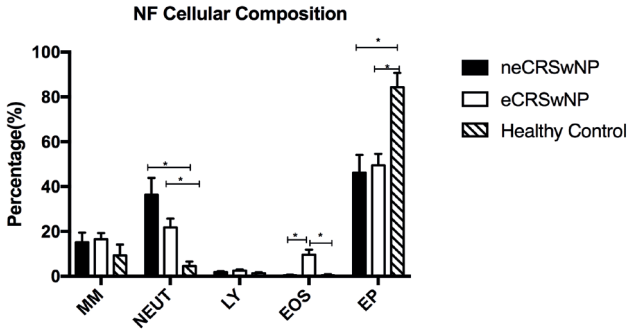

Figure 1. The cellular composition in nasal fluid (NF) of eCRSwNP (n = 61), neCRSwNP (n = 21) and healthy controls (n = 16). MM, monocyte/macrophage; NEUT, neutrophil; LY, lymphocyte; EOS, eosinophil; EP, epithelial. Shown as mean±SEM; *P < 0.05.

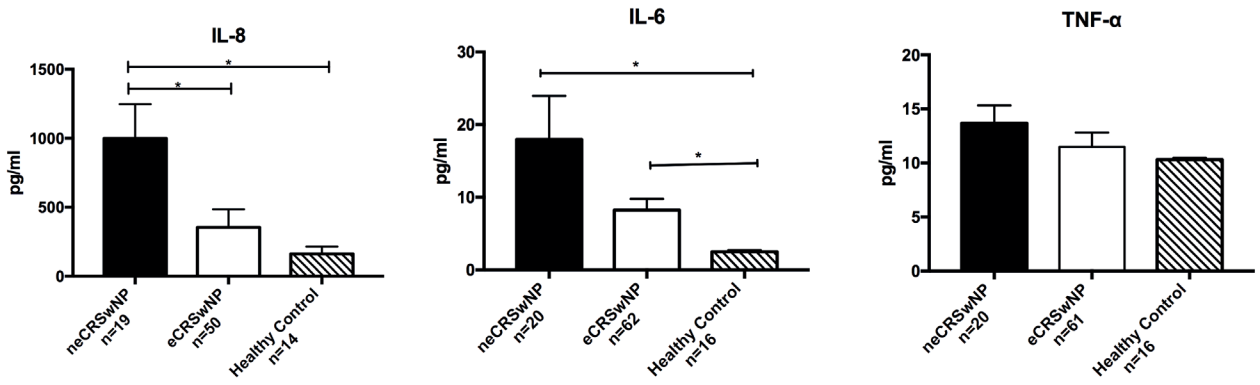

Figure 2. Cytokine (IL-8, IL-6, TNF-α) levels in nasal fluid of eCRSwNP, neCRSwNP and healthy controls. Shown as mean±SEM; *P < 0.05.

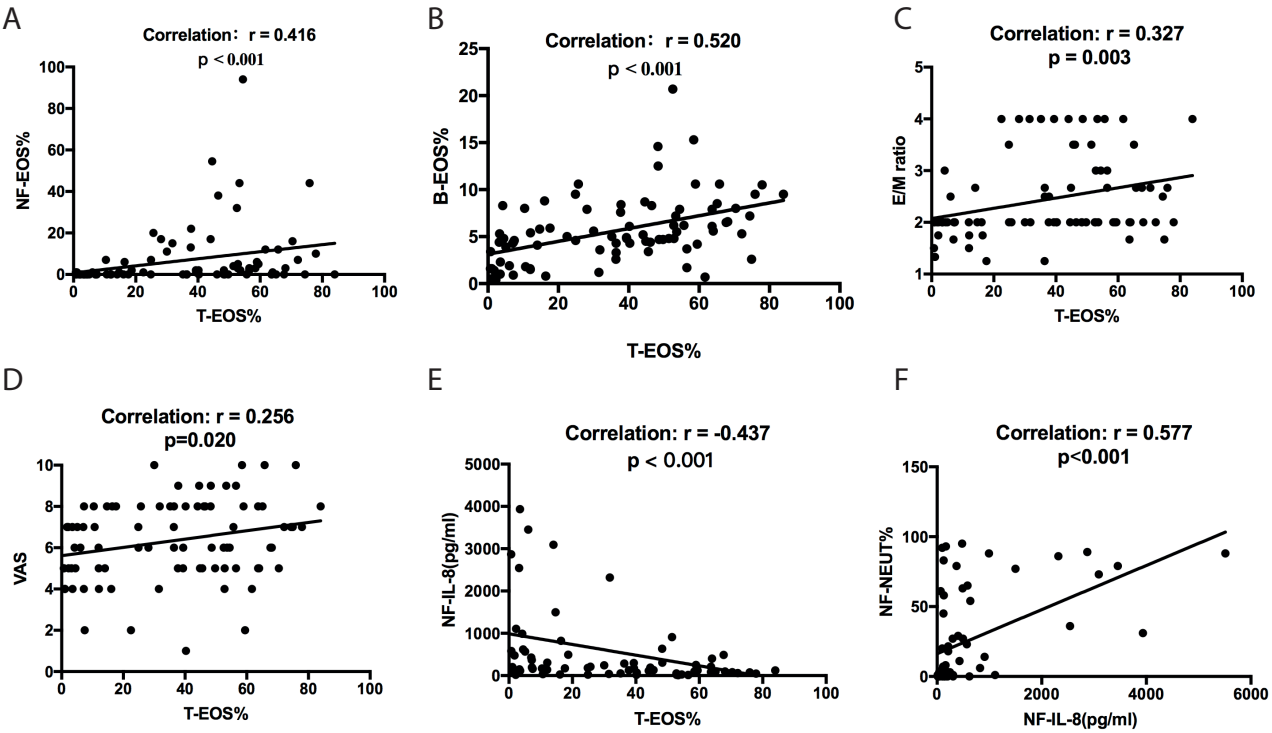

Figure 3. Correlation between tissue eosinophil percentage (T-EOS%) and nasal fluid eosinophil percentage (NF-EOS%), blood eosinophil percentage (B-EOS%), total ethmoid and maxillary sinus CT score ratio (E/M ratio), VAS score and IL-8 concentration in NF (NF-IL-8) were shown in (A)-(E). Correlation between IL-8 and neutrophil percentage in NF (F). Spearman correlation analysis was used.

Table 1. Compared with neCRSwNP, patients with eCRSwNP demonstrated higher concomitant asthma rate, higher VAS score, lower total maxillary sinus CT score, higher E/M ratio and higher peripheral blood eosinophil percentage.

Nasal fluid cytology and cytokine levels

The NF cellular composition of CRSwNP and healthy control was shown in Figure 1. Overall, the percentage of inflammatory cells was higher in CRSwNP group than in control group (51.55±38.86 vs 15.63±25.33, P = 0.001) and the percentage of epithelial cells was lower in CRSwNP group than in control group (48.45±38.86 vs 84.38±25.33, P = 0.001). Furthermore, the percentage of eosinophils was significantly higher in eCRSwNP than neCRSwNP (8.84±16.48 vs 0.48±1.54, P < 0.001) and controls (8.84±16.48 vs 0.44±1.75, P < 0.001). Meanwhile, the percentage of neutrophils was significantly higher in CRSwNP group than in control group (26.39±33.16 vs 4.50±8.25, P = 0.003). The mean percentage of neutrophils in neCRSwNP group was slightly higher than in eCRSwNP, but no statistical significance was attained (33.52±38.76 vs 23.93±30.98, P = 0.633). There was no significant difference between percentage of lymphocytes in CRSwNP group and control group (2.35±3.17 vs 1.38±1.78, P = 0.246).

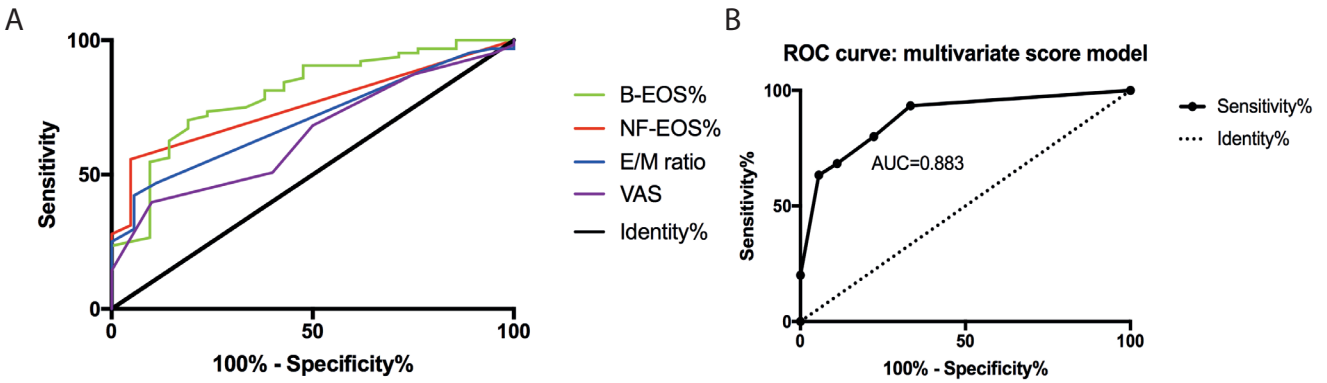

Figure 4. (A), Receiver operating characteristic (ROC) curves to predict eCRSwNP for single variable with nasal fluid eosinophil percentage (NF-EOS%), blood eosinophil percentage (B-EOS%), ethmoid and maxillary score ratio (E/M ratio) and VAS score; (B), ROC curve to predict eCRSwNP with the multivariate score model in Table 5. AUC, area under the ROC curve.

The levels of IL-6, IL-8 and TNF-α in nasal fluid of CRSwNP and healthy subjects were shown in Figure 2. The level of IL-6 was significantly greater in NF of CRSwNP group than control group (11.16±19.45 vs 2.51±0.95, P = 0.001). And IL-8 was significantly higher in neCRSwNP group than eCRSwNP group (969.00±1246.04 vs 429.46±929.65, P = 0.009) and control group (969.00±1246.04 vs 162.43±196.45, P = 0.006). There was no difference in level of TNF-α between CRSwNP group and control subjects (12.17±9.37 vs 10.30±0.76, P = 0.340). And the concentration of IL-10 in nasal fluid was below the detection limit (data not shown).

Correlation analysis and ROC curve

The correlation analysis demonstrated a positive correlation between the percentage of eosinophils in nasal polyp tissue and NF (r = 0.416, P < 0.001, Figure 3). Meanwhile, the tissue eosinophil percentage correlated positively with the peripheral blood eosinophil percentage (r = 0.520, P < 0.001), E/M ratio (r = 0.327, P = 0.003) and VAS score (r = 0.256, P = 0.020). However, the level of IL-8 in NF correlated negatively with tissue eosinophil percentage (r = -0.437, P < 0.001). In patients with CRSwNP, the level of IL-8 correlated positively with neutrophil percentage in NF (r = 0.577, P < 0.001).

The ROC curves for blood eosinophil percentage, NF eosinophil percentage, E/M ratio and VAS score to distinguish eCRSwNP from neCRSwNP were shown in Figure 4. The area under the curve (AUC) and 95% confidence interval (CI) of the four parameters (shown in Table 2) were 0.800 (95% CI= 0.692-0.908), 0.755 (95%CI = 0.650-0.860), 0.703 (95%CI = 0.584-0.822) and 0.648 (95%CI = 0.521-0.775), respectively. The optimal cutoff value of NF eosinophil percentage was 0.5% (sensitivity 61.3%, specificity 86.5%). The cutoff value of peripheral blood eosinophil

Table 2. Area under the ROC curves, sensitivity and specificity at the optimal cutoff value.

Predictors	AUC (95%CI)	Optimal cutoff value	Sensitivity	Specificity
B-EOS%	0.800 (0.692-0.908)	4.65	69.2	81.0
NF-EOS%	0.755 (0.650-0.860)	0.5	61.3	86.5
E/M ratio	0.703 (0.584-0.822)	2.25	46.2	88.9
VAS	0.648 (0.521-0.775)	5.5	68.3	50.0

B-EOS% = blood eosinophil percentage; NF-EOS% = nasal fluid eosinophil percentage; E/M ratio = total ethmoid sinus score/total maxillary sinus score; VAS = Overall discomfort evaluated by visual analogue scale; CI = confidence interval.

Table 3. Univariate logistic regression analysis of factors with eCRSwNP.

Factor	B	Odds ratio (95%CI)	Standard error	P value
B-EOS% ≥ 4.65%	2.309	10.066 (2.990-33.892)	0.619	< 0.001
NF-EOS% ≥ 0.5%	1.949	7.022 (2.102-23.453)	0.615	0.002
E/M ratio ≥ 2.25	1.954	7.059 (1.499-33.250)	0.791	0.013
VAS ≥5.5	0.765	2.150 (0.772-5.990)	0.523	0.143
Asthma	1.649	5.202 (1.112-24.344)	0.787	0.036

B-EOS% = blood eosinophil percentage; NF-EOS% = nasal fluid eosinophil percentage; E/M ratio = total ethmoid sinus score/total maxillary sinus score; VAS = Overall discomfort evaluated by visual analogue scale.

Table 4. Multivariate logistic regression analysis of factors with eCRSwNP.

Factor	B	Odds ratio (95%CI)	Standard error	P value
B-EOS% ≥ 4.65%	2.355	10.534 (2.415-45.941)	0.751	0.002
NF-EOS% ≥ 0.5%	1.753	5.773 (1.269-26.263)	0.773	0.023
E/M ratio ≥ 2.25	1.755	5.785 (1.013–33.045)	0.889	0.048
Asthma	-	-	-	0.197
Constant	-0.965	0.381	0.511	0.059

B-EOS% = blood eosinophil percentage; NF-EOS% = nasal fluid eosinophil percentage; E/M ratio = total ethmoid sinus score/total maxillary sinus score.

Table 5. The scoring model for the prediction of eCRSwNP.

Factor	Score
B-EOS% ≥ 4.65%	4
NF-EOS% ≥ 0.5%	3
E/M ratio≥ 2.25	3

B-EOS% = blood eosinophil percentage; NF-EOS% = nasal fluid eosinophil percentage; E/M ratio = total ethmoid sinus score/total maxillary sinus score.

percentage was 4.65% (sensitivity 69.2%, specificity 81.0%). The cutoff value of E/M ratio was 2.25 (sensitivity 46.2%, specificity 88.9%). The cutoff value of VAS score was 5.5 (sensitivity 68.3%, specificity 50.0%).

Logistic regression analysis and establishment of multivariate prediction model

To set up a multivariate prediction for eCRSwNP, we conducted logistic regression analysis to select associated parameters. For the convenience of clinical practice, we transformed NF eosinophil percentage, blood eosinophil percentage, E/M ratio and VAS score into bicategorized variables according to the optimal cutoff value. Then we conducted univariate logistic regression with the four variables and the presence of asthma. The coefficients, unadjusted odds ratios (ORs) and P value of each variable were shown in Table 3. And then variables with $P < 0.10$ in univariate logistic regression were inputted into multivariate logistic regression using forward stepwise selection and we identified NF eosinophil percentage ≥ 0.5%, blood eosinophil percentage ≥ 4.65%, and E/M ratio ≥ 2.25 as independent predictors for eCRSwNP (shown in Table 4). The Hosmer–Lemeshow test showed that the model fits the data well ($P = 0.496$). According to the regression coefficients, the multivariate score system for eCRSwNP was presented in Table 5. The ROC curve for the multivariate score system to diagnose eCRSwNP was shown in Figure 4(B) and the AUC was 0.883 (95%CI = 0.798-0.968). The sensitivity and specificity at different cutoff value was shown in Table S1 in the Online Repository. A cutoff value of 3.5 points had a sensitivity of 80% and specificity of 77.8%. The addition of nasal fluid eosinophil percentage ≥ 0.5% to the multivariate model improved its discrimination (IDI =10.5%).

Discussion

A recent retrospective study in asthma cohorts revealed that exposure to 4 or more prescriptions of oral corticosteroids was associated with greater odds adverse effects for osteoporosis, hypertension, obesity, type 2 diabetes, gastrointestinal ulcers/bleeds, fractures, and cataracts[22]. Considering the heterogeneity of CRS, eCRSwNP and neCRSwNP may respond distinctly to current therapy, especially oral corticosteroids. Therefore, distinguishing eCRSwNP from neCRSwNP in the initial maximal medical treatment before surgery is crucial for personalized medicine, which could minimize the side effects of medical therapy. Nasal fluid was widely used to detect airway inflammation as it is noninvasive and easy to perform. In this study, we compared the cytology and cytokine levels in NF of eCRSwNP, neCRSwNP and healthy controls. We also established a new preoperational multivariate prediction model for eCRSwNP with NF eosinophilia, blood eosinophilia and higher E/M ratio.

There were many kinds of methods to collect nasal secretion and we adopted the nasal washing machine in our study[18]. The machine decreased the interference of nasopharyngeal secretion effectively compared with traditional nasal lavage and was more convenient and comfortable for patients with better tolerance. After induction of fluid into nasal cavity, the recovery nasal fluid could be used to detect soluble cytokine levels and evaluate the cellular content after centrifugation and staining. There was no study focusing on the difference of nasal fluid between eCRSwNP and neCRSwNP. Therefore, our study intended to compare the differences of nasal fluid cytology and cytokine levels not only between CRSwNP and healthy subjects, but also between eCRSwNP and neCRSwNP. In the present study, we found the percentage of inflammatory cells was higher in NF of CRSwNP subjects than healthy control subjects. The percentage of eosinophils was significantly higher in eCRSwNP than neCRSwNP. Meanwhile, the neutrophil percentage was higher in CRSwNP, particularly in neCRSwNP. As for the cytokine levels, concentrations of IL-6 and IL-8 in NF were higher in CRSwNP than controls. The concentration of IL-8, a marker of neutrophilic inflammation, was significantly higher in NF of neCRSwNP than eCRSwNP, and correlated negatively with tissue eosinophil percentage and positively with neutrophil percentage in NF, which was similar to the results of a previous study in cystic fibrosis[23]. In addition, one previous study showed that nasal cytology associated with clinical comorbidities were identified

as predictors for relapse of nasal polyps[24]. Taken together, our results suggested nasal fluid could reflect nasal polyp tissue and clinical information, in agreement with other studies[25-27].

Previous studies have also shown that nasal fluid eosinophil and cytokine could be used for the diagnosis and monitoring of allergic rhinitis[28, 29] and help in the individualized treatment[30, 31]. Besides, De Corso et al.[32] suggested that patients of nonallergic rhinitis with nasal hypereosinophilia were at higher risk of development of nasal polyps and therefore in need of intensive treatment. With the concept of "the same airway, the same disease", nasal eosinophilia was also considered an alternative marker for eosinophilic asthma and better than blood eosinophil count in predicting sputum eosinophilia[33-35]. All those studies suggested that nasal fluid cytology and cytokine levels could be used as a quick, fast and non-invasive method to help in detecting and managing airway inflammation. Although NF analysis are not readily available in some hospitals now, we believe that the trend of personalizing CRSwNP regimen, such as using dupilumab[36], will bring more convenient instruments and procedures to analyze NF in our clinical practice. The NF test is a non-invasive and promising method for identifying eCRSwNP before starting the treatment.

Because the golden standard to diagnose eosinophilic CRSwNP was tissue eosinophilia, we could only achieve our goal by investigating the CRSwNP patients who underwent surgery to make histopathologic diagnosis. Moreover, most CRSwNP patients will undertake endoscopic sinus surgery because medical treatment cannot eradicate the nasal polyps and symptoms relapse. Therefore, the patients who underwent surgery could represent the characteristics of CRSwNP patients to some extent. We enrolled 86 consecutive CRSwNP patients who underwent surgery in our study to minimize the selection bias. In this cohort, 75.6% of enrolled patients (65/86) were eCRSwNP. According to the EPOS 2012[1], the eCRSwNP patients account for about 80% of all CRSwNP in Caucasian population. Our previous study showed that the proportion of eCRSwNP in all CRSwNP patients is 73.7% in Beijing, China[12]. Therefore, the patients enrolled in our study had the same eosinophilic-noneosinophilic distributing characteristics with CRSwNP patients in Chinese population and were highly representative. Thus, the multivariate predicting model for eCRSwNP established based on enrolled patients has substantial universality in the ordinary population.

Besides cytology and cytokines in NF, we also compared the clinical characteristics of eCRSwNP and neCRSwNP and found more frequent comorbid asthma, higher E/M ratio, higher VAS score and higher blood eosinophil percentage in eCRSwNP, which have also been mentioned in other studies. Correlation analysis showed that nasal fluid eosinophil percentage, peripheral blood eosinophil percentage, E/M ratio as well as VAS score correlated positively with nasal polyp tissue eosinophil percentage. Thus, we created 4 ROC curves for the four variables in the diagnosis of eCRSwNP (Figure 4). The association between eosinophilia in nasal fluid and nasal tissue has been proposed by some researchers[37, 38], but in this study, we firstly used NF eosinophil percentage as a predictor for eCRSwNP with a sensitivity of 54.8% and specificity of 94.6% at the cutoff value of 0.5%. The ability of blood eosinophil percentage in our study to predict eCRSwNP was higher[17], lower[15,39] or similar[40] than the results of previous studies, which could be explained by the different patient cohorts and different diagnostic criteria for eCRSwNP. Snidvongs et al.[41] suggested that the clinical use of blood eosinophilia was limited. Thus, Sakuma et al. established a scoring system with both sides of the disease, the presence of nasal polyp, higher CT shadow of ethmoid than maxillary sinus and blood eosinophilia to predict eCRS[16]. Meanwhile, Tao et al. combined blood eosinophil ratio and CT scores to predict uncontrolled CRS after endoscopic sinus surgery[42]. Based on our data and previous studies, we believed that establishing a multi-parameter prediction model is a better choice. Therefore, blood eosinophil percentage ≥ 4.65%, E/M ratio ≥ 2.25 and NF eosinophil percentage ≥ 0.5% were selected by multivariate logistic regression analysis to predict eCRSwNP. They were scored according to the coefficients in multivariate regression analysis (shown in Table 5) and the AUC of the score model was 0.883, indicating that the discrimination ability of multivariate score model was superior to each single parameter alone. A cutoff value at 3.5 demonstrated a sensitivity of 80.0% and a specificity of 77.8%. There were no neCRSwNP cases with the combination of blood eosinophil percentage ≥ 4.65%, NF eosinophil percentage ≥ 0.5% and E/M ratio ≥ 2.25. Meanwhile, the addition of nasal fluid eosinophil percentage ≥ 0.5% to the multivariate model improved its discrimination (IDI =10.5%). As shown in a study of prediction model for arteriosclerotic cardiovascular disease, variables with a relative IDI over 6% could bring significantly improved performance and should be included[43]. Therefore, combining NF eosinophilia with other variables could significantly improve the performance of the multivariate model in the prediction of eCRSwNP.

There are some limitations in our study. First, we only used H&E staining to analyze the histology of nasal polyp and Wright-Giemsa staining to analyze the cytology in the nasal fluid. However, Wright-Giemsa staining and May-Grünwald-Giemsa staining have been widely used to identify inflammatory cells in nasal fluid[25, 29, 30, 44]. May-Grünwald-Giemsa staining method also demonstrated similar results of eosinophil and granulocyte percentage compared with immunocytologic staining with monoclonal antibody[45]. H&E staining is sufficient to identify eosinophils, lymphocytes, and plasma cells and the key point of our article is the relationship of eosinophil percentage between nasal fluid and nasal polyp in CRSwNP. Many credible articles published before also used H&E staining to identify various inflammatory cells in CRSwNP[12, 13]. Hence our nasal cytology

and tissue histology results were generally acceptable. Second, this study was limited to a single medical center in Beijing. However, this may be an advantage to some extent because it was convenient to control confounding factors, such as technical protocols. We should conduct a nationwide multicenter study to validate the results in other cohorts, which needs to be further investigated in the future.

Conclusion

Cytology and cytokine levels in nasal fluid, as a nontraumatic method, could be used to detect, distinguish and manage airway inflammations. ECRSwNP, neCRSwNP and healthy controls demonstrate different cytology and cytokine profiles in nasal fluid. There was also a positive correlation between eosinophil percentage in nasal fluid and tissue eosinophil percentage. And we established a new preoperational multivariate prediction model for eCRSwNP with nasal fluid eosinophilia, blood eosinophilia and higher E/M ratio.

Acknowledgements

Funding sources for the study: Beijing Municipal Science & Technology Commission (Z171100001017219); China Postdoctoral Science Foundation(2018M641267); National Major Science & Technology Pillar Program during the Twelfth Five-year Plan Period (2012EP001004); Non-profit Central Research Institute Fund of Chinese Academy of Medical Sciences (2018PT32007); Education Reform Foundation of Peking Union Medical College (10023201800108).

Authorship contribution

WL designed this study; ZZZ, YJC and LRZ screened and collected sample; ZZZ, WQW and XYZ conducted experiments; ZZZ, WQW and XWW analyzed the data; ZZZ, WQW and XYZ drafted the manuscript; WL, XWW and YZ revised the manuscript; all the authors approved the final version of manuscript.

Conflict of interest

The authors declare no conflict of interest.

References

1. Fokkens WJ, Lund VJ, Mullol J, et al. European Position Paper on Rhinosinusitis and Nasal Polyps 2012. Rhinol Suppl. 2012;23:3 p preceding table of contents, 1-298.
2. Kountakis SE, Arango P, Bradley D, Wade ZK, Borish L. Molecular and cellular staging for the severity of chronic rhinosinusitis. Laryngoscope. 2004;114(11):1895-905.
3. Wen W, Liu W, Zhang L, et al. Increased neutrophilia in nasal polyps reduces the response to oral corticosteroid therapy. J Allergy Clin Immunol. 2012;129(6):1522-8 e5.
4. Lou H, Meng Y, Piao Y, Wang C, Zhang L, Bachert C. Predictive significance of tissue eosinophilia for nasal polyp recurrence in the Chinese population. Am J Rhinol Allergy. 2015;29(5):350-6.
5. Oakley GM, Christensen JM, Sacks R, Earls P, Harvey RJ. Characteristics of macrolide responders in persistent post-surgical rhinosinusitis. Rhinology. 2018;56(2):111-7.
6. Payne SC, Early SB, Huyett P, Han JK, Borish L, Steinke JW. Evidence for distinct histologic profile of nasal polyps with and without eosinophilia. Laryngoscope. 2011;121(10):2262-7.
7. Cao PP, Li HB, Wang BF, et al. Distinct immunopathologic characteristics of various types of chronic rhinosinusitis in adult Chinese. J Allergy Clin Immunol. 2009;124(3):478-84, 84 e1-2.
8. Thaitrakool W, Sukswai N, Keelawat S, et al. Histopathology of ethmoid mucosa versus polyp tissue in diagnosing eosinophilic mucin rhinosinusitis. Rhinology. 2019;57(1):67-72.
9. Wang X, Zhang N, Bo M, et al. Diversity of TH cytokine profiles in patients with chronic rhinosinusitis: A multicenter study in Europe, Asia, and Oceania. J Allergy Clin Immunol. 2016;138(5):1344-53.
10. Hellquist HB. Nasal polyps update. Histopathology. Allergy Asthma Proc. 1996;17(5):237-42.
11. Kim JW, Hong SL, Kim YK, Lee CH, Min YG, Rhee CS. Histological and immunological features of non-eosinophilic nasal polyps. Otolaryngol Head Neck Surg. 2007;137(6):925-30.
12. Wang W, Gao Y, Zhu Z, et al. Changes in the clinical and histological characteristics of Chinese chronic rhinosinusitis with nasal polyps over 11 years. Int Forum Allergy Rhinol. 2019;9(2):149-57.
13. Kim SJ, Lee KH, Kim SW, Cho JS, Park YK, Shin SY. Changes in histological features of nasal polyps in a Korean population over a 17-year period. Otolaryngol Head Neck Surg. 2013;149(3):431-7.
14. Katotomichelakis M, Tantilipikorn P, Holtappels G, et al. Inflammatory patterns in upper airway disease in the same geographical area may change over time. Am J Rhinol Allergy. 2013;27(5):354-60.
15. Hu Y, Cao PP, Liang GT, Cui YH, Liu Z. Diagnostic significance of blood eosinophil count in eosinophilic chronic rhinosinusitis with nasal polyps in Chinese adults. Laryngoscope. 2012;122(3):498-503.
16. Tokunaga T, Sakashita M, Haruna T, et al. Novel scoring system and algorithm for classifying chronic rhinosinusitis: the JESREC Study. Allergy. 2015;70(8):995-1003.
17. Meng Y, Lou H, Wang C, Zhang L. Predictive significance of computed tomography in eosinophilic chronic rhinosinusitis with nasal polyps. Int Forum Allergy Rhinol. 2016;6(8):812-9.
18. Zhang D, Xiao Y, Luo J, et al. Measurement of fractional exhaled nitric oxide and nasal nitric oxide in male patients with obstructive sleep apnea. Sleep Breath. 2018.
19. Wang WW, Lu DM, Zheng M, Zhang JG, Zhang B. TSLP regulates eotaxin-1 production by nasal epithelial cells from patients with eosinophilic CRSwNP. Rhinology. 2018;56(4):370-7.
20. Sullivan LM, Massaro JM, D'Agostino RB, Sr. Presentation of multivariate data for clinical use: The Framingham Study risk score functions. Stat Med. 2004;23(10):1631-60.
21. Pencina MJ, D'Agostino RB, Sr., D'Agostino RB, Jr., Vasan RS. Evaluating the added predictive ability of a new marker: from area under the ROC curve to reclassification and beyond. Stat Med. 2008;27(2):157-72; discussion 207-12.
22. Sullivan PW, Ghushchyan VH, Globe G, Schatz M. Oral corticosteroid exposure and adverse effects in asthmatic patients. J Allergy Clin Immunol. 2018;141(1):110-6 e7.
23. Pitrez PM, Brennan S, Turner S, Sly PD. Nasal wash as an alternative to bronchoalveolar lavage in detecting early pulmonary inflammation in children with cystic fibrosis. Respirology. 2005;10(2):177-82.
24. Gelardi M, Fiorella R, Fiorella ML, Russo C, Soleti P, Ciprandi G. Nasal-sinus polyposis: clinical-cytological grading and prognostic index of relapse. J Biol Regul Homeost Agents. 2009;23(3):181-8.
25. Bolard F, Gosset P, Lamblin C, Bergoin C, Tonnel AB, Wallaert B. Cell and cytokine profiles in nasal secretions from patients with nasal polyposis: effects of topical steroids and surgical treatment. Allergy. 2001;56(4):333-8.

26. Peric A, Vojvodic D, Radulovic V, Vukomanovic-Durdevic B, Miljanovic O. Correlation between cytokine levels in nasal fluid and eosinophil counts in nasal polyp tissue in asthmatic and non-asthmatic patients. Allergol Immunopath. 2011;39(3):133-9.
27. Di Lorenzo G, Drago A, Esposito Pellitteri M, et al. Measurement of inflammatory mediators of mast cells and eosinophils in native nasal lavage fluid in nasal polyposis. Int Arch Allergy Immunol. 2001;125(2):164-75.
28. Di Lorenzo G, Mansueto P, Melluso M, et al. Allergic rhinitis to grass pollen: measurement of inflammatory mediators of mast cell and eosinophils in native nasal fluid lavage and in serum out of and during pollen season. J Allergy Clin Immunol. 1997;100(6 Pt 1):832-7.
29. Miman MC, Uzun O, Gurses I, Kuku I, Ozturan O, Akarcay M. The sensitivity of nasal eosinophilia in allergic rhinitis. Eur Arch Otorhinolaryngol. 2007;264(9):1013-8.
30. Chen J, Zhou Y, Zhang L, et al. Individualized Treatment of Allergic Rhinitis According to Nasal Cytology. Allergy Asthma Immunol Res. 2017;9(5):403-9.
31. Howarth PH, Persson CG, Meltzer EO, Jacobson MR, Durham SR, Silkoff PE. Objective monitoring of nasal airway inflammation in rhinitis. J Allergy Clin Immunol. 2005;115(3 Suppl 1):S414-41.
32. De Corso E, Lucidi D, Battista M, et al. Prognostic value of nasal cytology and clinical factors in nasal polyps development in patients at risk: can the beginning predict the end? Int Forum Allergy Rhinol. 2017;7(9):861-7.
33. Amorim MM, Araruna A, Caetano LB, Cruz AC, Santoro LL, Fernandes AL. Nasal eosinophilia: an indicator of eosinophilic inflammation in asthma. Clin Exp Allergy. 2010;40(6):867-74.
34. Amorim MM, Fernandes PBL, Caetano LB, Dracoulakis S, Santoro IL, Fernandes ALG. Nasal lavage is better than blood count in predicting sputum eosinophilia. Clin Exp Allergy. 2015;45(5):1006-8.
35. de Farias CF, Amorim MM, Dracoulakis M, Caetano LB, Santoro IL, Fernandes AL. Nasal lavage, blood or sputum: Which is best for phenotyping asthma? Respirology. 2017;22(4):671-7.
36. Bachert C, Han JK, Desrosiers M, et al. Efficacy and safety of dupilumab in patients with severe chronic rhinosinusitis with nasal polyps (LIBERTY NP SINUS-24 and LIBERTY NP SINUS-52): results from two multicentre, randomised, double-blind, placebo-controlled, parallel-group phase 3 trials. Lancet. 2019;394(10209):1638-50.
37. Armengot M, Garin L, de Lamo M, Krause F, Carda C. Cytological and tissue eosinophilia correlations in nasal polyposis. Am J Rhinol Allergy. 2010;24(6):413-5.
38. Jankowski R, Persoons M, Foliguet B, Coffinet L, Thomas C, Verient-Montaut B. Eosinophil count in nasal secretions of subjects with and without nasal symptoms. Rhinology. 2000;38(1):23-32.
39. Zuo K, Guo J, Chen F, et al. Clinical characteristics and surrogate markers of eosinophilic chronic rhinosinusitis in Southern China. Eur Arch Otorhinolaryngol. 2014;271(9):2461-8.
40. Li Z, Han L, You QJ, et al. [Evaluation of blood eosinophil in the diagnosis of eosinophilic chronic rhinosinusitis]. Zhonghua Er Bi Yan Hou Tou Jing Wai Ke Za Zhi. 2016;51(7):528-32.
41. Snidvongs K, Lam M, Sacks R, et al. Structured histopathology profiling of chronic rhinosinusitis in routine practice. Int Forum Allergy Rhinol. 2012;2(5):376-85.
42. Tao X, Chen F, Sun Y, et al. Prediction models for postoperative uncontrolled chronic rhinosinusitis in daily practice. Laryngoscope. 2018;128(12):2673-80.
43. Yang X, Li J, Hu D, et al. Predicting the 10-Year Risks of Atherosclerotic Cardiovascular Disease in Chinese Population: The China-PAR Project (Prediction for ASCVD Risk in China). Circulation. 2016;134(19):1430-40.
44. Heffler E, Landi M, Caruso C, et al. Nasal cytology: Methodology with application to clinical practice and research. Clin Exp Allergy. 2018;48(9):1092-106.
45. Prat J, Xaubet A, Mullol J, et al. Immunocytologic analysis of nasal cells obtained by nasal lavage: a comparative study with a standard method of cell identification. Allergy. 1993;48(8):587-91.

Wei Lv, MD.
Department of Otolaryngology
Head and Neck Surgery
Peking Union Medical College Hospital
Chinese Academy of Medical Sciences
Peking Union Medical College; No.1 Shuaifuyuan, Wangfujing,
Dongcheng District
Beijing 100730
China.
Tel: 010-69156311
E-mail: lili20020615@sina.com

This paper contains supplementary materials online: at www.rhinologyjournal.org

SUPPLEMENTARY DATA

Table S1. Sensitivity, specificity and (1-Sensitivity)^2 + (1-Specificity)^2 at different cutoff points of multivariate scoring model.

Cutoff value	Sensitivity	Specificity	(1-Sensitivity)^2 + (1-Specificity)^2
1.5	93.3	66.7	0.115
3.5	80.0	77.8	0.089
5.0	68.3	88.9	0.113
6.5	63.3	94.4	0.138
8.5	20.0	100	0.640

Figure S1. Hematoxylin and eosin (H&E) of nasal polyps. Eosinophils and neutrophils are migrating from the lamina propria of nasal polyps through the epithelium.