International Archives of Allergy and Immunology

Clinical Allergy – Research Article

Int Arch Allergy Immunol 2020;181:853–861
DOI: 10.1159/000509211

Received: May 12, 2020
Accepted: June 6, 2020
Published online: July 20, 2020

Predictive and Diagnostic Value of Nasal Nitric Oxide in Eosinophilic Chronic Rhinosinusitis with Nasal Polyps

Hao Lv[a, b] Pei-Qiang Liu[a, b] Rong Xiang[a, b] Wei Zhang[a, b] Shi-Ming Chen[a, b] Yong-Gang Kong[a, b] Yu Xu[a, b]

[a]Department of Otolaryngology-Head and Neck Surgery, Renmin Hospital of Wuhan University, Wuhan, China; [b]Research Institute of Otolaryngology-Head and Neck Surgery, Renmin Hospital of Wuhan University, Wuhan, China

Keywords

Nasal nitric oxide · Eosinophilic chronic rhinosinusitis with nasal polyps · Predictor · Eosinophil · Computed tomography score · Immunoglobulin E · Cytokines · Logistic regression analysis · Receiver operating characteristic curves

Abstract

Background: A hallmark of eosinophilic chronic rhinosinusitis with nasal polyps (eCRSwNP) is mucosal eosinophil-predominant inflammation. Nasal nitric oxide (nNO) is a known biomarker of eosinophilic inflammation in the upper airway. However, the utility of nNO measurement in the upper airway remains controversial. The present study aimed to compare the use of other clinical parameters with nNO to prediagnose patients with eCRSwNP from Central China. ***Methods:*** From June 2019 to December 2019, 70 patients with CRSwNP undergoing endoscopic sinus surgery and 30 healthy subjects were enrolled. nNO measurements were performed in all of these subjects. Computed tomography scans, full blood count with differential analysis, and determination of total immunoglobulin E (total IgE) and plasma cytokines were performed before surgery. Receiver operating characteristic curves and logistic regression analysis were used to assess the predictive potential of the clinical parameters. ***Results:*** We recruited 24 patients with eCRSwNP and 46 with noneosinophilic CRSwNP (non-eCRSwNP). In patients with eCRSwNP, nNO levels were significantly higher than those in patients with non-eCRSwNP ($p < 0.0001$). Blood eosinophil percentages and counts, total IgE, and CT-derived ethmoid sinus and maxillary sinus ratio (E/M ratio) were all significantly higher compared with those in patients with non-eCRSwNP ($p < 0.05$). To diagnose eCRSwNP, the highest area under the curve (0.803) was determined for nNO. At a cutoff of >329 parts per billion (ppb), the sensitivity was 83.30% and the specificity was 71.70%. However, the levels of plasma cytokines Th1/Th2 were not significantly different between the histological types of CRSwNP ($p > 0.05$). ***Conclusion:*** Measurement of nNO is useful for the early diagnosis of eCRSwNP.

Edited by: H.-U. Simon, Bern.

karger@karger.com
www.karger.com/iaa

Yu Xu
Department of Otolaryngology-Head and Neck Surgery
Renmin Hospital of Wuhan University
238 Jiefang Rd., Wuhan 430060 (China)
xuy@whu.edu.cn

Introduction

Chronic rhinosinusitis with nasal polyps (CRSwNP), a chronic inflammatory disease occurring in the nasal-paranasal mucosa, is frequently encountered in the Department of Otolaryngology [1]. Its main symptoms include nasal congestion, anterior or posterior rhinorrhea, sense of olfaction, and facial pain/pressure, which last for at least 12 weeks and have a considerable impact on the patients' quality of life [2]. Several studies have shown that immune responses in patients with CRSwNP can vary across different geographical areas and populations with distinct racial backgrounds [3]. In Western populations, >80% of CRSwNP present with marked eosinophil-associated nasal mucosal inflammation [4], while over 50% of patients with CRSwNP in East Asia present with noneosinophilic inflammation [5, 6]. This heterogeneity allows CRSwNP to be divided into 2 distinct endotypes, namely, eosinophilic CRSwNP (eCRSwNP) and noneosinophilic CRSwNP (non-eCRSwNP) based on the degree of eosinophil infiltration in polyp pathological sections [7, 8]. The clinical features of patients with eCRSwNP include good steroid responsiveness, olfactory dysfunction, comorbid asthma, and high recurrence rate after surgery [9–11]. However, recent studies have found that the prevalence of eCRSwNP in East Asia has increased significantly in the past 20 years [12, 13]. Currently, eCRSwNP is the predominant CRSwNP subtype in Beijing, China [14]. Besides, over time, there has been a significant increase in eosinophilic inflammation associated with increased IgE production and Th2 response in patients from Central China with CRSwNP [15]. Therefore, to determine prognosis and long-term management strategies, Chinese rhinologists need to discriminate the phenotypes of patients with CRSwNP early.

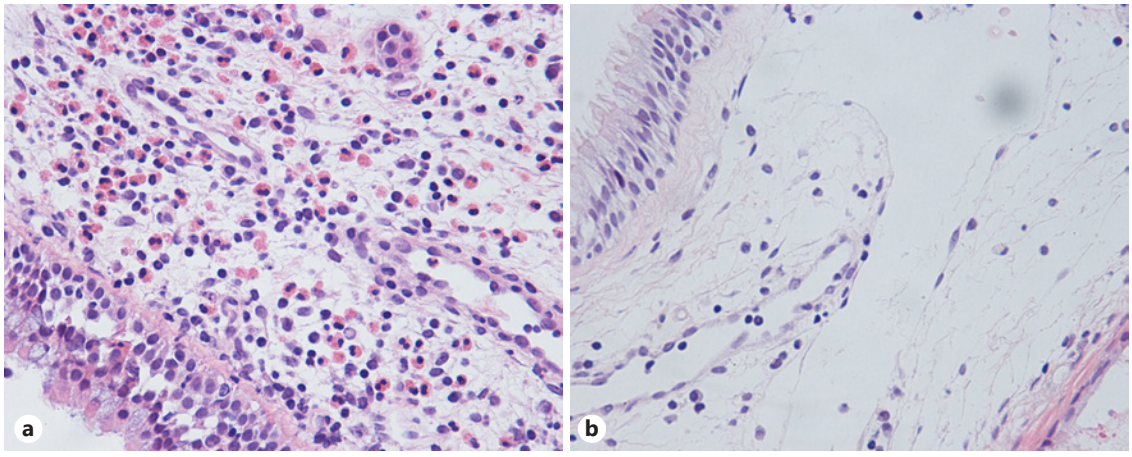
NO synthase (NOS) synthesizes nitric oxide (NO) from L-arginine and oxygen [16]. NO is produced mainly in the upper airway, especially in the paranasal sinus mucosa. NO plays a role in several different physiological and pathophysiological processes, including regulation of immunity, inflammation, blood flow, platelet function, and neurotransmission [17]. However, abnormal levels of NO are closely related to respiratory diseases [18]. According to clinical need, NO measurement can be divided into 2 categories. First, nasal NO (nNO) is collected from the upper airway using air exhaled from the nostrils. Second, to determine the NO levels in the lower airway, fractional exhaled NO (FeNO) is measured. Measurement of FeNO is an essential objective method to evaluate eosinophilic airway inflammation and is used for the diagnosis and management of asthma [19]. Rhinosinusitis status is believed to affect nNO levels, which are suggested to be involved in modulating cilia beating [20]. Thus, primary ciliary dyskinesia can be screened using nNO levels [21]. In addition, because nNO levels correlate well with symptom severity and radiographic staging, they have been used as a postoperative biomarker after sinus surgery for chronic rhinosinusitis (CRS) [22].

However, further research is required to develop nNO as a clinical marker of upper airway diseases. There are some limitations in the measurement of nNO that hinder its clinical application. First, in contrast to FeNO, researchers have not reached a consensus on the normal reference range of nNO. Second, nNO can be affected by various external and internal factors, making it less stable than FeNO. Previously, researchers have been exploring the changes in nNO levels in patients with CRSwNP. There is no doubt that nNO measurement will become an objective tool for diagnosing and monitoring patients with CRSwNP. However, few researchers have focused on the role of nNO detection in identifying different pathological types of CRSwNP. Thus, the present study aimed to explore the role of nNO detection in the diagnosis of eCRSwNP among patients from Central China with different types of CRSwNP, in comparison with other clinical data, to provide a guide for the clinical application of nNO levels.

Patients and Methods

Subjects

We conducted a cross-sectional, single-center study using data acquired from patients with CRSwNP, who had been subjected to endoscopic sinus surgery (ESS) between June 2019 and December 2019 at Renmin Hospital of Wuhan University. Thirty normal adult volunteers served as controls during the same period. In accordance with the diagnostic criteria recommended by the European Position Paper on Rhinosinusitis and Nasal Polyps [23], 70 patients were diagnosed with CRSwNP. The exclusion criteria comprised the following: the use of specific medications within 4 weeks before the inclusion visit (antibiotics, nasal rinsing, leukotriene receptor antagonists, antihistamines, and oral or local corticosteroids), systemic diseases that affect the nose (e.g., aspirin-exacerbated respiratory disease, Wegener granulomatosis primary ciliary dyskinesia, cystic fibrosis, and coagulation disorder), children under 18 years of age, significant psychological problems, pregnancy, lactation, and those unable to comply with the study protocol. Patients whose postoperative pathology report revealed fungal nasal-sinusitis, classic allergic fungal sinusitis, and nasal and paranasal sinus tumors were also excluded. The Ethics Committee of Renmin Hospital of Wuhan University approved this study, which was performed in accordance with the Helsinki Declaration. Written informed consent was provided by all subjects before data collection.



Color version available online

Fig. 1. Histological assessment of nasal polyps in eCRSwNP (**a**) and non-eCRSwNP (**b**). H&E staining. ×400. In eCRSwNP, infiltration of a large number of eosinophils under the mucosa was observed. eCRSwNP, eosinophilic chronic rhinosinusitis with nasal polyps; non-eCRSwNP, noneosinophilic chronic rhinosinusitis with nasal polyps.

Evaluation of Clinical Characteristics Preoperatively

Demographic data on patient sex, age, asthma, or allergic rhinitis were recorded as potential medical variables before surgery, and a senior otolaryngologist evaluated preoperative computed tomography (CT) scans in a blinded manner using the Lund-Mackay system to record sinusitis severity. Before ESS, the patients were subjected to routine peripheral blood tests (eosinophil percent and count), cellular immunoassays (plasma levels of Th1 cytokines [interleukin (IL)-2, tumor necrosis factor-alpha (TNF-α), interferon gamma (IFN-γ)], and Th2 cytokines [IL-4, -5, -6, and -10]), and assays for total IgE levels. We also assessed blood eosinophils of normal controls.

Nasal Nitric Oxide Measurement

To exclude the effects of other factors, such as time of day, diet, and sport, nNO was measured between 9 and 11 a.m. Before measurement, subjects rested for at least 30 min. In addition, the subjects were instructed not to eat nitrogen-rich food (e.g., animal offal, lettuce, spinach, and sausage) or use nasal decongestants within 1 h before the examination. A nanocoulomb nitric oxide analyzer (Sunvou, Wuxi, China) was used to measure the nNO levels. NO measurements were performed for all included participants based on the American Thoracic Society/European Respiratory Society guidelines [24]. In brief, a nasal olive was placed firmly in the nostrils and connected to the device through a central lumen. Subjects were asked to breathe normally. The aspiration flow rate was set at 5 mL/s. The nNO levels were measured continuously 3 times, and the mean of the 3 values was used for analysis.

Histological Examination

During ESS, tissue samples were obtained from all subjects, which were subjected to histological evaluation using standard techniques. Hematoxylin and eosin (H&E) staining was performed on 5-μm-thick sections, which were then observed under a light microscope at a ×400 magnification. All infiltrated inflammatory cells (lymphocytes, plasma cells, neutrophils, and eosinophils) were counted in 5 randomly selected high-magnification fields. The patients were classified as suffering from eCRSwNP when eosinophils were observed to comprise ≥10% of the total infiltrating cells; otherwise, they were classified as non-eCRSwNP (Fig. 1) [25].

Analysis of Statistical Data

SPSS software, version 24.0 (IBM Corp., Armonk, NY, USA), was used to perform all the statistical analyses. Data are presented as arithmetical mean values with standard deviation (SD). The statistical significance of differences was evaluated using an independent sample *t* test. The χ^2 test was used to evaluate the constituent ratios in both groups. As a widely used metric to assess clinical parameters' predictive values, receiver operating characteristic (ROC) curves were plotted to find the best cutoff point by calculating the sensitivity and specificity of the predictor. The Youden index, using the area under the ROC curve (AUC), could determine the diagnostic utility of each predictor.

Results

Subject Characteristics

In total, 70 patients with CRSwNP and 30 healthy controls were willing to participate in this study. Based on the histopathological examination, 24 patients were classified as the eCRSwNP group and 46 patients were classified as the non-eCRSwNP group. The 2 patient groups had a similar smoking history, sex ratio, and age distribution; however, the total IgE, history of asthma, and the blood eosinophil count and percentage were significantly higher in the eCRSwNP group compared with those in the non-eCRSwNP group. Table 1 shows the clinical and de-

Table 1. The demographic and clinical features of eCRSwNP patients and healthy subjects

	Normal (n = 30); 1	eCRSwNP (n = 24); 2	Non-eCRSwNP (n = 46); 3	p value (1 vs. 2)	p value (2 vs. 3)
Male/female	18/12	14/10	29/17	0.901	0.701
Age, years	38.5±14.7	39.3±12.7	42.6±15.6	0.872	0.340
Smoking, *n* (%)	4 (13.3)	4 (16.7)	11 (23.9)	0.732	0.483
Allergic rhinitis, *n* (%)	0 (0)	2 (8.3)	3 (6.5)	0.193	0.950
Asthma, *n* (%)	0 (0)	4 (16.7)	1 (2.2)	0.034	0.025
EOS count ($\times 10^9$/L)	0.15±0.10	0.32±0.20	0.19±0.16	0.001	0.011
EOS percentage, %	2.9±2.1	5.2±3.4	3.1±2.2	0.006	0.011
Total IgE, IU/mL	–	148.8±180.3	85.1±204.6	–	0.002
Maxillary sinus score	–	2.29±0.86	2.52±0.98	–	0.336
Posterior ethmoid score	–	2.63±0.97	2.13±1.05	–	0.059
Anterior ethmoid score	–	2.58±1.06	2.00±1.08	–	0.034
OMC score	–	2.21±1.62	2.33±1.22	–	0.764
Sphenoid sinus score	–	1.38±1.53	1.39±1.39	–	0.964
Frontal sinus score	–	1.29±1.49	1.72±1.62	–	0.286
Total score	–	12.38±5.39	12.02±5.15	–	0.766
E/M ratio	–	2.59±1.54	1.87±1.17	–	0.033

eCRSwNP, eosinophilic chronic rhinosinusitis with nasal polyps; non-eCRSwNP, noneosinophilic chronic rhinosinusitis with nasal polyps; EOS percentage, blood eosinophil percentage; EOS count, blood eosinophil count; Total IgE, total immunoglobulin E; OMC, osteomeatal complex; E/M ratio, ratio of the computed tomography scores for the ethmoid sinus and maxillary sinus.

Table 2. Levels of plasma cytokines (pg/mL) of Th1/Th2 cells in patients

Cytokines	eCRSwNP	Non-eCRSwNP	p value
IL-2	2.46±0.65	2.29±0.53	0.259
IL-4	2.74±0.78	2.61±0.78	0.522
IL-5	2.13±0.62	1.89±0.48	0.100
IL-6	5.31±2.19	4.18±1.85	0.220
IL-10	4.21±1.56	3.67±1.13	0.430
TNF-α	2.94±1.29	3.06±1.64	0.754
IFN-γ	2.51±0.84	2.71±1.25	0.471

eCRSwNP, eosinophilic chronic rhinosinusitis with nasal polyps; non-eCRSwNP, noneosinophilic chronic rhinosinusitis with nasal polyps; IL, interleukin; TNF-α, tumor necrosis factor-alpha; IFN-γ, interferon gamma.

mographic characteristics of the 2 groups of patients and healthy subjects.

Levels of Plasma Cytokines of Th1/Th2 Cells in Patients

Levels of plasma IL-2 (2.46 ± 0.65 vs. 2.29 ± 0.53 pg/mL; p = 0.259), IL-4 (2.74 ± 0.78 vs. 2.61 ± 0.78 pg/mL; p = 0.522), IL-5 (2.13 ± 0.62 vs. 1.89 ± 0.48 pg/mL; p = 0.100), IL-6 (5.31 ± 2.19 vs. 4.18 ± 1.85 pg/mL; p = 0.220), IL 10 (4.21 ± 1.56 vs. 3.67 ± 1.13 pg/mL; p = 0.430), TNF-α (2.94 ± 1.29 vs. 3.06 ± 1.64 pg/mL; p = 0.754), and IFN-γ (2.51 ± 0.84 vs. 2.71 ± 1.25 pg/mL; p = 0.471) were not significantly different between the 2 groups (Table 2).

Nasal NO Levels in the Groups

The average nNO levels were 469.8 ± 147.3 parts per billion (ppb) in healthy subjects, 341.8 ± 222.1 ppb in patients with CRSwNP, 268.9 ± 162.6 ppb in the non-eCRSwNP group, and 481.6 ± 255.5 ppb in the eCRSwNP group. Thus, we found that nNO levels in the CRSwNP group were significantly lower than those in the healthy group (p = 0.005) (Fig. 2a). The nNO levels in the eCRSwNP group were significantly higher than those in the non-eCRSwNP group (p < 0.0001). The non-eCRSwNP group showed significantly lower mean nNO levels compared with those in the healthy group (p < 0.0001). However, the nNO levels were not significantly different between the eCRSwNP group and the healthy controls (p = 0.833) (Fig. 2b).

Analysis by Logistic Regression

To identify the independent predictive factors associated with eCRSwNP, the stepwise forward method of logistic regression analysis was conducted based on a comparison between the 2 groups. Variables showing significant differences between the 2 subtypes were added into

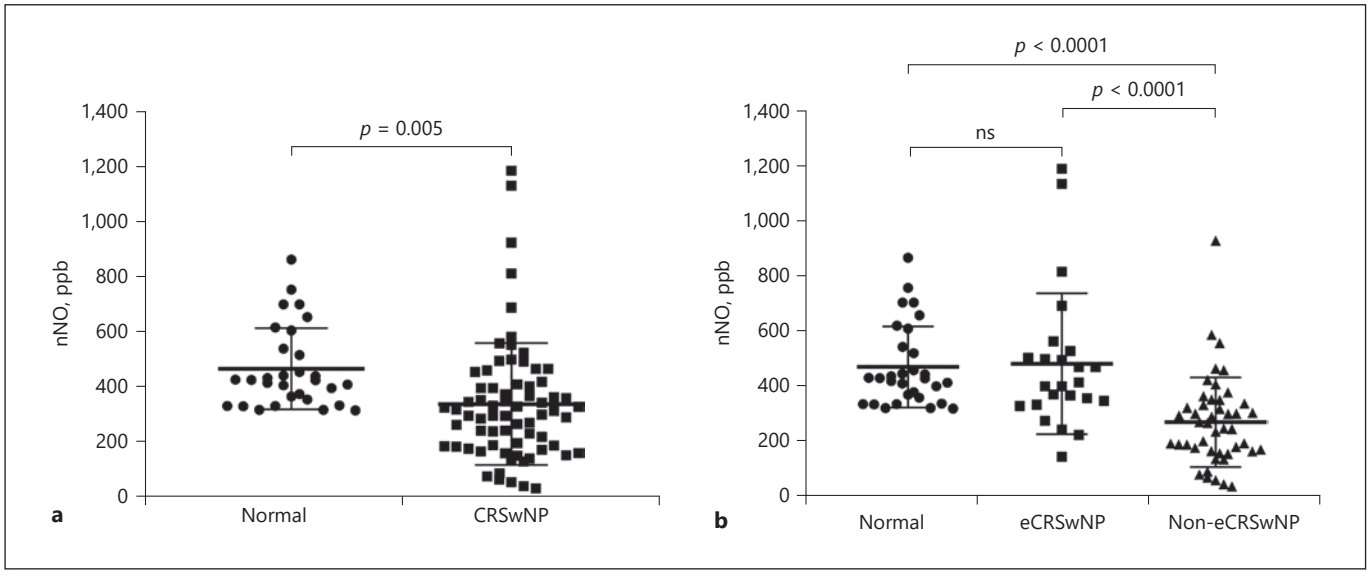


Fig. 2. The comparision of nNO levels between different groups. **a** Comparison of nNO levels between the normal and CRSwNP groups. **b** Comparison of nNO levels between the normal group and the different pathological types of CRSwNP groups. nNO, nasal nitric oxide; CRSwNP, chronic rhinosinusitis with nasal polyps; eCRSwNP, eosinophilic chronic rhinosinusitis with nasal polyps; non-eCRSwNP, noneosinophilic chronic rhinosinusitis with nasal polyps.

the model, e.g., the nNO level, blood eosinophil percentage and count, total IgE, and E/M ratio. Binary logistic analysis showed that eCRSwNP was associated significantly with the nNO level ($p = 0.002$; odds ratio [OR] = 1.737; 95% confidence interval [CI] = 1.025–2.942) and E/M ratio ($p = 0.040$; OR = 1.007; 95% CI = 1.002–1.011) (Table 3).

ROC Curve Evaluation

Table 4 shows the ROC curve analysis and AUC of factors associated with eCRSwNP. As a predictor for eCRSwNP (AUC = 0.803), the nNO level was highly accurate (Fig. 3).

Combination model 1 showed an AUC of 0.871, indicating that this model was the most accurate. However, at 0.749, the AUC of model 2 was not so accurate for eCRSwNP than that of nNO (Fig. 4).

Optimal Cutoff Point Determination

Table 5 shows the sensitivity and specificity, as well as the corresponding best cutoff point, for each predictor. To discriminate patients with eCRSwNP from those with non-eCRSwNP, the cutoffs, sensitivity, and specificity for each predictor were analyzed as follows: nNO: cutoff value = 329 ppb, sensitivity = 83.3%, specificity = 71.7%; total IgE: cutoff value = 105 IU/mL, sensitivity = 58.3%, specificity = 91.3%; blood eosinophil count: cutoff value = 0.2×10^9/L, sensitivity = 70.8%, specificity = 67.4%; blood eosinophil percentage: cutoff value = 3.2%, sensitivity = 66.7%, specificity = 67.4%; and E/M ratio: cutoff value = 2.0, sensitivity = 50.0%, specificity = 79.2%.

Table 3. Logistic regression analysis for identifying factors associated with eCRSwNP

Variables	OR	95% CI	*p* value
nNO	1.737	1.025–2.942	0.002
Total IgE	1.001	0.999–1.004	0.282
EOS count	1.232	0.732–2.886	0.320
EOS percentage	1.561	0.814–2.995	0.180
PE score	1.090	0.531–2.240	0.814
E/M ratio	1.007	1.002–1.011	0.040

eCRSwNP, eosinophilic chronic rhinosinusitis with nasal polyps; CI, confidence interval; OR, odds ratio; nNO, nasal nitric oxide; total IgE, total immunoglobulin E; EOS percentage, blood eosinophil percentage; EOS count, blood eosinophil count; PE, posterior ethmoid; E/M ratio, ratio of the computed tomography scores for the ethmoid sinus and maxillary sinus.

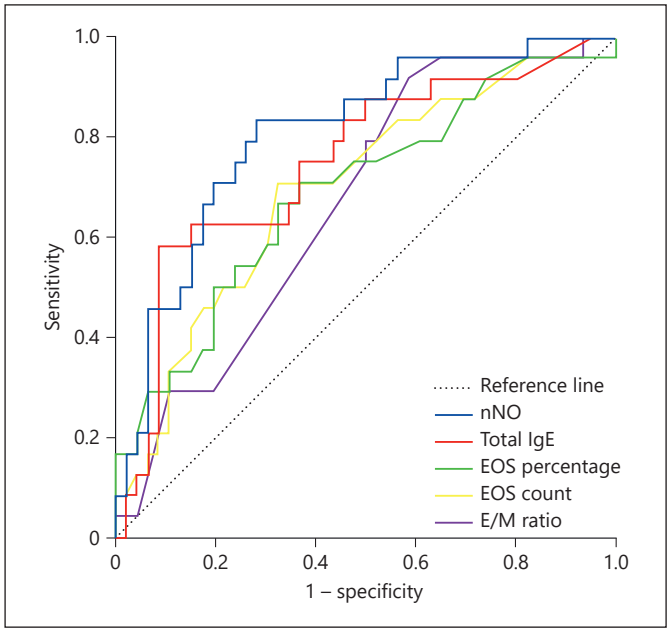


Fig. 3. ROC curves of predictive factors. ROC, receiver operating characteristic curves; nNO, nasal nitric oxide; total IgE, total immunoglobulin E; EOS percentage, blood eosinophil percentage; EOS count, blood eosinophil count; E/M ratio, ratio of the computed tomography scores for the ethmoid sinus and maxillary sinus.

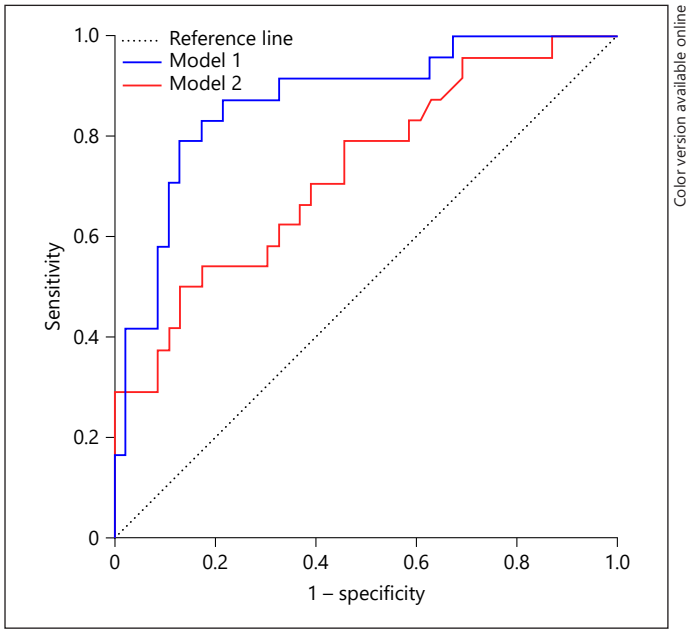


Fig. 4. ROC curves of model 1 (nNO included, blue line) and model 2 (nNO excluded, red line). ROC, receiver operating characteristic curves; nNO, nasal nitric oxide.

Table 4. ROC curve analysis of factors associated with eCRSwNP

Predictors	AUC	95% CI	*p* value
nNO	0.807	0.700–0.915	0.000
Total IgE	0.749	0.624–0.874	0.001
EOS count	0.694	0.562–0.826	0.008
EOS percentage	0.690	0.556–0.824	0.010
E/M ratio	0.667	0.538–0.795	0.023
Model 1	0.871	0.783–0.960	0.000
Model 2	0.749	0.627–0.871	0.001

eCRSwNP, eosinophilic chronic rhinosinusitis with nasal polyps; AUC, area under the ROC curve; nNO, nasal nitric oxide;total IgE, total immunoglobulin E; EOS percentage, blood eosinophil percentage; EOS count, blood eosinophil count; E/M ratio, ratio of the computed tomography scores for the ethmoid sinus and maxillary sinus; model 1, the combination model of 5 predictors (nNO, total IgE, EOS count, EOS percentage, and E/M ratio); model 2, the combination model of 4 predictors (nNO, total IgE, EOS count, EOS percentage, and E/M ratio).

Table 5. Sensitivity and specificity of clinical markers at the best cutoff point

Predictors	Cutoff point	Sensitivity	Specificity
nNO	329	0.833	0.717
Total IgE	105	0.583	0.913
EOS count	0.2	0.708	0.674
EOS percentage	3.2	0.667	0.674
E/M ratio	2	0.500	0.792

nNO, nasal nitric oxide; total IgE, total immunoglobulin E; EOS percentage, blood eosinophil percentage; EOS count, blood eosinophil count; E/M ratio, ratio of the computed tomography scores for the ethmoid sinus and maxillary sinus.

Discussion

Recently, the conception of precision medicine has promoted the in-depth study of the heterogeneity of CRSwNP, which can be classified as non-eCRSwNP or eCRSwNP. This classification is based on the percentage or absolute numbers of eosinophils in the nasal mucosa and the different responses to surgical and medical intervention [7, 26]. For the diagnosis of eCRSwNP, pathological evaluation remains the gold standard test; however, this is not applicable to patients who desire to nonsurgical treatment. Besides, the invasiveness and relatively high cost of this technology cannot be ignored. Thus, reliable and feasible biomarkers to identify subtypes of CRSwNP are clearly required. Successful identi-

fication of eCRSwNP biomarkers would facilitate subsequent clinical management of the disease.

The detection of nNO is a noninvasive, fast, safe, and repeatable method for upper airway inflammation, which has become increasingly valuable in the field of rhinology in recent years [27]. Frendø et al. [28] found that in patients with CRSwNP, nNO levels were significantly lower than those in the controls. A meta-analysis by Ambrosino et al. [29] also revealed that compared with healthy controls, CRSwNP patients showed significantly lower nNO levels. Similar to previous research, our results also showed that patients with CRSwNP had lower nNO levels compared with the healthy controls. The decrease in nNO levels was mainly caused by the occluded sinus ostia, which obstructed the ventilation of NO, and by decreased production of NO in the damaged nasal mucosa [30]. However, these previous studies did not perform the pathological typing of CRSwNP. In the present study, we found that nNO levels of patients with eCRSwNP were significantly higher compared with those of the patients with non-eCRSwNP. Compared with those all other parameters assessed, the AUC for the nNO level was the highest, at 0.803, indicating that nNO levels are highly predictive for eCRSwNP diagnosis. ECRSwNP is driven by eosinophilic inflammation through the Th2 inflammatory response [31]. In patients with asthma, Th2 cytokines, including IL-4, IL-5, and IL-13, can upregulate epithelial iNOS expression, which leads to increased NO concentrations [32]. Takeno et al.[33] revealed that in patients with eCRSwNP, nasal epithelial cells produce higher NO levels via simultaneous expression of different NOS isoforms (iNOS and eNOS). This could also explain the elevated NO levels reported here. The increased levels of nNO reflected the persistence of mucosal eosinophilic inflammation. However, Yoshida et al. [34] found that the preoperative nNO levels in the eCRSwNP group (n = 25) were significantly lower than those in the non-eCRSwNP group (n = 45) (42.3 ± 6.9 vs. 60.8 ± 5.2 ppb). The following points might explain the conflicting results. First, The NO analyzer we used was different from the one they used. Instrument differences can have a significant impact on nNO values. Second, we found that the average age of eCRSwNP patients in Yoshida's study was higher than that in our study (54.8 ± 2.9 vs. 39.3 ± 12.7). Previous studies have shown that age is an important factor affecting nNO levels. Third, the disease severity of patients with eCRSwNP may not be consistent across studies. This means that differences in NO production and ventilation status of the paranasal sinuses between the 2 studies may lead to different results. We also compared the 2 subgroups with the control group, separately. The nNO levels in the eCRSwNP group and the control group were not statistically different, while nNO levels were significantly lower in the non-eCRSwNP group than in the control group. In the patients with eCRSwNP, there is both obstructed sinus ventilation and enhanced NO production in the paranasal sinus mucosa, which might result in the lack of change in nNO levels. Combining the deductions from previous studies with our results, we hypothesized that the measured nNO levels reflect both the actual NO production and the ventilation status in the paranasal sinuses. Increased inflammation and obstruction of sinus ostium might lead to initial increase in nNO levels, followed by a reduction. Clearly, further studies are required to determine the changes in nNO levels in patients with eCRSwNP.

In addition, as a marker of eCRSwNP, blood eosinophil counts might also be used. The peripheral blood eosinophil count has been shown to be positively related to tissue infiltrating eosinophils [35–37]. Hu et al. [38] found that when the blood EOS count was $\geq 0.215 \times 10^9$/L, the sensitivity of diagnosing eCRSwNP was 74.2% and the specificity was 86.5%. We observed that in the diagnosis of eCRSwNP, peripheral blood eosinophil counts showed moderate accuracy. However, patients' peripheral blood eosinophil counts may be affected by various factors, including parasitic infections, allergies, autoimmune diseases, or adverse reactions to drugs [39]. Therefore, the predictive effect of blood eosinophil count on the diagnosis of eCRSwNP requires further study.

Our results indicated that the specificity of total serum IgE to distinguish the 2 subtypes of CRSwNP was the highest (91.3%), while the sensitivity of total IgE in this study was 58.3%. It was likely that the observed lower sensitivity and higher specificity of total IgE reflected the higher optimal cutoff point for total IgE employed in the present study. Similar to our research, Kambara et al. [40] showed that total IgE levels in patients with non-CRSwNP were significantly lower than those in patients with eCRSwNP. However, Ho et al. [41] demonstrated that total serum IgE had no significant association with eCRSwNP. The heterogeneous patient populations might partly account for the differences among the studies.

Researchers have attempted to identify a specific cytokine as an effective biomarker of eCRSwNP. In this respect, IL-5 in sinus mucosa has been proven to play a key role in the pathogenic mechanism of CRSwNP [42]. However, similar to the detection of tissue eosinophils, the clinical application of tissue cytokine detection is often subject to various restrictions. In the clinical setting, determination of plasma cytokines is relatively cost-effective and readily available. Therefore, we wanted to identify potential biomarkers of

eCRSwNP using blood cytokine measurements. The results of the present study showed no significant difference in the Th1/Th2 cytokine levels in the peripheral blood of the 2 subtypes, and the average levels were within the normal reference range. This result suggested that plasma cytokine levels in patients with CRSwNP were insufficient to reflect the inflammatory characteristics of sinus mucosa.

Previous studies have shown that a CT scan might help in the early diagnosis of chronic sinusitis [43, 44]. The CT scans of patients with eCRSwNP showed lesions predominantly in the ethmoid sinuses [43]. Meng et al. [45] suggested that an E/M ratio >2.59 demonstrated 94.2% sensitivity and 89.6% specificity for the diagnosis of eCRSwNP. In our study, the specificity and sensitivity of the E/M ratio were not high. The CT scores depended mainly on the subjective experience of the doctor, and patients' disease severity was not consistent across studies, which might partially explain the observed differences. However, imaging methods reflect morphological changes and did not demonstrate the pathophysiological process of polyps and the characteristics of inflammation in patients with eCRSwNP. Some patients might not show typical changes early in the disease process.

Another study showed that, in the diagnosis of eCRSwNP, a combined model consisting of several predictive indicators was superior to a single predictor model [46]. In this research, 2 combination predictor models were used. Model 1 had an AUC of 0.878, which was very accurate to predict eCRSwNP compared with nNO alone. This research showed that a predictive model comprising nNO levels, clinical features, imaging, and blood tests could help otolaryngologists accurately identify patients with eCRSwNP.

In summary, in the present study, patients with eCRSwNP were characterized clinically by high nNO levels, high peripheral eosinophil count and percentage, high total IgE, ethmoid sinus-dominant opacification on CT scans, and comorbid asthma. Currently, there is no consensus concerning the method and instruments for measuring nNO. We believe that nNO measurement is an objective, effective, convenient, and noninvasive solution for the accurate typing of CRSwNP. However, the present study was limited by its small sample size; therefore, a larger-scale multi-center study should be performed in the future.

Conclusion

This study shows that the detection of nNO is the most useful single test for early diagnosis of eCRSwNP before mucosal biopsy. In addition, we demonstrate that a predictive model comprising nNO levels, clinical features, imaging, and blood tests could help otolaryngologists accurately identify patients with eCRSwNP. However, a large-scale multi-center study is required to determine the changes in nNO levels in patients with CRSwNP.

Acknowledgement

The authors thank all participants in this study for their enthusiastic cooperation.

Statement of Ethics

The Ethics Committee of Renmin Hospital of Wuhan University approved this study. Written informed consent was provided by all subjects before data collection.

Conflict of Interest Statement

The authors declare that they have no conflicts of interest.

Funding Sources

This study was supported by the National Natural Science Foundation of China (No. 81770986).

Author Contributions

H.L. designed the study and wrote the manuscript. P.Q.L., R.X., and W.Z. performed the clinical tests and collected the data. S.M.C. and Y.-G.K. performed the statistical analysis. Y.X. supervised the study and critically revised the manuscript.

References

1 Saito DM, Innes AL, Pletcher SD. Rheologic properties of sinonasal mucus in patients with chronic sinusitis. Am J Rhinol Allergy. 2010 Jan–Feb;24(1):1–5.

2 Lou H, Zhang N, Bachert C, Zhang L. Highlights of eosinophilic chronic rhinosinusitis with nasal polyps in definition, prognosis, and advancement. Int Forum Allergy Rhinol. 2018 Nov;8(11):1218–25.

3 Wang X, Zhang N, Bo M, Holtappels G, Zheng M, Lou H, 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 Nov;138(5):1344–53.

4 Van Zele T, Claeys S, Gevaert P, Van Maele G, Holtappels G, Van Cauwenberge P, et al. Differentiation of chronic sinus diseases by measurement of inflammatory mediators. Allergy. 2006 Nov;61(11):1280–9.

5 Zhang N, Van Zele T, Perez-Novo C, Van Bruaene N, Holtappels G, DeRuyck N, et al. Different types of T-effector cells orchestrate mucosal inflammation in chronic sinus disease. J Allergy Clin Immunol. 2008 Nov; 122(5):961–8.
6 Ikeda K, Shiozawa A, Ono N, Kusunoki T, Hirotsu M, Homma H, et al. Subclassification of chronic rhinosinusitis with nasal polyp based on eosinophil and neutrophil. Laryngoscope. 2013 Nov;123(11):e1–9.
7 Akdis CA, Bachert C, Cingi C, Dykewicz MS, Hellings PW, Naclerio RM, et al. Endotypes and phenotypes of chronic rhinosinusitis: a PRACTALL document of the European Academy of Allergy and Clinical Immunology and the American Academy of Allergy, Asthma & Immunology. J Allergy Clin Immunol. 2013 Jun;131(6):1479–90.
8 Gurrola J 2nd, Borish L. Chronic rhinosinusitis: endotypes, biomarkers, and treatment response. J Allergy Clin Immunol. 2017 Dec; 140(6):1499–508.
9 Haruna S, Shimada C, Ozawa M, Fukami S, Moriyama H. A study of poor responders for long-term, low-dose macrolide administration for chronic sinusitis. Rhinology. 2009 Mar;47(1):66–71.
10 Hauser LJ, Chandra RK, Li P, Turner JH. Role of tissue eosinophils in chronic rhinosinusitis-associated olfactory loss. Int Forum Allergy Rhinol. 2017 Oct;7(10):957–62.
11 Vlaminck S, Vauterin T, Hellings PW, Jorissen M, Acke F, Van Cauwenberge P, et al. The importance of local eosinophilia in the surgical outcome of chronic rhinosinusitis: a 3-year prospective observational study. Am J Rhinol Allergy. 2014 May–Jun;28(3):260–4.
12 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 Sep;149(3):431–7.
13 Katotomichelakis M, Tantilipikorn P, Holtappels G, De Ruyck N, Feng L, Van Zele T, et al. Inflammatory patterns in upper airway disease in the same geographical area may change over time. Am J Rhinol Allergy. 2013 Sep–Oct;27(5):354–60.
14 Wang W, Gao Y, Zhu Z, Zha Y, Wang X, Qi F, et al. Changes in the clinical and histological characteristics of Chinese chronic rhinosinusitis with nasal polyps over 11 years. Int Forum Allergy Rhinol. 2019 Feb;9(2):149–57.
15 Jiang WX, Cao PP, Li ZY, Zhai GT, Liao B, Lu X, et al. A retrospective study of changes of histopathology of nasal polyps in adult Chinese in central China. Rhinology. 2019 Aug 1; 57(4):261–7.
16 Naraghi M, Deroee AF, Ebrahimkhani M, Kiani S, Dehpour A. Nitric oxide: a new concept in chronic sinusitis pathogenesis. Am J Otolaryngol. 2007 Sep–Oct;28(5):334–7.
17 Ferraro VA, Zanconato S, Baraldi E, Carraro S. Nitric oxide and biological mediators in pediatric chronic rhinosinusitis and asthma. J Clin Med. 2019 Oct;8(11):1783.
18 Deroee AF, Naraghi M, Sontou AF, Ebrahimkhani MR, Dehpour AR. Nitric oxide metabolites as biomarkers for follow-up after chronic rhinosinusitis surgery. Am J Rhinol Allergy. 2009 Mar–Apr;23(2):159–61.
19 Ricciardolo FL, Sorbello V, Ciprandi G. FeNO as biomarker for asthma phenotyping and management. Allergy Asthma Proc. 2015 Jan–Feb;36(1):e1–8.
20 Ragab SM, Lund VJ, Saleh HA, Scadding G. Nasal nitric oxide in objective evaluation of chronic rhinosinusitis therapy. Allergy. 2006 Jun;61(6):717–24.
21 Shapiro AJ, Josephson M, Rosenfeld M, Yilmaz O, Davis SD, Polineni D, et al. Accuracy of nasal nitric oxide measurement as a diagnostic test for primary ciliary dyskinesia. A systematic review and meta-analysis. Ann Am Thorac Soc. 2017 Jul;14(7):1184–96.
22 Fu CH, Huang CC, Chen YW, Chang PH, Lee TJ. Nasal nitric oxide in relation to quality-of-life improvements after endoscopic sinus surgery. Am J Rhinol Allergy. 2015 Nov–Dec; 29(6):e187–91.
23 Fokkens WJ, Lund VJ, Mullol J, Bachert C, Alobid I, Baroody F, et al. European position paper on rhinosinusitis and nasal polyps 2012. Rhinol Suppl. 2012 Mar;23:3.
24 Dweik RA, Boggs PB, Erzurum SC, Irvin CG, Leigh MW, Lundberg JO, et al. An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (FENO) for clinical applications. Am J Respir Crit Care Med. 2011 Sep;184(5):602–15.
25 Cao PP, Li HB, Wang BF, Wang SB, You XJ, Cui YH, et al. Distinct immunopathologic characteristics of various types of chronic rhinosinusitis in adult Chinese. J Allergy Clin Immunol. 2009 Sep;124(3):478–84.
26 Czerny MS, Namin A, Gratton MA, Antisdel JL. Histopathological and clinical analysis of chronic rhinosinusitis by subtype. Int Forum Allergy Rhinol. 2014 Jun;4(6):463–9.
27 Rimmer J, Hellings P, Lund VJ, Alobid I, Beale T, Dassi C, et al. European position paper on diagnostic tools in rhinology. Rhinology. 2019 Jul 25;57(Suppl S28):1–41.
28 Frendø M, Håkansson K, Schwer S, Ravn AT, Meteran H, Porsbjerg C, et al. Exhaled and nasal nitric oxide in chronic rhinosinusitis patients with nasal polyps in primary care. Rhinology. 2018 Mar 1;56(1):59–64.
29 Ambrosino P, Molino A, Spedicato GA, Parrella P, Formisano R, Motta A, et al. Nasal nitric oxide in chronic rhinosinusitis with or without nasal polyps: a systematic review with meta-analysis. J Clin Med. 2020 Jan 11;9(1): 200.
30 Colantonio D, Brouillette L, Parikh A, Scadding GK. Paradoxical low nasal nitric oxide in nasal polyposis. Clin Exp Allergy. 2002 May; 32(5):698–701.
31 Jonstam K, Swanson BN, Mannent LP, Cardell LO, Tian N, Wang Y, et al. Dupilumab reduces local type 2 pro-inflammatory biomarkers in chronic rhinosinusitis with nasal polyposis. Allergy. 2019 Apr;74(4):743–52.
32 Mahr TA, Malka J, Spahn JD. Inflammometry in pediatric asthma: a review of fractional exhaled nitric oxide in clinical practice. Allergy Asthma Proc. 2013 May–Jun;34(3):210–9.
33 Takeno S, Taruya T, Ueda T, Noda N, Hirakawa K. Increased exhaled nitric oxide and its oxidation metabolism in eosinophilic chronic rhinosinusitis. Auris Nasus Larynx. 2013 Oct; 40(5):458–64.
34 Yoshida K, Takabayashi T, Imoto Y, Sakashita M, Narita N, Fujieda S. Reduced nasal nitric oxide levels in patients with eosinophilic chronic rhinosinusitis. Allergol Int. 2019 Apr; 68(2):225–32.
35 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 Dec; 137(6):925–30.
36 Ishitoya J, Sakuma Y, Tsukuda M. Eosinophilic chronic rhinosinusitis in Japan. Allergol Int. 2010 Sep;59(3):239–45.
37 Kountakis SE, Arango P, Bradley D, Wade ZK, Borish L. Molecular and cellular staging for the severity of chronic rhinosinusitis. Laryngoscope. 2004 Nov;114(11):1895–905.
38 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 Mar;122(3):498–503.
39 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 Sep–Oct;29(5):350–6.
40 Kambara R, Minami T, Akazawa H, Tsuji F, Sasaki T, Inohara H, et al. Lower airway inflammation in eosinophilic chronic rhinosinusitis as determined by exhaled nitric oxide. Int Arch Allergy Immunol. 2017;173(4):225–32.
41 Ho J, Hamizan AW, Alvarado R, Rimmer J, Sewell WA, Harvey RJ. Systemic predictors of eosinophilic chronic rhinosinusitis. Am J Rhinol Allergy. 2018 Jul;32(4):252–7.
42 Wei B, Liu F, Zhang J, Liu Y, Du J, Liu S, et al. Multivariate analysis of inflammatory endotypes in recurrent nasal polyposis in a Chinese population. Rhinology. 2018 Sep 1;56(3):216–26.
43 Wang ET, Zheng Y, Liu PF, Guo LJ. Eosinophilic chronic rhinosinusitis in East Asians. World J Clin Cases. 2014 Dec 16;2(12):873–82.
44 Zuo L, Koozechian MS, Chen LL. Characterization of reactive nitrogen species in allergic asthma. Ann Allergy Asthma Immunol. 2014 Jan;112(1):18–22.
45 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 Aug; 6(8):812–9.
46 Sakuma Y, Ishitoya J, Komatsu M, Shiono O, Hirama M, Yamashita Y, et al. New clinical diagnostic criteria for eosinophilic chronic rhinosinusitis. Auris Nasus Larynx. 2011 Oct; 38(5):583–8.