Serum metabolomics identifies uric acid as a possible novel biomarker for predicting recurrence of chronic rhinosinusitis with nasal polyps*

Shaobing Xie[1,2,3,4], Caixia Zhang[1,2,3,4], Zhihai Xie[1,2,3,4], Junyi Zhang[1,2,3,4], Hua Zhang[1,2,3,4], Weihong Jiang[1,2,3,4]

Rhinology 61: 6, 541- 551, 2023
https://doi.org/10.4193/Rhin23.236

[1] Department of Otolaryngology Head and Neck Surgery, Xiangya Hospital of Central South University, Changsha, People's Republic of China
[2] Hunan Province Key Laboratory of Otolaryngology Critical Diseases, Xiangya Hospital of Central South University, Changsha, People's Republic of China
[3] National Clinical Research Center for Geriatric Disorders, Xiangya Hospital of Central South University, Changsha, People's Republic of China
[4] Anatomy Laboratory of Division of Nose and Cranial Base, Clinical Anatomy Center of Xiangya Hospital, Central South University, Changsha, People's Republic of China

***Received for publication:** July 9, 2023
Accepted: August 20, 2023

Abstract

Background: Metabolomics has proven to be a valuable tool in gaining new insights into disease progression and prognosis, the specific metabolic alterations in the serum of recurrent chronic rhinosinusitis with nasal polyps (CRSwNP) patients remain unknown. This study aims to explore the serum metabolomic profiles of recurrent CRSwNP and identify potential predictive biomarkers.

Methods: A prospective, single-center study was conducted on CRSwNP patients prior to endoscopic sinus surgery. Serum samples were subjected to untargeted metabolomic profiling. Patients were followed up for over 2 years and categorized into recurrence and non-recurrence groups. Metabolite differences between the two groups were compared, and the identified differentially regulated metabolites were subsequently validated in a large clinical cohort.

Results: 67 CRSwNP patients completed the follow-up schedule, with 47 classified into the non-recurrent group and 20 into the recurrent group. Significant differences were found in the metabolomic profiles between both groups, and serum uric acid (SUA) showed promising predictive potential for postoperative recurrence in both positive and negative ion models. A validation cohort comprising 398 non-recurrent and 142 recurrent CRSwNP patients was recruited, and a significant elevation in SUA levels was observed in recurrent cases. Patients were stratified into tertiles based on the distribution of baseline SUA levels. Multivariate Cox regression analysis showed that higher tertiles of SUA were associated with an increased risk of CRSwNP recurrence compared to lower tertiles, even after adjusting for potential confounding factors. The receiver operating characteristic curve and Kaplan-Meier survival analysis highlighted that elevated SUA levels exhibited potential predictive values for postoperative recurrence.

Conclusion: Serum metabolic signatures might predict postoperative recurrence in CRSwNP patients. Increased SUA concentrations were found to be associated with a higher risk of future postoperative recurrence in CRSwNP, independent of traditional risk factors.

Key words: chronic rhinosinusitis with nasal polyps, uric acid, metabolomics, biomarker, recurrence

Introduction

Chronic rhinosinusitis (CRS) is a persistent inflammatory condition characterized by inflammation in the nasal and paranasal sinus mucosa[1, 2]. Patients diagnosed with CRS often experi-

ence significant effects on their physical, social, and emotional health[3, 4]. CRS is phenotypically classified into two subtypes: CRS with nasal polyps (CRSwNP) and CRS without nasal polyps (CRSsNP)[5-7]. Currently, emerging evidence indicates that CRSwNP exhibits more complex biological mechanisms and tissue heterogeneity, leading to a poorer prognosis and an increased risk of recurrence[8, 9]. Functional endoscopic sinus surgery (FESS) is the primary treatment approach for patients with chronic rhinosinusitis (CRS) who do not respond adequately to conservative management[10]. However, a significant proportion of patients experience early recurrence of symptoms, and the rate of revision surgery has been reported to range from 20% to 50%[11, 12]. Therefore, it is of utmost importance to accurately identify the risk factors for recurrence and identify CRSwNP patients at a high risk of relapse before undergoing FESS treatment. This information will greatly assist in guiding postoperative management and facilitating personalized precision therapy.

Metabolomics, an advancing field of omics research, has become a valuable tool for comprehending the metabolic changes linked to airway inflammatory diseases[13, 14]. Through the analysis of metabolites in different biological samples, previous studies have uncovered specific patterns of metabolites that are associated with disease characteristics, severity, and treatment outcomes in conditions like asthma and allergic rhinitis[15, 16]. These investigations have revealed disruptions in energy metabolism, lipid metabolism, oxidative stress, and inflammatory pathways, shedding light on the intricate relationship between metabolic dysregulation and the inflammatory response in these diseases[17, 18]. However, limited research has utilized serum untargeted metabolomics to investigate the underlying pathophysiological mechanisms of CRSwNP, particularly in recurrent cases.

To address this knowledge gap, we conducted a prospective observational cohort study, and employed untargeted metabolomic profiling to identify potential serum metabolites for predicting CRSwNP recurrence. Our findings revealed that patients who experienced recurrence and those who did not have distinct differences in their baseline metabolomic profiles. Specifically, we discovered that the levels of serum uric acid (SUA) were associated with the recurrence of CRSwNP after surgery. Furthermore, we validated these results in a larger cohort and confirmed that serum SUA levels showed promising predictive value for CRSwNP recurrence.

Materials and methods

Patients and settings

This prospective, single-center study initially recruited a total of 70 CRSwNP patients who treat with FESS between June 2018 and October 2018. The study protocol was approved by the Human Ethical Committee at Xiangya Hospital of Central South University (Approval No. 201809632). Before participating in the study, all patients provided written informed consent. CRSwNP was diagnosed based on the guidelines of the European Position Paper on Rhinosinusitis and Nasal Polyps 2012[19]. The criteria for excluding participants in this study were as follows: incomplete clinical data; presence of fungal sinusitis, allergic fungal rhinosinusitis, or benign or malignant tumors in the sinonasal area; recent use of corticosteroids, antibiotics, or other immune-regulating drugs within 1 month prior to recruitment; current acute inflammation; history of previous radiotherapy; age below 18 years or above 70 years; and severe heart, kidney, or other organ dysfunction. The baseline characteristics and clinical data were collected before FESS including gender, age, body mass index (BMI), smoking, alcohol consumption, duration of disease, allergic rhinitis and asthma comorbidity, visual analog scale (VAS) value, Lund-MacKay score, and Lund-Kennedy score.
For the validation cohort, we enrolled a substantial sample of 597 patients diagnosed with CRSwNP between January 2017 and December 2021. We ensured that the inclusion and exclusion criteria were identical to those used for the discovery cohort. Furthermore, we collected the same clinical data as the discovery cohort and additionally obtained additional parameters, such as blood pressure, fasting blood glucose (FBG), SUA, and creatinine.

Follow-up and postoperative recurrence assessment

FESS was performed in compliance with uniform standards by three senior surgeons. After surgery, all patients in both the discovery and validation cohorts who underwent postoperative treatment were instructed to follow a standardized regimen. This regimen included daily nasal saline irrigation, administration of antibiotics, application of topical corticosteroids, and periodic endoscopic debridement. Regular follow-up appointments were scheduled to monitor the patients' progress, which involved conducting endoscopic examinations. The recurrence of CRSwNP was defined by the reappearance of clinical symptoms, endoscopic signs, and/or computed tomography (CT) evidence persisting for at least 2 months despite receiving the rescue regimen of antibiotics and oral steroids, as previously described[20, 21]. All patients were followed up for more than 2 years, then they were categorized into two groups: the recurrence group and the non-recurrence group.

Serum sample collection and preparation

Serum samples were collected from patients with CRSwNP in the discovery cohort, following a methodology previously described[22]. These serum samples were stored at room temperature for 1 h, then centrifuged at 4 °C (3000 rpm for 10 min). The resulting supernatants were collected and stored in equal aliquots

Table 1. Characteristics of CRSwNP patients in the discovery cohort.

	Non-recurrence group	Recurrence group	P value
Number, (n)	47	20	
Gender, (Male/female)	29 (61.7)	14 (70.0)	
Age, years	45.0 (35.0, 52.0)	42.0 (27.3, 46.8)	0.325
BMI, kg/m^2	23.0 (20.8, 25.0)	23.0 (21.3, 24.9)	0.863
Smoker, n (%)	11 (23.4)	6 (30.0)	
Drinker, n (%)	7 (14.9)	4 (20.0)	
Duration of disease, months	36.0 (12.0, 72.0)	48.0 (27.0, 72.0)	0.823
Allergic rhinitis, n (%)	9 (19.1)	7 (35.0)	
Asthma, n (%)	5 (10.6)	3 (15.0)	
VAS	9.0 (8.0,1.0)	8.5 (7.0, 10.0)	0.536
Lund-MacKay score	13.0 (11.0, 15.0)	13.5 (10.3, 15.0)	0.830
Lund-Kennedy score	7.0 (6.0, 9.0)	8.0 (6.3, 8.8)	0.609

CRSwNP, chronic rhinosinusitis with nasal polyps; BMI, body mass index; VAS, visual analogue score.

at -80 °C for subsequent untargeted metabolomics analysis. To prepare the serum samples for analysis using ultra-high-performance liquid chromatography-mass spectrometry (UHPLC-MS), 100 µL of each serum sample was mixed with 300 µL of methanol containing an internal standard (L-2-Chlorophenylalanine, 2 µg/mL). After a 30 s vortex, the samples were sonicated for 10 min in an ice-water bath. Subsequently, the samples were incubated at -40 °C for 1 hour and then centrifuged at 12000 rpm for 15 min at 4 °C. Finally, 100 µL of the resulting supernatant was transferred to a fresh glass vial for UHPLC-MS analysis(23, 24). To assess the reproducibility and reliability of the UHPLC-MS analytical system, a quality control (QC) sample was prepared and utilized as described in a previous study to monitor deviations of the analytical results from the pool mixtures[25, 26].

Untargeted metabolomic profiling

Nontargeted global metabolomic profiling was performed on a 1290 Infinity series UHPLC System (Waters Corporation, Milford, MA, USA) following appropriate chromatographic and mass spectrometry conditions as our previous study described[24, 26]. To obtain a more comprehensive and detailed view of the metabolic profile, covering different types of metabolic products. The serum samples were subjected to analysis in both positive ion mode and negative ion mode. During this process, the acquisition software (Analyst TF 1.7, AB Sciex, Framingham, MA, USA) continuously evaluated the full scan survey MS data and collected the corresponding MS spectra.

Data processing and analysis

The MS raw data files (.wiff) were converted to the mzXML format using Proteo Wizard and processed using the R package

Table 2. Characteristics of CRSwNP patients between the two groups in the validation cohort.

	Non-recurrence group	Recurrence group	P value
Number	398	142	
Gender-male, n (%)	270 (67.8)	90 (63.4)	0.352
Age, years	45.0 (32.0, 54.0)	45.0 (30.0, 55.0)	0.918
BMI, kg/m^2	23.0 (21.1, 25.3)	22.7 (21.0, 25.5)	0.989
Smoking, n (%)	101 (25.4)	50 (35.2)	0.029
Alcohol consumption, n (%)	68 (17.1)	21 (22.6)	0.599
Duration of disease, months	24.0 (10.0, 72.0)	48.0 (16.5, 120.0)	0.029
Allergic rhinitis, n (%)	82 (20.6)	44 (31.0)	0.015
Asthma, n (%)	34 (8.5)	19 (13.4)	0.102
VAS	8.0 (8.0, 10.0)	8.0 (7.0, 9.0)	0.084
Lund-MacKay score	13.0 (11.0, 15.0)	13.0 (11.0, 14.0)	0.510
Lund-Kennedy score	7.0 (6.0, 8.0)	7.0 (6.0, 8.0)	0.907
Diabetes mellitus, n (%)	65 (16.3)	24 (16.9)	0.792
Hypertension, n (%)	84 (21.1)	30 (21.1)	1.000
Hyperuricemia, n (%)	85 (21.4)	78 (54.9)	<0.001
Uric acid lowering agents, n (%)	25 (6.3)	26 (18.3)	<0.001
Uric acid, mg/dL	5.7±1.2	6.6±1.5	<0.001
Creatinine, µmol/L	78.0 (67.0, 88.1)	77.5 (39.1, 87.1)	0.788
SBP, mmHg	126.0 (116.0, 137.0)	122.0 (113.0, 135.0)	0.203
DBP, mmHg	80.0 (72.0, 86.0)	78.0 (72.0, 83.3)	0.602
FBG, mmol/L	4.9 (4.5, 5.4)	4.8 (4.5, 5.3)	0.646

CRSwNP, chronic rhinosinusitis with nasal polyps; BMI, body mass index; VAS, visual analogue score; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBG, fasting blood glucose.

XCMS V3.2. The processing steps involved peak deconvolution, alignment, and integration, as previously described(26, 27). To identify potential biomarkers contributing to the metabolic differences between the two groups, orthogonal partial least squares discriminant analysis (OPLS-DA) was performed. Heat maps were generated to visualize the metabolic changes associated with significant shifts in metabolites (variable importance for project (VIP) >1.0 and P <0.05), and those differentially expressed metabolites meeting the criteria of P values <0.05 and fold changes >1.5 were displayed in volcano plots. The predictive values of potential metabolites for the recurrence of CRSwNP were evaluated using receiver operating characteristic (ROC) analysis and Kaplan-Meier survival analysis. Additionally, metabolic pathway analysis was conducted to gain insight into the underlying metabolic mechanisms associated with postoperative recurrence. All the analyses were performed in both ion modes.

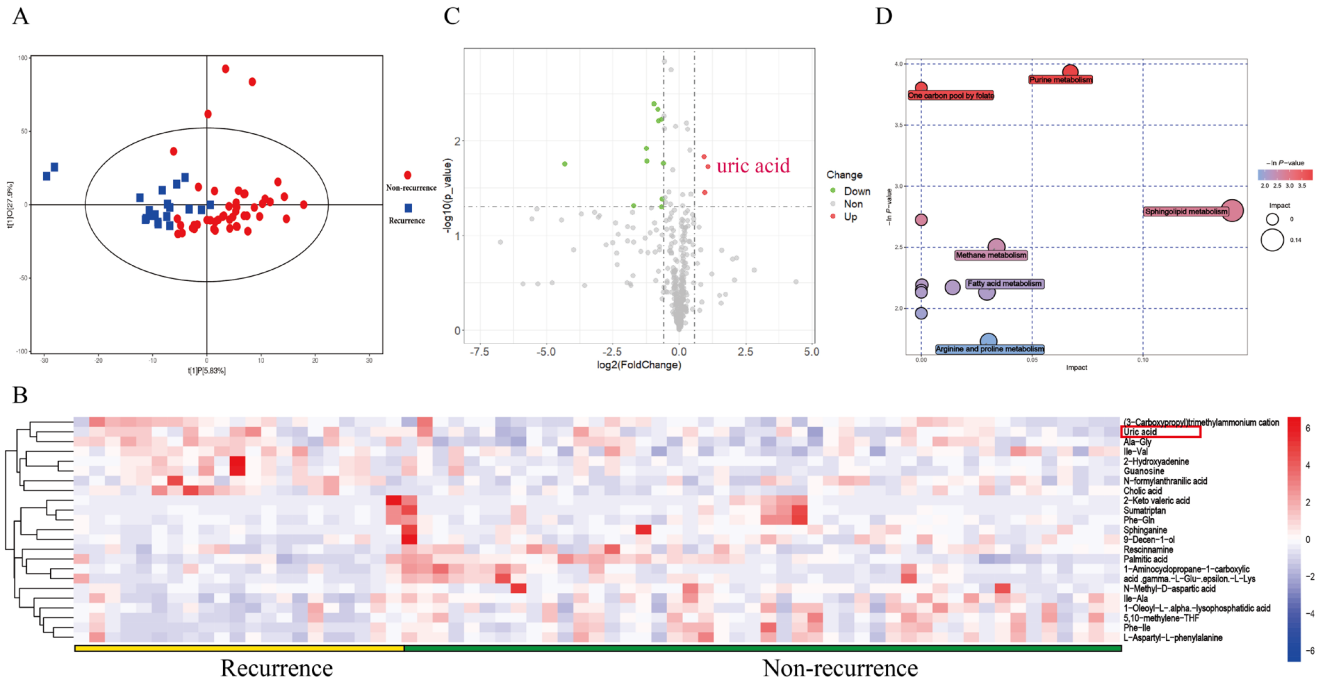

Figure 1. Serum metabolomic profiling of CRSwNP between recurrence and non-recurrence groups in positive ion model. (A) OPLS-DA model; (B) heat map of constructed using 23 serum metabolites (VIP>1, P<0.05); (C) volcano plot with differently expressed serum metabolites (fold changes >1.5, P<0.05); (D) Metabolic pathway bubble chart. CRSwNP, chronic rhinosinusitis with nasal polyps; OPLS-DA, orthogonal partial least squares discriminant analysis; VIP, variable importance for project.

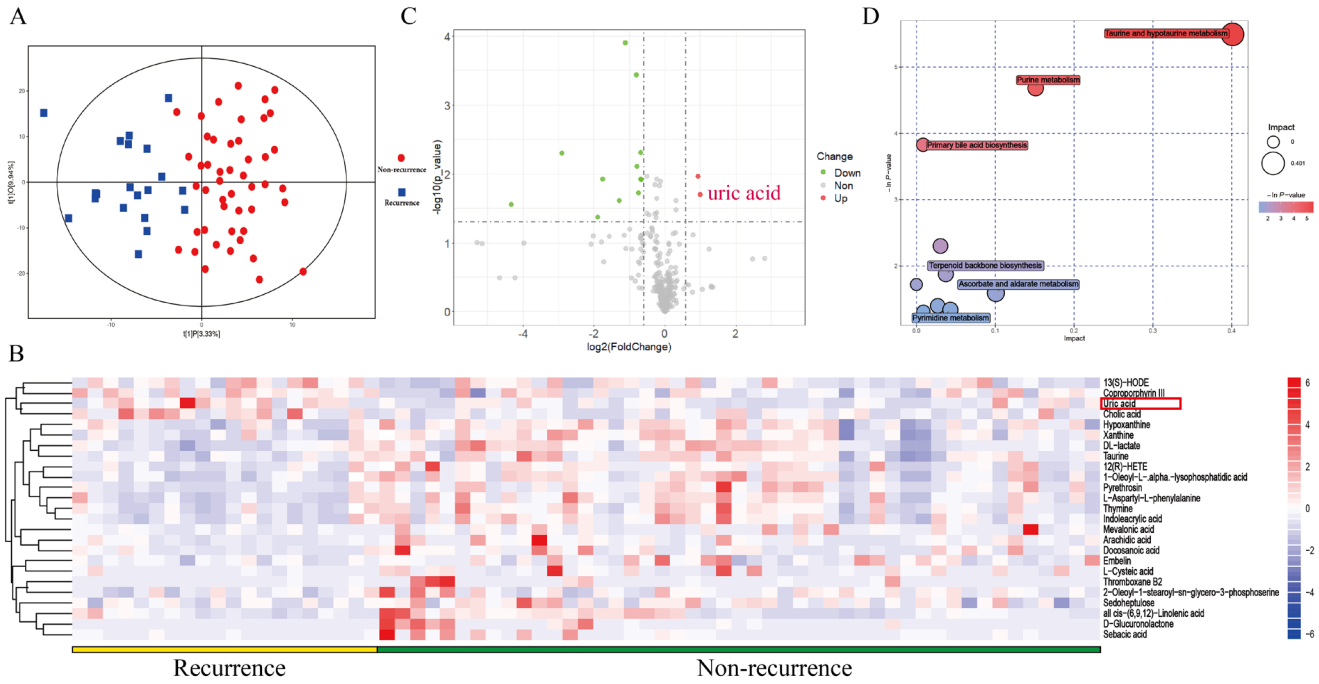

Figure 2. Serum metabolomic profiling of CRSwNP between recurrence and non-recurrence groups in negative ion model. (A) OPLS-DA model; (B) heat map of constructed using 23 serum metabolites (VIP>1, P<0.05); (C) volcano plot with differently expressed serum metabolites (fold changes >1.5, P<0.05); (D) Metabolic pathway bubble chart. CRSwNP, chronic rhinosinusitis with nasal polyps; OPLS-DA, orthogonal partial least squares discriminant analysis; VIP, variable importance for project.

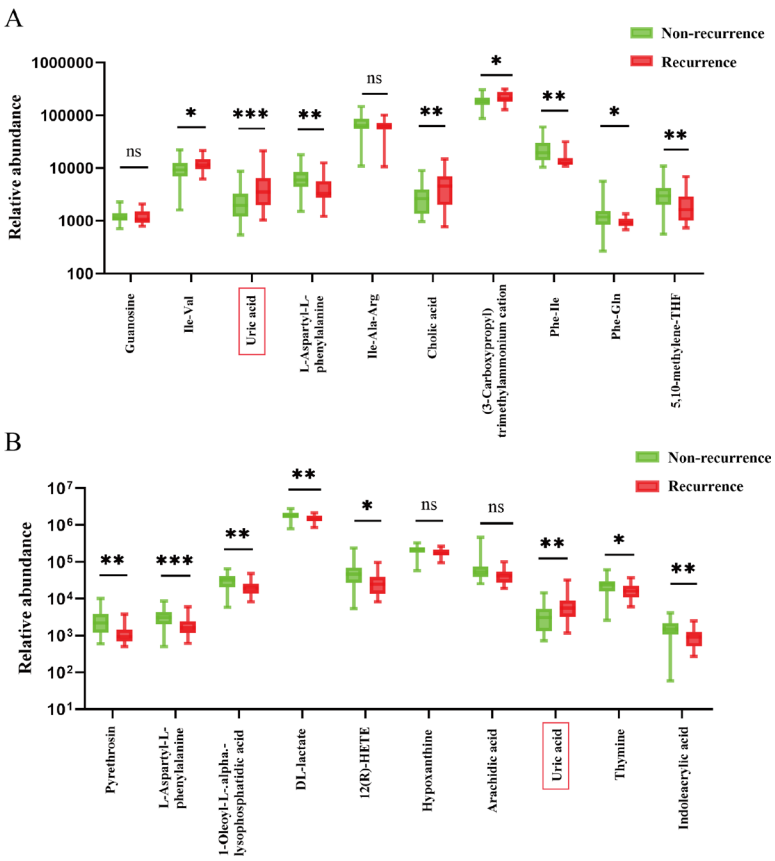

Figure 3. Relative abundance of top 10 differently expressed metabolites between recurrence and non-recurrence groups in both ion models. (A) positive ion model; (B) negative ion model. *P<0.05, **P<0.01, ***P<0.001, ns, no significance.

Statistical analysis

Categorical variables were presented as numbers and percentages and compared using the chi-squared test. Quantitative variables with a normal distribution were reported as mean ± standard deviation and compared using Student's t-test or one-way analysis of variance (ANOVA). For variables that did not follow a normal distribution, median and interquartile ranges (IQRs) were provided, and the comparison was performed using the Mann-Whitney U test or the Kruskal-Wallis H test.

In the validation cohort, CRSwNP patients were divided into three groups based on tertiles of baseline SUA levels. Kaplan-Meier survival analysis was conducted to assess the associations between the different SUA tertiles and the risk of CRSwNP recurrence. Cox regression analysis was performed to estimate the associations between the different SUA tertiles and the risk of postoperative recurrence in different adjusted models. Additionally, both univariate and multivariate Cox proportional hazards models were computed, considering SUA as a continuous variable. ROC curves were constructed to evaluate the potential of SUA in predicting postoperative recurrence. Statistical significance was considered at a two-tailed P-value of less than 0.05. All statistical analyses were performed using SPSS version 23.0 (IBM, Chicago, IL, USA).

Results

Serum metabolomic profiling between non-recurrence and recurrence groups

In the discovery cohort, a total of 67 CRSwNP patients completed the whole follow-up schedule, and 3 patients lost to follow-up. Among them, 47 patients were included in the non-recurrence group, and the other 20 patients were classified into the recurrence group. No significant difference was observed in baseline characteristics between the two groups (Table 1).

In Figure 1, the positive ion mode analysis results are presented. The OPLS-DA model demonstrates a clear separation in metabolic profiles between the non-recurrence and recurrence groups, indicating distinct metabolic clustering. Figure 1B displays a total of 23 metabolites, including 8 upregulated and 15 downregulated metabolites, which were identified as potential markers for distinguishing CRSwNP recurrence. Furthermore, Figure 1C highlights the more significant metabolites among them. The metabolic pathway analysis results in Figure 1D indicate that purine metabolism, one-carbon pool by folate, and sphingolipid metabolism are the main metabolic pathways involved in CRSwNP recurrence. Figure 2 exhibits the negative ion mode analysis results. A total of 25 metabolites, including 4 upregu-

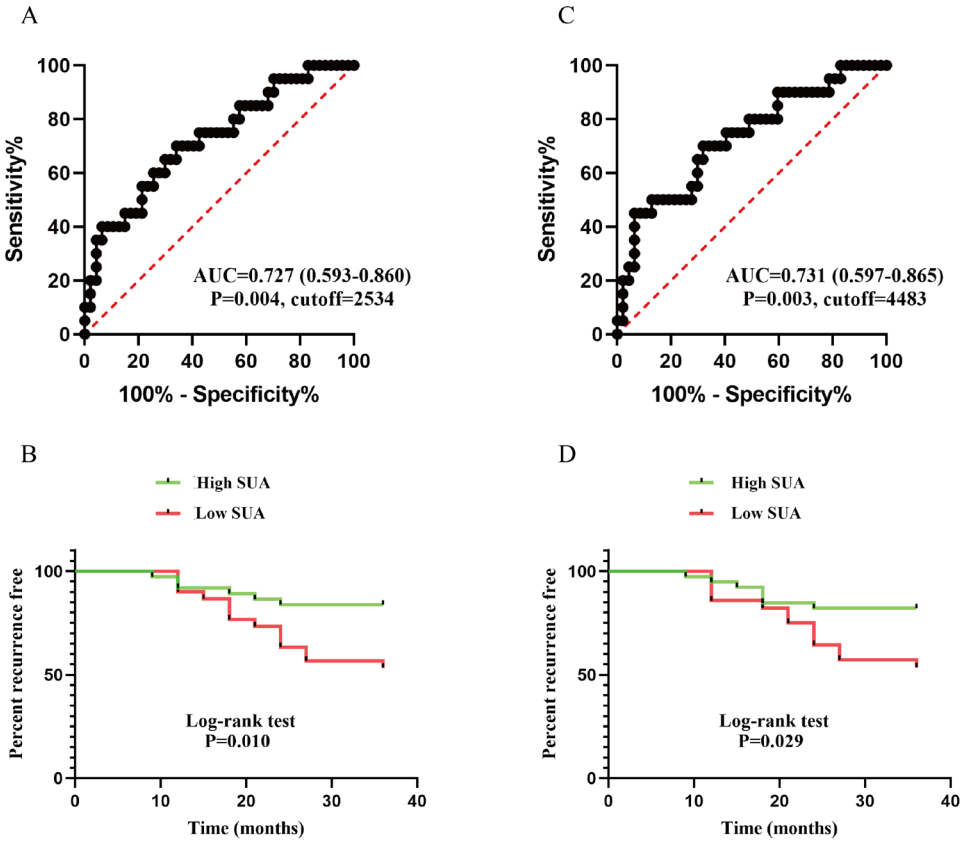

Figure 4. The ROC curve and Kaplan-Meier survival analysis exploring the potential values of SUA in predicting postoperative recurrence in both ion models. (A-B) positive ion model; (C-D) negative ion model. High and low SUA were defined on the cutoff values of SUA relative abundance. ROC, receiver operating characteristic; SUA, serum uric acid.

lated and 21 downregulated metabolites were significantly expressed between the two groups. The most affected pathways were taurine and hypotaurine metabolism, purine metabolism, and primary bile acid biosynthesis.

Serum metabolomic identifies SUA as biomarker for CRSwNP recurrence

Figure 3 presents the relative abundances of the top 10 differentially expressed metabolites between the non-recurrence and recurrence groups in both ion models. Interestingly, our findings revealed a significant elevation in SUA levels in the recurrence group compared to the non-recurrence group in both ion models. Moreover, the ROC curves demonstrated that SUA levels showed promising predictive values for postoperative recurrence of CRSwNP. Additionally, the Kaplan-Meier survival analysis revealed that CRSwNP patients with higher baseline SUA levels had a higher risk of future recurrence, as shown in Figure 4.

Elevated SUA increases the risk of CRSwNP recurrence

To validate the predictive ability of SUA observed in the serum metabolomic analysis, we established a validation cohort with a large sample size. After a minimum follow-up period of 2 years, a total of 540 patients were finally included in the analysis, and 57 patients lost to follow-up (Table S1). Among these patients, 142 experienced postoperative recurrence of CRSwNP, while the remaining 398 patients did not experience recurrence. Table 2 summarizes the characteristics of CRSwNP patients between the two groups. The patients in the recurrence group presented longer duration of disease, higher rates of allergic rhinitis, hypertension, hyperuricemia, and consumption of uric acid lowering agents, and higher SUA levels in comparison with the non-recurrence group.

Based on SUA tertiles distribution, patients were further classified into three groups: first tertile ≤ 5.35 mg/dL, second tertile 5.36-6.46 mg/dL and third tertile ≥ 6.47. The demographics and clinical characteristics of three groups were displayed in Table 3. Our findings revealed that patients with higher SUA levels were more likely to be male, have a higher BMI, be smokers, exhibit higher creatinine levels, have elevated blood pressure, experience a shorter follow-up time, and have a higher rate of postoperative recurrence. However, no statistical differences were observed in other variables among the three groups. In Table 4, both unadjusted and adjusted Cox regression analysis

Table 3. Demographics and disease characteristics of patients based on tertiles of serum uric acid levels.

	Serum uric acid, mg/dL			P value
	≤5.35	5.36-6.46 (HR, 95%CI)	≥6.47 (HR, 95%CI)	
Number	182	178	180	
Gender-male, n (%)	91 (50.0)	133 (74.7)	136 (75.6)	<0.001
Age, years	46.5 (33.0, 55.0)	43.0 (29.8, 55.0)	45.0 (32.0, 53.8)	0.543
BMI, kg/m^2	22.1 (20.7, 24.1) **, ***	22.6 (20.8, 25.4) *, ***	24.4 (21.9,26.3) *, **	<0.001
Alcohol consumption, n (%)	22 (12.1)	30 (16.9)	37 (20.6)	0.093
Smoking, n (%)	38 (20.9)	49 (27.5)	64 (35.6)	0.008
Duration of disease, months	24.0 (12.0, 96.0)	30.5 (12.0, 72.0)	30.5 (12.0, 81.0)	0.660
Allergic rhinitis, n (%)	50 (27.5)	34 (19.1)	42 (23.3)	0.181
Asthma, n (%)	15 (8.2)	17 (9.6)	21 (11.7)	0.543
VAS	8.0 (7.0, 9.0)	9.0 (8.0, 10.0)	8.0 (8.0, 9.0)	0.090
Lund-MacKay score	13.0 (11.0, 15.0)	13.0 (11.0, 15.0)	13.0 (11.0, 15.0)	0.804
Lund-Kennedy score	7.0 (6.0, 8.0)	7.0 (6.0, 9.0)	7.0 (6.0, 8.0)	0.137
Diabetes mellitus, n (%)	37 (20.3)	21 (11.8)	31 (17.2)	0.088
Hypertension, n (%)	38 (20.9)	37 (20.8)	39 (21.7)	0.975
Hyperuricemia, n (%)	1 (0.5)	27 (15.2)	135 (75.0)	<0.001
Uric acid lowering agents, n (%)	1 (0.5)	14 (7.9)	36 (20.0)	<0.001
Uric acid, mg/dL	4.7 (4.2, 5.1) **, ***	5.8 (5.6, 6.2) *, ***	7.3 (6.8, 7.9) *, **	<0.001
Creatinine, μmol/L	70.0 (61.0, 82.0) **, ***	80.0 (69.0, 89.0) *, ***	82.0 (72.3, 90.7) *, **	<0.001
SBP, mmHg	123.0 (113.0, 135.3) ***	124.0 (116.0, 134.0) ***	128.0 (116.0, 142.0) *, **	0.035
DBP, mmHg	79.5 (72.0, 85.0) ***	79.0 (72.0, 83.0) ***	80.0 (73.0, 90.0) *, **	0.030
FBG, mmol/L	5.0 (4.6, 5.5)	4.8 (4.5, 5.3)	4.9 (4.5, 5.3)	0.153
Follow-up time, months	43.0 (36.0, 54.0) ***	48.0 (36.0, 54.0) *, ***	36.0 (24.0, 52.5) *, **	<0.001
Postoperative recurrence, n (%)	31 (17.0)	36 (20.2)	75 (41.7)	<0.001

CRSwNP, chronic rhinosinusitis with nasal polyps; BMI, body mass index; VAS, visual analogue score; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBG, fasting blood glucose. *P<0.05 vs SUA ≤ 5.35 mg/dL; ** P<0.05 vs SUA 5.36-6.46 mg/dL; *** P<0.05 vs SUA ≥6.47 mg/dL.

suggested that SUA levels were associated with CRSwNP recurrence. The Kaplan-Meier survival curves demonstrated that the third tertile of SUA levels had a higher risk of postoperative recurrence than the other two tertiles (Figure 5A).

To further investigate the associations between SUA levels and the risk of postoperative recurrence in CRSwNP, we conducted univariate and multivariate Cox regression analyses. The variables that showed significant differences in Table 2 were included in the analyses, with SUA considered as a continuous variable. The results presented in Table 5 indicated a significant correlation between SUA levels and the risk of future CRSwNP recurrence. Additionally, the duration of disease and the presence of allergic rhinitis were found to be associated with a higher risk of postoperative recurrence. ROC curves highlighted that SUA level was a potential biomarker for predicting the postoperative recurrence, and the cutoff value was 6.9 mg/dL (sensitivity=45.1%, specificity=85.4%, Figure 5B).

Discussion

To the best of our knowledge, this is the first prospective study to utilize serum metabolomics to investigate biomarkers for predicting the recurrence of CRSwNP and validate their predictive values in a large clinical cohort. Our study revealed that baseline serum metabolic signatures were significantly associated with future postoperative recurrence. Among these potential metabolites, SUA levels demonstrated promising predictive ability for recurrence in both positive and negative ion models. Moreover, by recruiting a validation cohort, we observed that CRSwNP patients with elevated baseline SUA levels were at a higher risk of postoperative recurrence, independent of traditional risk factors. These findings highlight the potential of SUA as a predictive biomarker for CRSwNP recurrence and underscore the importance of considering metabolic signatures in the clinical management of this condition.

Extensive research efforts have been focused on comprehen-

Table 4. Cox regression analysis for CRSwNP recurrence based on tertiles of SUA levels.

	Serum uric acid, mg/dL				
	≤5.35	5.36-6.46	P value	≥6.47	P value
Unadjusted	Ref	1.173 (0.725-1.896)	0.516	2.772 (1.824-4.124)	<0.001
Model 1	Ref	1.193 (0.724-1.963)	0.489	3.151 (2.023-4.910)	<0.001
Model 2	Ref	1.253 (0.756-2.075)	0.382	3.110 (1.987-4.867)	<0.001
Model 3	Ref	1.248 (0.750-2.078)	0.394	3.362 (2.113-5.349)	<0.001

Model 1: Adjusted for gender, age, BMI, alcohol consumption and smoking; Model 2: Adjusted for gender, age, BMI, alcohol consumption, smoking, duration of disease, VAS, Lund-MacKay score, and Lund-Kennedy score; Model 3: Adjusted for gender, age, BMI, alcohol consumption, smoking, VAS, Lund-MacKay score, Lund-Kennedy score, allergic rhinitis, asthma, diabetes mellitus, hypertension, and creatinine. CRSwNP, chronic rhinosinusitis with nasal polyps; SUA, serum uric acid; HR, hazard ratio; CI, confidence interval.

Table 5. Univariate and multivariate Cox regression analysis for CRSwNP recurrence.

Variables	Univariate		Multivariate	
	HR (95%CI)	P value	HR (95%CI)	P value
Duration of disease, months	1.002 (1.000-1.004)	0.033	1.002 (1.000-1.004)	0.021
Smoking				
Yes	1.311 (0.922-1.864)	0.132	1.168 (0.818-1.667)	0.392
No (ref.)				
Allergic rhinitis				
Yes	1.519 (1.064-2.168)	0.021	1.852 (1.283-2.672)	0.001
No (ref.)				
Uric acid, mg/dL	1.521 (1.355-1.706)	<0.001	1.569 (1.391-1.770)	<0.001

CRSwNP, chronic rhinosinusitis with nasal polyps; HR, hazard ratio; CI, confidence interval.

ding the pathogenesis of recurrent CRSwNP. However, the mechanisms underlying postoperative recurrence remain complex and not fully elucidated. Consequently, there is a pressing need to develop a practical and reliable approach for identifying objective biomarkers that can accurately predict the likelihood of recurrence. Prior studies have highlighted various factors, including surgeon-related factors, anatomic variances, tissue endotype and phenotype, and coexisting conditions, as major contributors to disease recurrence in CRSwNP[28, 29]. More recently, there has been growing interest in peripheral blood biomarkers and serum metabolites as potential predictors for prognosis and recurrence in CRSwNP patients. These biomarkers have garnered attention due to their objective nature, simplicity, convenience, and minimally invasive nature[30-32]. In our previous study, we discovered distinctive serum metabolic signatures in patients with CRSwNP compared to healthy individuals, and several differently expressed metabolites contributed to its diagnosis and were associated with tissue endotype which providing insights into the underlying pathophysiological mechanisms of the condition[22]. However, the serum metabolomics profiles in recurrent CRSwNP patients were still unclear. In this study, we conducted a prospective study with 67 CRSwNP patients, and found that baseline serum metabolic signatures could predict postoperative recurrence. We also observed that purine metabolism was the most affected pathway in serum of CRSwNP patients who were more likely to suffer postoperative recurrence. These suggested that serum purine metabolism was closely involved in the pathophysiological mechanisms of recurrent CRSwNP. Previous studies suggested that alterations in purine metabolism, including dysregulation of purine nucleotide synthesis could lead to excessive adenosine production and dysregulated immune responses[33, 34]. Moreover, alterations in purine metabolism might influence the activation of immune cells, cytokine production, and tissue remodeling processes, thereby aggravating the inflammation and tissue pathology-mediated recurrence[33, 35].

Our metabolomics findings further support the association between baseline SUA levels and the risk of future recurrence in CRSwNP. The statistical analysis results from our validation cohort demonstrated a significant correlation, indicating that

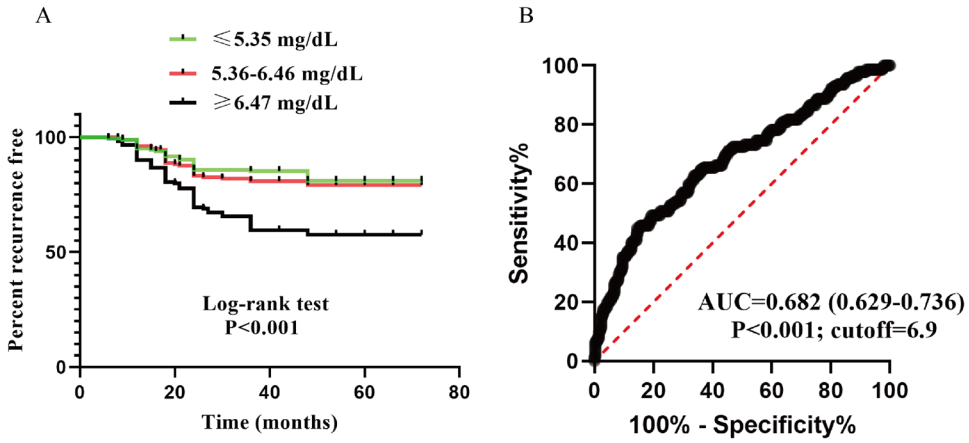

Figure 5. The potential values of SUA in predicting postoperative recurrence in validation cohort. (A) Kaplan-Meier survival analysis; (B) ROC curve. ROC, receiver operating characteristic; SUA, serum uric acid.

higher baseline SUA levels were associated with an increased risk of postoperative recurrence. Moreover, our analysis revealed that SUA could serve as an independent biomarker for predicting CRSwNP recurrence. Uric acid has been recognized to possess significant proinflammatory properties, and its accumulation and activation of downstream pathways have been implicated in promoting the inflammatory stress response and exacerbating disease progression in various inflammatory conditions[36, 37]. Previous publications suggested that high SUA levels were associated with the incidence of asthma and its disease severity, and elevated baseline SUA concentration could predict the acute exacerbation. In a recent study by Li et al.[38], metabolomic profiling was conducted on sinonasal tissue samples, revealing elevated levels of uric acid in CRS patients and its association with different CRS subtypes. These findings emphasize the potential role of uric acid in the development of CRSwNP and its contribution to tissue heterogeneity. It is well-established that the severe inflammatory cell infiltration and excessive accumulation of proinflammatory cytokines are the major pathogenic mechanisms contributing to tissue heterogeneity in recurrent CRSwNP[10, 39]. It is noteworthy that the activation of the purine pathway and the accumulation of SUA can promote the accumulation of innate immune cells and the production of pro-inflammatory cytokines, thereby exacerbating Th2 inflammatory responses and tissue eosinophilia. Experimental and clinical studies demonstrated that hyperuricemia could contribute to the dysregulation of immune and inflammatory processes through the release of epithelial-derived cytokines, which played play a crucial role in promoting inflammatory responses[40]. Furthermore, uric acid has been found to be involved in the process of epithelial-mesenchymal transition (EMT), and elevated tissue levels of uric acid can exacerbate EMT[41]. The dysregulation of EMT, driven by increased uric acid levels, is involved in various pathological conditions including tissue fibrosis and remodeling[42]. Collectively, we propose a hypothesis that elevated levels of SUA may enhance the activation of immune cells and the production of proinflammatory cytokines. This, in turn, can result in increased infiltration of inflammatory cells in nasal tissues and exacerbate the inflammatory processes. Additionally, it may promote EMT, which can lead to impaired epithelial barrier function. The combined effects of these mechanisms may contribute to the recurrence of CRSwNP. Further research is needed to validate this hypothesis and unravel the precise underlying mechanisms involved.

Another interesting finding in this study was that duration of disease and accompanying allergic rhinitis were risk factors for CRSwNP recurrence, which was in consistent with most previous conclusion[43, 44]. Patients with longer disease duration may be more likely to receive inadequate or incomplete treatment, which can contribute to the persistence or recurrence of the condition[45]. Furthermore, patients with longer disease duration often exhibit more pronounced tissue histopathological alterations, such as increased tissue eosinophilia, tissue EMT, and tissue remodeling. These changes in the nasal and sinus tissues can further perpetuate the inflammatory response and contribute to the recurrence of CRSwNP[45, 46]. The presence of allergic rhinitis has been confirmed as a potential risk factor for the occurrence and development of CRSwNP[47]. Furthermore, CRSwNP patients who also have allergic rhinitis exhibit more severe tissue inflammation and tissue heterogeneity[47, 48]. The ongoing allergic inflammation disrupts the function of the nasal epithelial barrier, leading to tissue remodeling and the growth of nasal polyps. These factors ultimately contribute to the recurrence of CRSwNP[43, 48].

This study has several limitations. Firstly, the sample size in the discovery cohort for the serum metabolomics study was limited,

which may have restricted the identification of differential serum metabolites. Secondly, SUA can vary over time and are influenced by various external factors, which may introduce variability in the analysis results, and affect the sensitivity and specificity for predicting the recurrence. Thirdly, the study population was recruited solely from a single medical center, with a homogeneous ethnicity and region, potentially leading to selection bias and limiting the generalizability of the findings. Fourthly, the follow-up period in this study was limited to 2 years, and further research is needed to investigate the long-term predictive value of serum metabolites for CRSwNP recurrence. Lastly, our study did not evaluate and adjust for other traditional factors due to the lack of access to data or incomplete data, such as aspirin-exacerbated respiratory disease, that may be associated with the risk of postoperative recurrence but were not included in our analysis.

Conclusion

This pioneering study introduces a novel approach for predicting the recurrence of CRSwNP using a serum metabolomics-based model. The results demonstrate that baseline serum metabolic signatures are significantly linked to future postoperative recurrence. Notably, SUA levels exhibit promising predictive ability for recurrence and have been validated in a larger patient cohort. Additionally, the study identifies the duration of disease and accompanying allergic rhinitis as risk factors for CRSwNP recurrence. Taken together, the findings emphasize the potential of SUA as a predictive biomarker for CRSwNP recurrence and underscore the importance of considering metabolic signatures in the clinical management of this condition. This research provides valuable insights that may enhance patient care and guide personalized treatment strategies for CRSwNP.

Authorship contribution

SX wrote the paper. CZ, ZX and JZ performed research and analyzed data. HZ and WJ designed and supervised the research. All authors critically read the paper and approved the manuscript.

Acknowledgement

We are grateful for the help of Shanghai BIOTREE Biomedical Technology Co., Ltd. (Shanghai, China) in the metabolite detection and data analysis.

Conflict of interest

There are no patents, products in development or marketed products to declare. Authors on this manuscript have no relevant financial or other relationships to disclose.

Funding

This research was supported by the National Natural Science Foundation of China (No.82171118), the Natural Science Foundation of Hunan Province (2022JJ30327 and 2023JJ41004) and the Natural Science Foundation of Changsha (kq2208391).

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Hua Zhang, MD, PhD, Professor
Department of Otolaryngology Head and Neck Surgery
Xiangya Hospital of Central South University
87 Xiangya Road
Kaifu District, Changsha
People's Republic of China, 410008
E-mail: entxy@126.com

Weihong Jiang, MD, PhD, Professor
Department of Otolaryngology Head and Neck Surgery
Xiangya Hospital of Central South University
87 Xiangya Road
Kaifu District, Changsha
People's Republic of China, 410008
E-mail: jiangwh68@126.com

This manuscript contains online supplementary material

SUPPLEMENTARY MATERIAL

Table S1. Demographic data of the CRSwNP patients.

	Overall patients	Included patients	Excluded patients
Number	597	540	57
Gender-male, n (%)	395 (66.2)	360 (66.7)	35 (61.4)
Age, years	45.0 (32.0, 55.0)	45.0 (32.0, 54.8)	44.4±12.6
BMI, kg/m^2	22.9 (21.1, 25.3)	22.9 (21.1, 25.3)	21.7 (20.0, 23.9)
Smoking, n (%)	168 (28.1)	151 (28.0)	17 (29.8)
Alcohol consumption, n (%)	103 (17.3)	89 (16.5)	14 (24.6)
Duration of disease, months	24.0 (12.0, 72.0)	24.0 (12.0, 84.0)	12.0 (6.0, 36.0)
Allergic rhinitis, n (%)	140 (23.5)	126 (23.3)	14 (24.6)
Asthma, n (%)	59 (9.9)	53 (9.8)	6 (10.5)
VAS	8.0 (8.0, 1.0)	8.0 (7.0, 10.0)	9.0 (8.0, 9.0)
Lund-MacKay score	13.0 (11.0, 15.0)	13.0 (11.0, 15.0)	14.0 (11.0, 15.0)
Lund-Kennedy score	7.0 (6.0, 8.0)	7.0 (6.0, 8.0)	7.0 (5.0, 8.0)
Diabetes mellitus, n (%)	96 (16.1)	89 (16.5)	7 (12.3)
Hypertension, n (%)	129 (21.6)	114 (21.1)	15 (26.3)
Hyperuricemia, n (%)	177 (29.6)	163 (30.2)	14 (24.6)
Uric acid lowering agents, n (%)	58 (9.7)	51 (9.4)	7 (12.3)
Uric acid, mg/dL	5.8 (5.0, 6.8)	5.8 (5.1, 6.8)	5.7±1.2
Creatinine, μmol/L	77.0 (65.3, 87.0)	78.0 (67.0, 88.0)	63.4±15.9
SBP, mmHg	126.0 (115.5, 137.0)	125.0 (115.3, 136.0)	130.4±18.9
DBP, mmHg	80.0 (72.0, 86.0)	80.0 (74.0, 88.0)	80.6±11.1
FBG, mmol/L	4.9 (4.5, 5.4)	4.9 (4.6, 5.4)	4.7 (4.3, 5.5)