Heliyon 10 (2024) e24295

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Abnormal S100A8 expression associates with postoperative recurrence in chronic rhinosinusitis with nasal polyps

Xiaocong Deng *, Yingbin Zhao, Di Wu, Yong Qian **

Department of Head and Neck Surgery, Hainan Cancer Hospital, Haikou, Hainan, China

ARTICLE INFO

Keywords:
Chronic rhinosinusitis with nasal polyps
S100A8
Recurrence
Biomarker

ABSTRACT

Objective: To investigate the role of S100A8 in chronic rhinosinusitis with nasal polyps (CRSwNP) and assess its value in predicting disease recurrence after surgery.
Methods: Thirty healthy controls (HC), 30 patients with chronic rhinosinusitis without nasal polyp (CRSsNP), and 60 patients with CRSwNP were enrolled. Serum S100A8 concentration was measured by ELISA. Immunohistochemistry (IHC), western blotting (WB), and reverse transcription-polymerase chain reaction (RT-PCR)were performed to examine tissue expression levels of S100A8. The potential values of S100A8 in predicting postoperative recurrence of CRSwNP were assessed by the receiver operating characteristic (ROC)curve.
Results: Serum S100A8 concentrations in the CRSwNP group were higher than the HC group and the CRSsNP group , especially in the recurrent CRSwNP group ($P < 0.05$). Serum S100A8 levels were positively correlated with peripheral blood eosinophil numbers ($r = 0.263$, $P = 0.043$) and percentages ($r = 0.336$, $P = 0.009$), tissue eosinophil percentages ($r = 0.273$, $P = 0.035$), VAS score ($r = 0.385$, $P = 0.002$) and Lund-Kennedy score ($r = 0.283$, $P = 0.029$). IHC, WB, and RT-PCR results showed tissue S100A8 expression was significantly enhanced in the CRSwNP group, especially in the recurrence group ($P < 0.05$). Binary regression analysis showed that serum S100A8 concentration and tissue eosinophil percentage were correlated with postoperative recurrence of CRSwNP. ROC curve analysis showed that compared with tissue eosinophil percentage, the S100A8 level had a higher value for postoperative recurrence of CRSwNP.
Conclusion: Serum and tissue S100A8 levels were elevated in patients with CRSwNP , especially in the recurrent CRSwNP patients, and were correlated with the degree of peripheral blood and tissue eosinophilic inflammation. S100A8 seemed to be a potential objective biomarker to predict the postoperative recurrence of CRSwNP.

1. Introduction

Chronic rhinosinusitis (CRS) is a common otolaryngology disease characterized by chronic inflammatory changes in the mucosa of the nasal cavity and sinuses, with a prevalence of about 4%–8% in China and increasing yearly [1–3]. Currently, CRS could be further classified into two phenotypes, CRS with nasal polyp (CRSwNP) and CRS without nasal polyp (CRSsNP), based on the presence or absence of polyps under nasal endoscopy [4,5]. In contrast to CRSsNP, CRSwNP is characterized by T helper 2 (Th2) type inflammation

* Corresponding author.
** Corresponding author.
E-mail addresses: entdxc@163.com (X. Deng), yfykqkqy@163.com (Y. Qian).

https://doi.org/10.1016/j.heliyon.2024.e24295
Received 20 April 2023; Received in revised form 4 January 2024; Accepted 5 January 2024
Available online 6 January 2024

and eosinophil infiltration as the main pathological feature, and the degree of eosinophil infiltration in the polyp tissue was found to be associated with the poor treatment outcomes and increase the risk of recurrence after surgery [6–8].Therefore, exploring strategies to prevent the postoperative recurrence of CRSwNP and identifying objective biomarkers for predicting its recurrence is extremely crucial. Although previous studies mentioned that the nasal microbiome, serum metabolites, and sinus CT score could be used to assess postoperative recurrence, the use of these indicators was limited by their lack of objectivity in clinical applications [9–11]. Thus, exploring an objective and reliable biomarker to predict the postoperative recurrence of CRSwNP is of great importance to guide disease treatment and achieve precise treatment.

S100A8, a member of the calcium-binding protein family, is secreted by a variety of immune cells, such as eosinophils, neutrophils, and macrophages, and is essential for cell proliferation, migration, and differentiation [12–14]. Previous studies showed that S100A8 could be involved in the development of various airway inflammatory diseases and autoimmune diseases by binding to its receptor and promoting the differentiation of Th2 cells and the production of related type 2 cytokines [13,15]. A previous study showed that serum S100A8 levels were significantly increased in asthma patients and its levels could potentially be used as a biomarker for asthma [16]. Another publication demonstrated that serum S100A8 levels were elevated in patients with inflammatory bowel disease and its levels were associated with disease recurrence [17]. However, the role of S100A8 in CRSwNP has not been reported. Therefore, this study aims to investigate the role of S100A8 in CRSwNP and the potential value of predicting postoperative recurrence.

2. Materials and methods

2.1. Participants and setting

Ninety patients with CRS who were hospitalized in Hainan Cancer Hospital between February 2019 and January 2020 were included, including 30 CRSsNP patients and 60 CRSwNP patients. The inclusion criteria for the healthy control (HC) group were as follows: 1)18 $<$ age $<$70; 2)undergoing nasal bone fracture or nasal benign tumor in our institution HC group and CRS group exclusion criteria were listed as follows: 1) fungal sinusitis, posterior nasal polyp, sinus cyst, aspirin triad, and acute respiratory infection; 2) other inflammatory diseases, autoimmune diseases, and immunodeficiency-like diseases; 3) severe underlying disease or severe organic cardiovascular disease; 4) use of antibiotics, steroid hormones, antihistamines, and leukotriene receptor antagonists within the last 1 month; 5) age $<$18 years or $>$70 years. The study was approved by the hospital ethics committee, and all patients were informed and signed the consent form.

2.2. Collection of serum specimens and determination of S100A8 concentration

Five milliliters of venous blood were collected and allowed to stand at room temperature for 1 h, then centrifuged at 3000 rpm for 5 min at 4 °C, followed by aspiration of the supernatant into a 15 mL centrifuge tube, then centrifuged at 3000 rpm for 3 min, and finally stored in a −80 °C refrigerator for subsequent use. The collected serum was thawed at room temperature and the concentration of S100A8 in the serum was measured using a commercial ELISA kit (CUSABIO, Wuhan, China) according to the commercial instructions.

2.3. Immunohistochemical (IHC)

Specimens were obtained during surgery and placed in formaldehyde fixative for paraffin embedding and partly in liquid nitrogen for subsequent experiments. A 4 μm section was excised from the paraffin-embedded tissue. For IHC staining, sections were incubated with primary antibodies to S100A8 (Affinity Biosciences, Changzhou, China) overnight at 4 °C. Thereafter, the sections were incubated with biotinylated secondary antibodies (Affinity Biosciences, Changzhou, China) for 30 min at 37 °C, and 3′3-diaminobenzidine (DAB) was used for visualization. The representative images were selected in each group, and tissue S100A8 protein expression levels were represented as the average optical density (IOD/Area) (HPFs, 200 ×).

2.4. Western blotting (WB)

Tissue was removed in liquid nitrogen and proteins were extracted. The operation was as described previously [18]. Total proteins of tissues were extracted in a mixture of RIPA lysis buffer with protease inhibitors. Equal amounts of proteins were electrophoresed on SDS-PAGE in 10 % Tris-glycine gels and transferred to PVDF membranes (Millipore, Massachusetts, USA). The membranes were closed with 5 % skim milk for 1 h at room temperature and incubated overnight with primary antibodies against S100A8 and Tubulin (Affinity Biosciences, Changzhou, China). After the membranes were incubated with secondary antibodies and developed using ECL Ultrasensitive Luminol. Band intensities were quantified by the Image system.

2.5. Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was prepared from tissues using TriZol reagent (Invitrogen). complementary DNA (cDNA) was synthesized using reverse transcriptase (Invitrogen). mRNA expression was determined using an ABI PRISM 7500 detection system (Applied Biosystems, Foster City, CA) and a SYBR Green qPCR kit (Accurate Biology, Hunan, China). Primers were selected based on published data after normalizing the average transcript levels of genes to GAPDH. The primer sequences are as follows.: S100A8, Forward primer (5′–3′) GATCTGCACAGGCATGGTCGT, Reverse primer (5′–3′) AACAGAGTGCCTCGGGACAGGA; GAPDH, Forward primer (5′–3′)

TGCACTTACCTGTGCTCCCACTCCTG, Reverse primer (5′–3′) ACAGGGCAAGCCCACCCCTTCTCTA.

2.6. Statistical analysis

Continuous variables were expressed as mean ± standard deviation, and comparisons between two groups were conducted using Student's -T test, and among three groups with one-way ANOVA. Correlations between serum S100A8 levels and clinical variables were explored by correlation analysis. Binary regression analysis and the operating characteristic curve (ROC) were used to analyze the predictive value of serum S100A8 on postoperative recurrence of CRSwNP. $P < 0.05$ was considered a statistically significant difference.

3. Results

3.1. Comparison of clinical data of participants

One hundred and twenty patients were included in this study. The clinical data of all participants are shown in Table 1. Peripheral blood eosinophil count and percentage, VAS score, and Lund-Kennedy score are significantly higher in the CRSwNP group than in the HC and CRSsNP groups ($P < 0.05$). Gender, age, and body mass index (BMI) exhibit no statistical difference among the three groups ($P > 0.05$). As shown in Table 2, the percentages of peripheral blood and tissue eosinophils were significantly higher in the recurrent CRSwNP group than in the primary CRSwNP group ($P < 0.05$).

3.2. Serum S100A8 expression in CRSwNP and correlation with clinical variables

As shown in Fig. 1, S100A8 levels were significantly higher in the CRSsNP and CRSwNP groups ($P < 0.05$) (Fig. 1A). In addition, the expression of S100A8 was significantly upregulated in the recurrent CRSwNP compared with that in the primary CRSwNP group (Fig. 1B). The correlation analysis showed that serum S100A8 levels were positively correlated with peripheral blood eosinophil number and percentage (Fig. 2A–B), tissue eosinophil percentage, VAS score, and Lund-Kennedy score (Fig. 2D–F),but serum S100A8 levels did not correlate significantly with tissue eosinophil counts (Fig. 2C).

3.3. Evaluation of tissue S100A8 expression in CRSwNP

The IHC results showed that the positive number of cells stained with S100A8 was significantly increased in the CRSwNP group compared with the HC group and CRSsNP group (Fig. 3A). In contrast to primary CRSwNP, the S100A8 expression enhanced in the recurrent CRSwNP group (Fig. 3B). In addition, WB analysis suggested that tissue S100A8 levels were higher in the CRSwNP group (Fig. 4A), especially in the recurrent CRSwNP group (Fig. 4B). RT-PCR likewise revealed that tissue S100A8 mRNA levels were higher in the CRSwNP group compared to the HC group and the CRSsNP group (Fig. 5A), especially in the recurrent CRSwNP group (Fig. 5B).

3.4. Assessment of the values of serum and tissue S100A8 in predicting CRSwNP recurrence

These variables with the statistical difference in Table 2 and serum S100A8 were included in binary regression analysis. The results in Table 3 showed that tissue eosinophil percentage and serum S100A8 were correlated with postoperative recurrence in patients with CRSwNP. The ROC curve indicated that serum S100A8 exhibited potential value and reliability in predicting postoperative recurrence in CRSwNP (Fig. 6). The detailed data are presented in Table 4. Furthermore, we further suggested that tissue S100A8 mRNA level was a potential indicator in predicting postoperative recurrence in CRSwNP (Fig. 7).

4. Discussion

In the present study, we found that serum S100A8 levels were increased in the serum samples of CRSwNP patients, especially in the recurrent CRSwNP cases. The elevated circulating S100A8 levels were positively correlated with the peripheral blood and tissue

Table 1
Comparison of clinical data among three groups.

Variables	HC group n = 30	CRSsNP group n = 30	CRSwNP group n = 60	P-valve
Gender, male/female	18/12	20/10	34/26	0.659
Age, year	43.0 ± 12.3	41.6 ± 12.7	41.7 ± 13.5	0.889
BMI, kg/m^2	24.6 ± 2.9	25.1 ± 3.1	25.4 ± 3.5	0.300
Blood eosinophil count, $10^6/L$	102.0 ± 33.7	149.8 ± 56.0	236.5 ± 114.2	<0.001
Blood eosinophil percentage, %	2.3 ± 0.3	3.1 ± 0.7	6.3 ± 1.4	<0.001
VAS score	–	5.2 ± 1.5	6.9 ± 2.2	<0.001
Lund-Kennedy score	–	4.2 ± 1.5	7.7 ± 2.5	<0.001

HC, healthy control; CRSsNP, chronic rhinosinusitis without nasal polyp; CRSwNP, chronic rhinosinusitis with nasal polyp; BMI, body mass index; VAS, visual analogue scale.

Table 2
Comparison of clinical data between the two groups.

Variables	Primary CRSwNP group n = 32	Recurrent CRSwNP group n = 28	P-value
Gender, male/female	21/11	13/15	0.134
Age, year	39.3 ± 12.8	43.4 ± 14.0	0.242
BMI, kg/m^2	24.7 ± 2.6	26.2 ± 4.2	0.058
Blood eosinophil count, 10^6/L	210.1 ± 109.8	266.7 ± 113.6	0.055
Blood eosinophil percentage, %	5.9 ± 1.6	6.8 ± 1.0	0.016
Tissue eosinophil count, n/HPF	25.4 ± 11.5	27.8 ± 9.9	0.397
Tissue eosinophil percentage, %	9.7 ± 3.4	12.5 ± 5.1	0.013
VAS score	7.0 ± 2.1	6.7 ± 2.3	0.624
Lund-Kennedy score	7.6 ± 2.4	7.8 ± 2.7	0.719

CRSsNP, chronic rhinosinusitis without nasal polyp; CRSwNP, chronic rhinosinusitis with nasal polyp; BMI, Body mass index; VAS, visual analogue scale; HPF, high-power field.

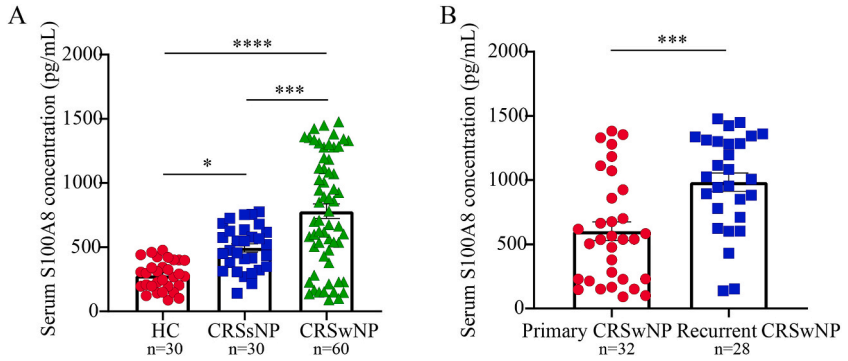

Fig. 1. Serum S100A8 levels were increased in CRSwNP group. (A) Serum expression level of S100A8 among the three groups. (B) Serum expression level of S100A8 between primary CRSwNP group and recurrent CRSwNP group. *P < 0.05, ***P < 0.001, ****P < 0.0001. HC, healthy control; CRSsNP, chronic rhinosinusitis without nasal polyp; CRSwNP, chronic rhinosinusitis with nasal polyp.

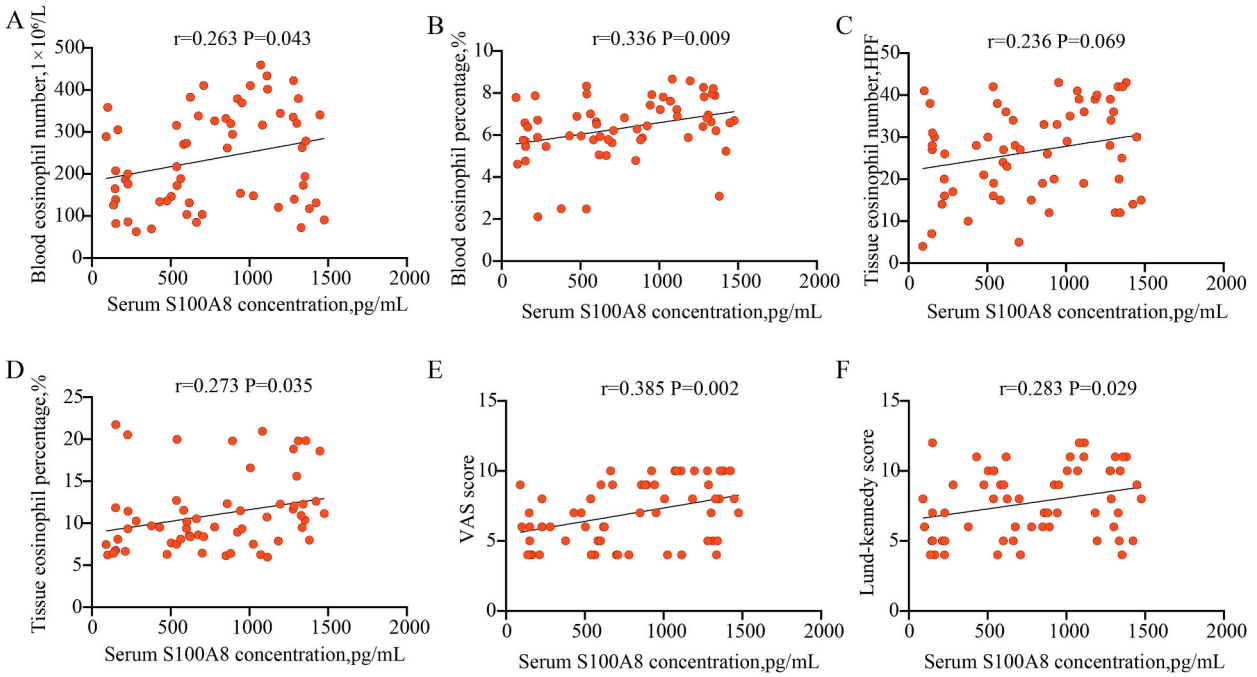

Fig. 2. Correlation between serum S100A8 levels and clinical variable. (A) Blood eosinophil number, (B) Blood eosinophil percentage, (C) Tissue eosinophil number, (D) Tissue eosinophil percentage, (E) VAS score, (F) TNSS score.

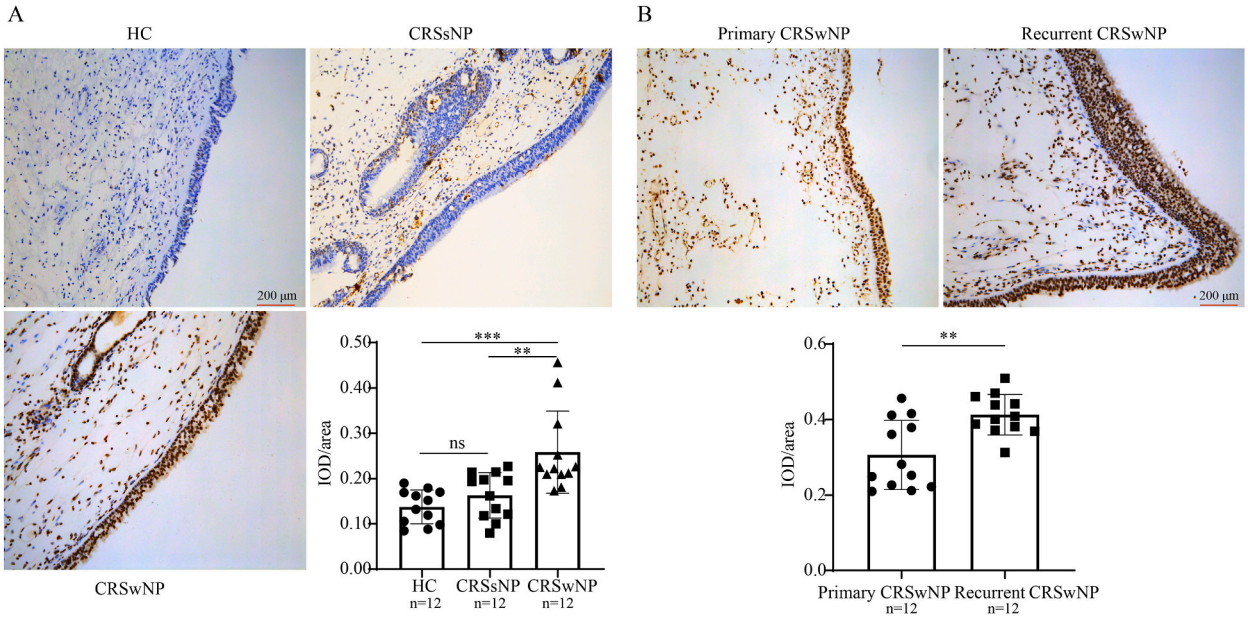

Fig. 3. IHC staining of S100A8 in different groups. S100A8 protein expression was significantly enhanced in the CRSwNP group(A), especially in the recurrent group(B), and concentrated in the epithelial region of the nasal mucosa (200 ×). **P < 0.01, ***P < 0.001, ns, no significance. IHC, immunohistochemistry. HC, healthy control; CRSsNP, chronic rhinosinusitis without nasal polyp; CRSwNP, chronic rhinosinusitis with nasal polyp. IOD/Area, average optical density.

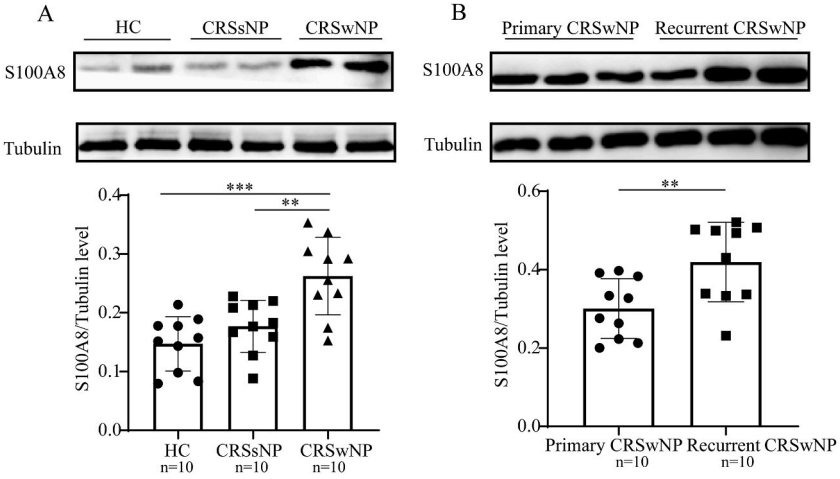

Fig. 4. S100A8 protein level in different groups. WB result showed that S100A8 protein expression was significantly enhanced in the CRSwNP group (A), especially in the recurrent CRSwNP group (B). **P < 0.01, ***P < 0.001. WB, western blotting; HC, healthy control; CRSsNP, chronic rhinosinusitis without nasal polyp; CRSwNP, chronic rhinosinusitis with nasal polyp.

eosinophil percentages, and clinical disease severity. The tissue expression levels were consistent with the serological findings. Moreover, we demonstrated that both serum and tissue S100A8 levels showed potential values in predicting postoperative recurrence. These results suggested that S100A8 was involved in the pathological mechanism, and it might serve as a novel biomarker for monitoring the severity and predicting postoperative recurrence in patients with CRSwNP.

S100A8 is a crucial protein in eosinophils and macrophages and acts as an important component of the innate immune system [19, 20]. Previous studies proved that S100A8 was a potent ligand for Toll-like receptor 4 (TLR4), which mediated pathway activation promotes Th2 cell differentiation and release of type 2 cytokines [21–24]. Recent publications found that serum S100A8 concentrations were significantly elevated in asthma patients, and their levels were positively correlated with the severity of the disease [25]. In another study, tissue S100A8 levels were also found to be increased in inflammatory bowel diseases, especially in active inflammatory bowel diseases [26]. Intriguingly, the serum S100A8 levels were found to be increased in patients with systemic lupus

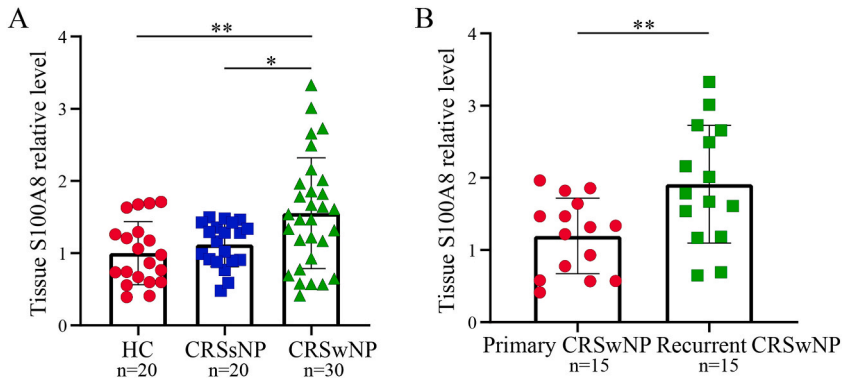

Fig. 5. S100A8 mRNA level in different groups. RT-PCR results showed that compared to the HC group and CRSsNP group, S100A8 level was significantly enhanced in the CRSwNP group(A), especially in the recurrent CRSwNP group(B). *P < 0.05, ***P < 0.001. RT-PCR, reverse transcription-polymerase chain reaction; HC, healthy control; CRSsNP, chronic rhinosinusitis without nasal polyp; CRSwNP, chronic rhinosinusitis with nasal polyp.

Table 3
Binary regression analysis of clinical variables associated with postoperative recurrence of CRSwNP.

Clinical variables	OR	95%CI	P-value
Blood eosinophil percentage, %	1.596	0.960–2.652	0.071
Tissue eosinophil percentage, %	1.176	1.017–1.359	0.029
SerunS100A8, pg/mL	2.012	1.001–2.035	0.004

CRSwNP, chronic rhinosinusitis with nasal polyp; OR, odd ratio; CI, confidence interval.

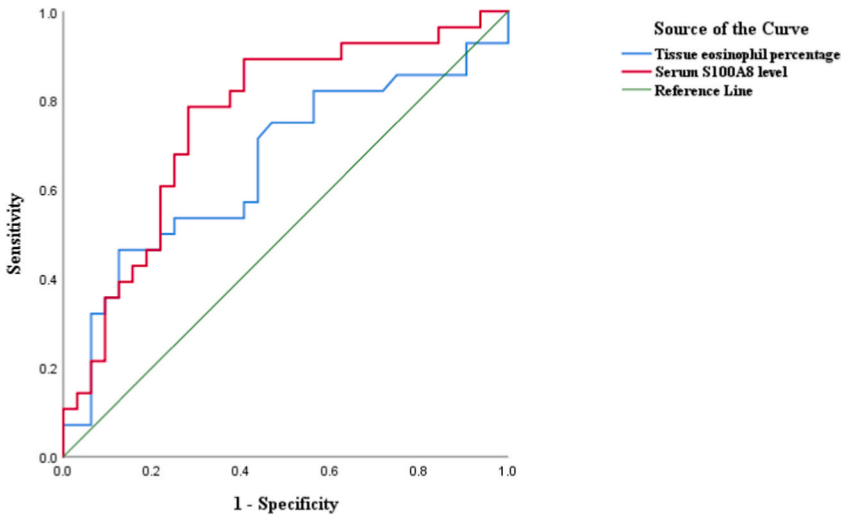

Fig. 6. ROC curves evaluating the predictive values of serum S100A8 levels and tissue eosinophil percentage for recurrence of CRSwNP. ROC, receiver operating characteristics; CRSwNP, chronic rhinosinusitis with nasal polyp.

Table 4
ROC curve analysis to explore the predictive value.

Clinical variables	AUC	95%CI	Sensitivity	Specificity	P-value	Cut-off value
Tissue eosinophil percentage, %	0.655	0.631–0.882	0.46	0.88	0.039	11.52
Serum S100A8, pg/mL	0.757	0.512–0.799	0.79	0.72	0.001	705.19

ROC, Receiver operating characteristic; AUC, area under the curve; CI, confidence interval.

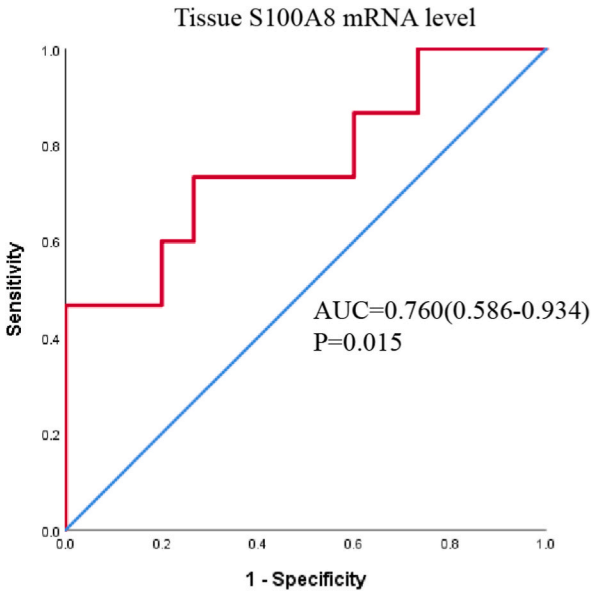

Fig. 7. ROC curves evaluating the predictive values of tissue S100A8 levels for recurrence of CRSwNP. ROC, receiver operating characteristics; CRSwNP, chronic rhinosinusitis with nasal polyp.

erythematosus, and decreased significantly after treatment, suggesting that S100A8 could be used as a reference indicator of disease activity and help guide disease treatment and prognosis assessment [27]. In addition, Previous studies have shown that the degree of tissue type 2 inflammation and eosinophilic inflammation were significantly correlated with CRSwNP disease severity [28,29]. Interestingly, Waddell et al. [30] S100A8 could be involved in disease progression by interacting with eosinophil chemokines, thereby promoting migratory aggregation of eosinophils. These studies implied that the changes in S100A8 levels were closely related to the development of inflammatory diseases, but the relationship between S100A8 and CRSwNP is currently unclear. Our results demonstrated that serum and tissue S100A8 levels were significantly higher in the CRSwNP group and its serum levels were positively correlated with the peripheral blood and tissue eosinophil ratios, VAS scores, and Lund-Kennedy scores. These results suggested that altered serum S100A8 concentrations were involved in the development of CRSwNP and might be associated with tissue eosinophilic inflammation, but the specific pathological mechanisms need to be further investigated.

Currently, although surgical treatment is one of the effective treatment modalities to improve clinical symptoms in patients with CRSwNP, not all patients achieve good prognoses due to its uncertain etiology and high disease heterogeneity [31]. It was well established that the degree of eosinophil infiltration and type 2 inflammation in the mucosa profoundly influenced the risk of CRSwNP recurrence after surgery [32–34].In the present study, we found that serum and tissue S100A8 levels were significantly upregulated in patients with recurrent CRSwNP compared to patients with primary CRSwNP. Moreover, serum S100A8 levels were positively correlated with peripheral blood and tissue eosinophil percentages. ROC curves further indicated that both serum and tissue S100A8 exhibited potential values in predicting postoperative recurrence of CRSwNP. Accumulating evidence showed that S100A8 promoted the release of Th2 cytokines and eosinophil chemotaxis through binding to the receptor TLR4, which contributed to the development of tissue Th2 inflammation and eosinophilia [35,36]. Therefore, we hypothesized that high levels of S100A8 could exacerbate the migratory infiltration of eosinophils into nasal mucosal tissues by promoting Th2 cell differentiation and cytokine production, aggravating type 2 inflammation and eosinophilia in nasal mucosal tissues and increasing the risk of postoperative recurrence of CRSwNP.

In summary, serum and tissue S100A8 levels were significantly increased in CRSwNP patients and correlated with postoperative recurrence. Serum S100A8 might be used as a novel and objective biomarker for predicting the postoperative recurrence of CRSwNP. However, the findings of this study are yet to be confirmed by further large-sample, multicenter studies.

Ethics approval statement

This study complies with the Declaration of Helsinki and was approved by the ethics committee of Hainan Cancer Hospital.

Data availability statement

Data will be made available on request.

The following is the Supplementary data to this article.

CRediT authorship contribution statement

Xiaocong Deng: Writing - original draft, Investigation. **Yingbin Zhao:** Methodology. **Di Wu:** Validation. **Yong Qian:** Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e24295.

References

[1] X. Wang, Y. Sima, Y. Zhao, N. Zhang, M. Zheng, K. Du, et al., Endotypes of chronic rhinosinusitis based on inflammatory and remodeling factors, J. Allergy Clin. Immunol. (2022), https://doi.org/10.1016/j.jaci.2022.10.010.

[2] W.J. Fokkens, A.S. Viskens, V. Backer, D. Conti, E. De Corso, P. Gevaert, et al., EPOS/EUFOREA update on indication and evaluation of biologics in chronic rhinosinusitis with nasal polyps 2023, Rhinology 61 (3) (2023) 194–202, https://doi.org/10.4193/Rhin22.489.

[3] A.R. Sedaghat, W.J. Fokkens, V.J. Lund, P.W. Hellings, R.C. Kern, S. Reitsma, et al., Consensus criteria for chronic rhinosinusitis disease control: an international Delphi Study, Rhinology 61 (6) (2023) 519–530, https://doi.org/10.4193/Rhin23.335.

[4] W.J. Fokkens, V.J. Lund, J. Mullol, C. Bachert, I. Alobid, F. Baroody, et al., European position paper on rhinosinusitis and nasal polyps 2012, Rhinology 50 (2012) 1–298.

[5] G.K. Scadding, G.W. Scadding, Biologics for chronic rhinosinusitis with nasal polyps (CRSwNP), J. Allergy Clin. Immunol. 149 (3) (2022) 895–897, https://doi.org/10.1016/j.jaci.2021.10.029.

[6] D.H. Kim, J.S. Han, G.J. Kim, M.A. Basurrah, S.H. Hwang, Clinical predictors of polyps recurring in patients with chronic rhinosinusitis and nasal polyps: a systematic review and meta-analysis, Rhinology 61 (6) (2023) 482–497, https://doi.org/10.4193/Rhin23.136.

[7] P.W. Wu, C.H. Chiu, Y.L. Huang, Y.S. Fan, T.J. Lee, C.C. Huang, et al., Tissue eosinophilia and computed tomography features in paediatric chronic rhinosinusitis with nasal polyps requiring revision surgery, Rhinology 61 (3) (2023) 348–357, https://doi.org/10.4193/Rhin22.435.

[8] S.P. Ramkumar, L. Marks, D. Lal, M.J. Marino, Outcomes of limited versus extensive surgery for chronic rhinosinusitis: a systematic review and meta-analysis, International forum of allergy & rhinology. 13 (11) (2023) 2096–2100, https://doi.org/10.1002/alr.23178.

[9] W.G. Gan, H.T. Zhang, F.J. Yang, S.X. Liu, F. Liu, J. Meng, The influence of nasal bacterial microbiome diversity on the pathogenesis and prognosis of chronic rhinosinusitis patients with polyps, Eur Arch Oto-Rhino-L. 278 (4) (2021) 1075–1088, https://doi.org/10.1007/s00405-020-06370-4.

[10] M. Lilja, A. Koskinen, A. Julkunen-Iivari, A. Makitie, J. Numminen, M. Rautiainen, et al., Radiological score of computed tomography scans predicts revision surgery for chronic rhinosinusitis, Acta Otorhinolaryngo 42 (1) (2022) 63–74, https://doi.org/10.14639/0392-100x-N1561.

[11] S. Xie, C. Zhang, Z. Xie, J. Zhang, H. Zhang, W. Jiang, Serum metabolomics identifies uric acid as a possible novel biomarker for predicting recurrence of chronic rhinosinusitis with nasal polyps, Rhinology 61 (6) (2023) 541–551, https://doi.org/10.4193/Rhin23.236.

[12] S.Y. Lim, A.E. Yuzhalin, A.N. Gordon-Weeks, R.J. Muschel, Tumor-infiltrating monocytes/macrophages promote tumor invasion and migration by upregulating S100A8 and S100A9 expression in cancer cells, Oncogene 35 (44) (2016) 5735–5745, https://doi.org/10.1038/onc.2016.107.

[13] Y. Huang, M. Wang, Y. Hong, X. Bu, G. Luan, Y. Wang, et al., Reduced expression of antimicrobial protein secretory leukoprotease inhibitor and clusterin in chronic rhinosinusitis with nasal polyps, J Immunol Res 2021 (2021) 1057186, https://doi.org/10.1155/2021/1057186.

[14] A. Abtin, L. Eckhart, R. Glaser, R. Gmeiner, M. Mildner, E. Tschachler, The antimicrobial heterodimer S100A8/S100A9 (calprotectin) is upregulated by bacterial flagellin in human epidermal keratinocytes, J. Invest. Dermatol. 130 (10) (2010) 2423–2430, https://doi.org/10.1038/jid.2010.158.

[15] G. Sreejit, A. Abdel-Latif, B. Athmanathan, R. Annabathula, A. Dhyani, S.K. Noothi, et al., Neutrophil-Derived S100a8/A9 amplify granulopoiesis after myocardial infarction, Circulation 141 (13) (2020) 1080–1094, https://doi.org/10.1161/CIRCULATIONAHA.119.043833.

[16] Y.G. Lee, J. Hong, P.H. Lee, J. Lee, S.W. Park, D. Kim, et al., Serum calprotectin is a potential marker in patients with asthma, J Korean Med Sci 35 (43) (2020). ARTN e36210.3346/jkms.2020.35.e362.

[17] C. Kessel, M. Lavric, T. Weinhage, M. Brueckner, S. de Roock, J. Dabritz, et al., Serum biomarkers confirming stable remission in inflammatory bowel disease, Sci Rep-Uk 11 (1) (2021). ARTN 669010.1038/s41598-021-86251-w.

[18] R.W. Liu, J.T. Du, J. Zhou, B. Zhong, L. Ba, J. Zhang, et al., Elevated microRNA-21 is a brake of inflammation involved in the development of nasal polyps, Front. Immunol. 12 (2021). ARTN 53048810.3389/fimmu.2021.530488.

[19] E.M. Wilkerson, M.W. Johansson, A.S. Hebert, M.S. Westphall, S.K. Mathur, N.N. Jarjour, et al., The peripheral blood eosinophil proteome, J. Proteome Res. 15 (5) (2016) 1524–1533, https://doi.org/10.1021/acs.jproteome.6b00006.

[20] F. Wu, Y.T. Zhang, F. Teng, H.H. Li, S.B. Guo, S100a8/a9 contributes to sepsis-induced cardiomyopathy by activating ERK1/2-Drp1-mediated mitochondrial fission and respiratory dysfunction, Int. Immunopharm. 115 (2023) 109716, https://doi.org/10.1016/j.intimp.2023.109716.

[21] J.M. Ehrchen, C. Sunderkotter, D. Foell, T. Vogl, J. Roth, The endogenous Toll-like receptor 4 agonist S100A8/S100A9 (calprotectin) as innate amplifier of infection, autoimmunity, and cancer, J. Leukoc. Biol. 86 (3) (2009) 557–566, https://doi.org/10.1189/jlb.1008647.

[22] B. Ma, S.S. Athari, E. Mehrabi Nasab, L. Zhao, PI3K/AKT/mTOR and TLR4/MyD88/NF-kappaB signaling inhibitors attenuate pathological mechanisms of allergic asthma, Inflammation 44 (5) (2021) 1895–1907, https://doi.org/10.1007/s10753-021-01466-3.

[23] J. Li, L. Zhang, X. Chen, D. Chen, X. Hua, F. Bian, et al., Pollen/TLR4 innate immunity signaling initiates IL-33/ST2/Th2 pathways in allergic inflammation, Sci. Rep. 6 (2016) 36150, https://doi.org/10.1038/srep36150.

[24] A.M. Tan, H.C. Chen, P. Pochard, S.C. Eisenbarth, C.A. Herrick, H.K. Bottomly, TLR4 signaling in stromal cells is critical for the initiation of allergic Th2 responses to inhaled antigen, J. Immunol. 184 (7) (2010) 3535–3544, https://doi.org/10.4049/jimmunol.0900340.

[25] D.H. Kim, E. Choi, J.S. Lee, N.R. Lee, S.Y. Baek, A. Gu, et al., House dust mite allergen regulates constitutive apoptosis of normal and asthmatic neutrophils via toll-like receptor 4, PLoS One 10 (5) (2015). ARTN e012598310.1371/journal.pone.0125983.

[26] T.A. Kopi, A.A. Kadijani, H. Parsian, S. Shahrokh, H.A. Aghdaei, A. Mirzaei, et al., The value of mRNA expression of S100A8 and S100A9 as blood-based biomarkers of inflammatory bowel disease, Arab J Gastroenterol 20 (3) (2019) 135–140, https://doi.org/10.1016/j.ajg.2019.07.002.

[27] J.W. Kim, J.Y. Jung, S.W. Lee, W.Y. Baek, H.A. Kim, C.H. Suh, S100A8 in serum, urine, and saliva as a potential biomarker for systemic lupus erythematosus, Front. Immunol. (2022) 13. ARTN 88620910.3389/fimmu.2022.886209.

[28] Y. Yao, Z.C. Wang, J.X. Liu, J. Ma, C.L. Chen, Y.K. Deng, et al., Increased expression of TIPE2 in alternatively activated macrophages is associated with eosinophilic inflammation and disease severity in chronic rhinosinusitis with nasal polyps, Int Forum Allergy Rhinol 7 (10) (2017) 963–972, https://doi.org/10.1002/alr.21984.
[29] A.H. Khan, I. Gouia, S. Kamat, R. Johnson, M. Small, J. Siddall, Prevalence and severity distribution of type 2 inflammation-related comorbidities among patients with asthma, chronic rhinosinusitis with nasal polyps, and atopic dermatitis, Lung 201 (1) (2023) 57–63, https://doi.org/10.1007/s00408-023-00603-z.
[30] A. Waddell, R. Ahrens, Y.T. Tsai, J.D. Sherrill, L.A. Denson, K.A. Steinbrecher, et al., Intestinal CCL11 and eosinophilic inflammation is regulated by myeloid cell-specific RelA/p65 in mice, J. Immunol. 190 (9) (2013) 4773–4785, https://doi.org/10.4049/jimmunol.1200057.
[31] P.C. Tsai, T.J. Lee, P.H. Chang, C.H. Fu, Role of serum eosinophil cationic protein in distinct endotypes of chronic rhinosinusitis, Rhinology (2023), https://doi.org/10.4193/Rhin23.170.
[32] K.Z. Zhu, C. He, Z. Li, P.J. Wang, S.X. Wen, K.X. Wen, et al., Development and multicenter validation of a novel radiomics-based model for identifying eosinophilic chronic rhinosinusitis with nasal polyps, Rhinology 61 (2) (2023) 132–143, https://doi.org/10.4193/Rhin22.361.
[33] S. Jo, S.H. Lee, H.R. Jo, S. Weon, C. Jeon, M.K. Park, et al., Eosinophil-derived TGFβ1 controls the new bone formation in chronic rhinosinusitis with nasal polyps, Rhinology 61 (4) (2023) 338–347, https://doi.org/10.4193/Rhin22.439.
[34] H. Zhang, S. Xie, R. Fan, F. Wang, Z. Xie, W. Jiang, Elevated ALCAM expression associated with endotypes and postoperative recurrence in chronic rhinosinusitis with nasal polyps, J. Inflamm. Res. 15 (2022) 1063–1077, https://doi.org/10.2147/jir.S350609.
[35] T. Kato, H. Kouzaki, K. Matsumoto, J. Hosoi, T. Shimizu, The effect of calprotectin on TSLP and IL-25 production from airway epithelial cells, Allergol. Int. 66 (2) (2017) 281–289, https://doi.org/10.1016/j.alit.2016.06.011.
[36] H.A. Kim, J.H. Han, W.J. Kim, H.J. Noh, J.M. An, H. Yim, et al., TLR4 endogenous ligand S100a8/A9 levels in adult-onset still's disease and their association with disease activity and clinical manifestations, Int. J. Mol. Sci. 17 (8) (2016). ARTN 134210.3390/ijms17081342.