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Predictive Significance of Charcot-Leyden Crystal Protein in Nasal Secretions in Recurrent Chronic Rhinosinusitis with Nasal Polyps

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Keywords

Chronic rhinosinusitis with nasal polyp · Recurrence · Charcot-Leyden crystal · Receiver operating characteristic curves · Eosinophil

Abstract

Introduction: The recurrence occurs frequently among patients with chronic rhinosinusitis with nasal polyps (CRSwNP), and predictors that could be conveniently detected during practice in outpatient service are needed. ***Objective:*** We aimed to illustrate that the concentration of Charcot-Leyden crystal (CLC) in nasal secretions can effectively and noninvasively predict polyp recurrence. ***Methods:*** 108 patients with CRSwNP were divided into recurrence (n = 68) and recurrence-free (n = 40) groups. Preoperative CLC concentrations in nasal secretions were collected and detected by ELISA. Polyp tissues were harvested during biopsy or endoscopic sinus surgery and were evaluated for inflammatory cells by histopathological staining. Demographic information and the clinical characteristics of each patient were reviewed for associations with recurrence. Binary logistic regression analysis was performed to determine predictive factors for polyp recurrence. Receiver operating characteristic (ROC) curves and the Youden index were performed to determine their predictive values. Survival analysis was performed to compare recurrence risk of patients with different CLC concentrations. ***Results:*** Sixty-eight (62.96%) patients developed recurrence during a 12- to 33-month postoperative follow-up. CLC concentrations in nasal secretions were positively correlated with eosinophil percent in polyp tissue and peripheral blood and were significantly higher in patients of the recurrence group than in the patients of the recurrence-free group (p < 0.001). Binary logistic regression and ROC curve demonstrated that CLC protein in nasal secretions is predictive of polyp recurrence. According to the Youden index, a CLC concentration of 34.24 ng/mL can predict postoperative polyp recurrence with 92.6% sensitivity and 87.5% specificity. Patients with CLC concentrations higher than the cutoff value yielded a higher risk of recurrence (p < 0.001, HR = 11.31, 95% CI: 6.41–19.98). ***Conclusions:*** CLC protein in nasal secretions may serve as a promising noninvasive biomarker to predict CRSwNP recurrence.

Introduction

Chronic rhinosinusitis with nasal polyps (CRSwNP) describes a spectrum of heterogeneous diseases characterized by a massive infiltration of inflammatory cells,

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defects in the epithelial barrier, and the presence of edematous masses of inflamed tissue, significantly affecting patients' quality of life [1–5]. First-line therapy includes the use of intranasal and/or oral corticosteroids, and endoscopic sinus surgery (ESS) is recommended when first-line therapy fails [1, 6–11]. However, recurrence appears in over 50% patients worldwide who have undergone ESS, including the most radical procedure, suggesting that persistent medical care is needed after ESS [6, 11–13].

Thus, the identification of predictors for recurrence is crucial for better disease management. Tissue eosinophilia has been proven to be the most reliable predictor [3, 8, 12, 14, 15]. Lou et al. [3, 12] reported that a percentage of tissue eosinophils higher than 27% predicted recurrence with promising accuracy, and extremely eosinophilic nasal polyps exhibited a recurrence rate of over 90%. Moreover, previous studies suggested peripheral blood eosinophil levels as an alternative predictor for recurrence [7, 8, 14–21]. However, the procedure for tissue harvesting is invasive and inevitably associated with trauma to mucosa and an increased risk of bleeding and infection [7, 12, 22]. Therefore, there is a need for biomarkers that can be collected and detected noninvasively before the ESS or during the postoperative follow-up in outpatient department, such as Charcot-Leyden crystal (CLC) protein [23–25].

Elevated CLC protein levels were observed in eosinophilic diseases including atopic dermatitis, parasitic infections, and eosinophilic airway diseases such as aspirin-induced asthma, allergic rhinitis, and chronic rhinosinusitis [24, 26–31]. Persson et al. [32] have reported that crystallized CLCs are key features of severe eosinophilic inflammatory airway diseases, including asthma and CRSwNP. In our recently published study, we have confirmed a promising predictive significance of mRNA level of CLC in polyp tissue for eosinophilic CRSwNP and in nasal brushing for polyp recurrence [33, 34]. In addition, our previous study reported CLC protein concentrations in nasal secretions were significantly elevated in patients with better response to oral glucocorticoids (GCs) and higher percentage of tissue eosinophilia [35].

As the association between CLC protein concentrations in nasal secretions and the recurrence of nasal polyp has not been investigated, the purpose of our study was to determine the significance of CLC protein concentrations in nasal secretions in predicting recurrent CRSwNP, which we believe would bring further understanding to the current knowledge for CLC and CRSwNP.

Materials and Methods

Study Design and Patients

This was a single-center, retrospective study using data collected prospectively from patients with bilateral nasal polyps who had undergone ESS at Beijing TongRen Hospital from October 2016 to June 2018. The study was approved by the Ethics Committee of Beijing TongRen Hospital, and all the patients provided written informed consent before the collection of data. Diagnosis of CRSwNP was strictly confirmed according to the European Position Paper on Rhinosinusitis and Nasal Polyps (EPOS) 2012 [1]. The medical records of 108 patients (69 men and 39 women), age 18–69 years (mean ± SD: 44.75 ± 12.11 years), were reviewed and analyzed for preoperative demographic characteristics and medical history, including smoking history, comorbid asthma, comorbid allergic rhinitis, comorbid atopy, 4 main symptoms (nasal obstruction, rhinorrhea, facial pain/headache, and olfactory disorder), 2 sinonasal CT scan parameters (the Lund-Mackay score and the total ethmoid/maxillary sinus score [E/M] ratio of the Lund-Mackay score), preoperative endoscopic nasal polyp score, and percentages of eosinophils and neutrophils in peripheral blood [36–43].

Seventy-seven of the 108 patients were the same as the eligible patients of our previous study (12 were excluded for the uncompleted follow-up or the cancel of surgery due to personal issues), and the other 31 were newly recruited participants [35]. Their related data were used for analysis in this study.

None of the patients had been treated with GCs, immunomodulatory agents, or antibiotics within 4 weeks before enrollment. Patients with fungal sinusitis, allergic fungal rhinosinusitis, cystic fibrosis, or primary ciliary dyskinesia were excluded. None of the patients were known to have aspirin-exacerbated respiratory disease.

Clinical Assessment

A total of 15 descriptors, comprising demographic information, clinical characteristics, and laboratory data, were reviewed and are shown in Table 1. Asthma was diagnosed according to the 2014 Global Initiative for Asthma's definition, and allergic rhinitis was diagnosed according to the 2008 Allergic Rhinitis and its Impact on Asthma criteria [36, 37]. Atopy was evaluated by measurement of serum-specific IgE (Phadia, Uppsala, Sweden; cutoff value, 0.35 kUA/L) [38]. Peripheral blood data were obtained from routine blood tests. Self-report symptoms were evaluated using a 0–10 point visual analog scale (VAS) system, where 0 indicated the absence of any symptoms and 10 indicated the presence of the most severe symptoms [1]. CT scan findings were evaluated by the Lund-Mackay staging system, where for frontal, sphenoid, maxillary, and anterior and posterior ethmoid sinuses at each side, 0 (no opacification), 1 (partial opacification), and 2 (total opacification) were used; where for ostiomeatal complex at each side, 0 (not occluded) and 2 (occluded) were used, and the E/M ratio of the Lund-Mackay score (0–8) was calculated [39–41]. The size of the polyps in each side of the nasal cavity was measured endoscopically by using the nasal polyp size score (NPSS, 0–4, a higher score representing a larger size of polyps, see online suppl. Table 1; for all online suppl. material, see www.karger.com/doi/10.1159/000510120) [42, 43].

The ESS and postoperative follow-up for each enrolled patient were performed by the same surgeon (C.W.), who was blinded to all laboratory data. A total of 108 patients with CRSwNP were en-

Table 1. Demographic information and clinical characteristics of recurrence and recurrence-free groups

	Recurrence (n = 68)	Recurrence-free (n = 40)	p value
Sex (male/female)	41/27	28/12	0.861
Age, years (mean±SD)	43.03±12.38	47.68±11.19	0.054
Asthma (Y/N)	34/34	8/32	0.002
Allergic rhinitis (Y/N)	22/46	7/33	0.117
Atopy (Y/N)	45/23	16/24	0.010
Smoking history (Y/N)	12/56	7/33	>0.999
Nasal obstruction (median; IQR)	7.0; 6.0–8.0	7.0; 6.0–8.0	0.844
Rhinorrhea (median; IQR)	6.0; 4.0–7.0	5.0; 3.0–6.8	0.108
Facial pain/headache (median; IQR)	0; 0–3.0	0; 0–3.8	0.743
Olfactory disorder (median; IQR)	5.0; 4.0–10.0	4.0; 3.0–8.8	0.300
Lund-Mackay score (median; IQR)	19.0; 16.0–22.0	17.0; 14.3–20.0	0.133
Lund-Mackay score E/M ratio (median; IQR)	2.3; 2.0–3.5	2.0; 1.4–3.0	0.004
Nasal polyp size score (median; IQR)	5.0; 4.0–6.0	5.0; 4.0–6.0	0.534
Eosinophils% (peripheral blood) (median; IQR)	6.25; 4.65–9.23	3.35; 1.65–5.58	<0.001
Neutrophils% (peripheral blood) (median; IQR)	54.35; 49.25–60.18	56.60; 48.85–63.15	0.620

IQR, interquartile range; E/M ratio, total ethmoid/maxillary sinus score ratio.

rolled. Seventy-seven patients who had participated in the previous study underwent ESS 2 weeks after the enrollment (once finishing the 2-week GC therapy) [35], whereas the other 31 patients with shorter-than-2-week GC therapy received the ESS procedure once the medical treatment was finished. All patients were followed up after ESS in the first week, the second week, the first month, the third month, and once every 3 months thereafter for 12–33 months [1, 44]. All other experimental procedures were performed by 2 individuals who were blinded to the follow-up results (D.W. and B.Y.). During follow-up, medical treatment was applied as recommended by the EPOS 2012 guideline [1]. Briefly, intranasal budesonide nasal spray (Rhinocort Aqua, AstraZeneca, Sweden) 128 μg twice a day was routinely used for 3 months until symptom control was achieved. All patients were also treated with nasal irrigation daily and clarithromycin 250 mg daily for 3 months after ESS. Intranasal steroid therapy was started again, as recommended, if nasal polyps or mucosal edema were observed during follow-up. In cases where the symptoms were inadequately controlled, oral GCs and/or larger doses of intranasal GCs were administered, and clarithromycin 250 mg daily was administered to patients with returned mucopurulent secretions [1, 45, 46]. The definition of recurrence was as follows: endoscopic evidence of returned polyposis, together with 1 or more bothersome symptoms (nasal obstruction, rhinorrhea, headache/facial pain, smell abnormalities, and sleep disturbance/fatigue) lasting at least 1 week even after receiving appropriate intranasal GC treatment [1, 3, 12, 44].

Collection of Nasal Secretions

Nasal secretions were collected bilaterally from each patient once enrolled in the study, as described by Watelet et al. [47]. Briefly, these secretions were obtained by inserting a scissored postoperative sinus sponge pack Merocel® (Medtronic Xomed, Jacksonville, FL, USA) in middle meatus of each nostril parallel to the sagittal plane for 5 min. The secretion was extracted from the sponge by the addition of 1 mL of 0.9% normal saline. All sponges were stored at 4°C for 2–24 h and then transferred to a 5-mL BD syringe (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) and were centrifuged at 1,500 *g* for 15 min at 4°C. The supernatants were separated and stored in aliquots at −80°C until CLC protein concentration analysis.

Enzyme-Linked Immunosorbent Assays

The CLC protein concentration in nasal secretions was estimated using commercial ELISA kits (Cloud-Clone Corp., Wuhan, China). This kit measures both crystallized and non-crystallized CLC proteins. The detection range for CLC protein is 0.3–20 ng/mL. Before the assay, all samples were diluted 50-fold with 0.9% normal saline; the assay was conducted strictly according to the manufacturer's instructions. The assay was repeated using a stock solution for samples with a CLC protein concentration that was lower than the detection limit.

Histological Evaluation

Polyp tissue samples were collected via biopsy before GC therapy (for the patients who received complete or incomplete GC therapy) or the ESS procedure (for the patients who took no medical treatment before ESS). Histological criteria were determined by hematoxylin and eosin staining, as previously described [48]. Briefly, tissue samples were dehydrated, embedded in paraffin, and cut at a thickness of 5 μm using a Leica RM2235 cryostat (Leica Microsystems, Bannockburn, IL, USA). All sections were examined by optical microscopy at a magnification of ×400, and absolute numbers and percentages of eosinophils, neutrophils, plasma cells, and lymphocytes were calculated (data for neutrophil, plasma cells, and lymphocytes are not shown) [3, 35]. For each section, the absolute numbers and percentages of each cell type were recorded as the mean of 3 non-overlapping regions; the final evaluation of each patient's tissue sample was recorded as the mean of 5 sections. Inflammatory cells were counted by 2 pathologists who were blinded to the study design.

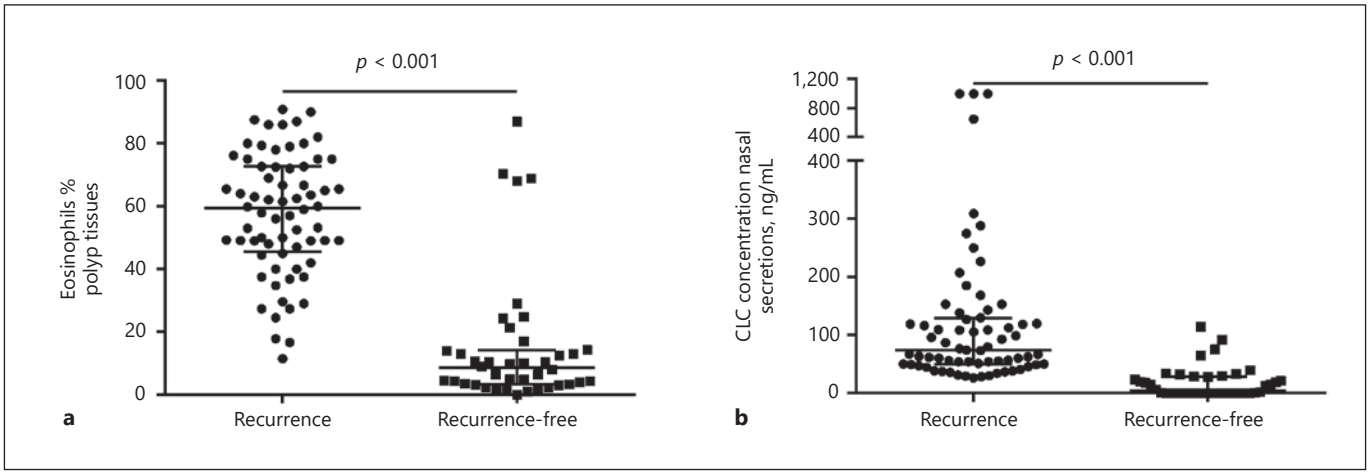

Fig. 1. Differences in histological evaluations and laboratory data between patients from the recurrence ($n = 68$) and recurrence-free ($n = 40$) groups. **a** Percentage of eosinophils in polyp tissues. **b** Charcot-Leyden crystal (CLC) protein concentration in nasal secretions.

Statistical Analysis

All the data are expressed as the median and interquartile range (IQR), except age, which is expressed as the mean and SD, and binary variables, which are expressed as numbers. Statistical analysis was performed using GraphPad Prism 6 (GraphPad Software Inc., LA Jolla, CA, USA). All parametric variants were analyzed using a Student's *t* test, and nonparametric variables were tested using the Mann-Whitney U test. Binary variables were analyzed by a χ^2 or Fisher's exact test. Correlations between 2 variables were analyzed using Spearman's rank correlation coefficient. Binary logistic regression was used to find potential predictors of polyp recurrence. Receiver operating characteristic (ROC) curves were generated to determine the optimal cutoff point using SPSS (version 24.0; IBM Corp., Armonk, NY, USA). The ability of the predictor to predict response to GC therapy was determined by the area under the ROC curve (AUC) and the optimal cutoff point was determined by the Youden index. Comparisons of AUCs were performed using MedCalc statistical software package (version 15.2; Ostend, Belgium). Survival analysis was estimated by the Kaplan-Meier method, and a hazard ratio (HR) and 95% confidence interval (CI) for recurrence were calculated via a stratified log-rank test. A p value <0.05 was considered statistically significant. All data were analyzed using 2-tailed tests.

Results

Patient Characteristics

Overall, 68 out of 108 patients were identified by polyp recurrence and classified into a recurrence group, whereas 40 patients showed no sign of recurrence and were classified into a recurrence-free group. All demographic information and clinical characteristics are shown in Table 1. Results indicated that patients from the recurrence group had a higher comorbid rate of asthma ($p = 0.002$) and atopy ($p = 0.010$). Results of clinical characteristics and examinations revealed that patients in the recurrence group had a significantly higher E/M ratio ($p = 0.004$) and a higher percentage of eosinophils in the peripheral blood ($p < 0.001$).

Conversely, there was no significant difference found in sex, age, comorbid allergic rhinitis, or smoking history. The severity of disease showed no significant difference between recurrence and recurrence-free groups when measured either by symptoms (including nasal obstruction, rhinorrhea, facial pain, and olfactory disorder), by Lund-Mackay scores based on CT scans, or by preoperative NPSS values. In addition, the percentage of neutrophils in peripheral blood was not significantly different between the 2 groups.

Histological Evaluations and Laboratory Data of Patients

On histological evaluation, the recurrence group displayed a higher percentage of tissue eosinophils ($p < 0.001$, Fig. 1a) and lower percentages of neutrophils ($p < 0.001$) (data not shown) compared to the recurrence-free group. The average CLC protein concentration in nasal secretions was significantly higher in the recurrence group (median: 74.05 ng/mL, IQR: 50.15–129.4 ng/mL) than in the recurrence-free group (median: 4.06 ng/mL, IQR: 0.3–28.51 ng/mL; $p < 0.001$; Fig. 1b).

Determination of the Predictive Parameters for CRSwNP Recurrence

All metrics found to be significantly different based on between-group comparison analysis, including comorbid

Table 2. Binary logistic regression analysis to determine risk factors for recurrence

Parameters	OR	95% CI	*p* value
CLC protein concentration in nasal secretions	1.039	1.011–1.067	0.006
Eosinophils% in polyp tissue	1.061	1.023–1.101	0.002

OR, odds ratio; CI, confidence interval; CLC, Charcot-Leyden crystal.

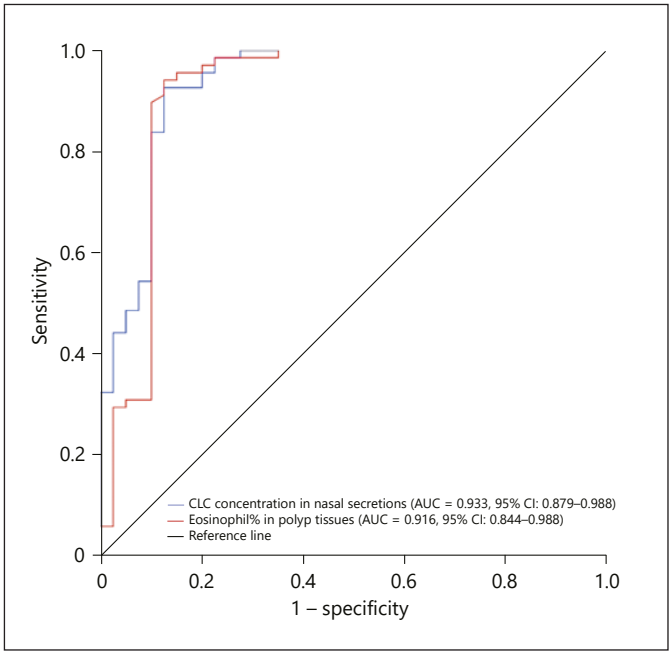

Fig. 2. Receiver operating characteristic (ROC) curves of the Charcot-Leyden crystal (CLC) protein concentration in nasal secretions (blue line) and percentage of eosinophils in polyp tissue samples (red line). The area under the curve (AUC) for CLC protein concentration in nasal secretions was 0.933 and the AUC for the percentage of eosinophils in the polyp tissue samples was 0.916.

Color version available online

Table 3. Five highest Youden indexes and determination of the optimal cutoff value for each marker

Cutoff value	Sensitivity	Specificity	Youden index
CLC protein concentration in nasal secretions, ng/mL			
34.243	0.926	0.875	0.801
35.359	0.912	0.875	0.787
33.863	0.926	0.850	0.776
36.804	0.897	0.875	0.772
28.700	0.985	0.775	0.760
Eosinophils% in polyp tissues			
26.125	0.941	0.875	0.816
24.400	0.956	0.850	0.806
27.370	0.926	0.875	0.801
29.315	0.897	0.900	0.797
24.700	0.941	0.850	0.791

asthma, comorbid atopy, the E/M ratio, percentage of eosinophils in peripheral blood, percentage of tissue eosinophils and neutrophils, and the CLC protein concentration in nasal secretions were entered into the binary logistic regression model. Only the CLC protein concentration in nasal secretions (p = 0.006, odds ratio 1.039, 95% CI: 1.011–1.067) and the percentage of eosinophils in polyp tissue samples (p = 0.002, odds ratio 1.061, 95% CI: 1.023–1.101) were identified as potential predictors of postoperative recurrence in patients with CRSwNP (Table 2).

Evaluation of the Predictive Significance of Parameters for CRSwNP Recurrence

To analyze the predictive significance of the 2 parameters for the recurrence of CRSwNP, ROC curves were generated and are shown in Figure 2. The area under the curves (AUCs) for CLC protein concentration in nasal secretions and percentage of tissue eosinophils were 0.933 (95% CI: 0.879–0.988) and 0.916 (95% CI: 0.844–0.988), respectively, indicating that both the CLC protein concentration in nasal secretions and the percentages of eosinophils in polyp tissues presented high predictive values for recurrence. A comparison of ROC curves for the 2 variables further indicated that there was no significant difference between the AUCs for CLC protein concentrations and percentages of tissue eosinophils (p = 0.665).

The optimal cutoff point for each parameter was identified by the maximal Youden index (sensitivity + specificity − 1), and the 5 highest Youden indexes for each parameter were demonstrated in Table 3 in descending order. Results demonstrated that the optimal cutoff value for CLC protein concentration in nasal secretions was 34.243 ng/mL (with Youden index = 0.801, sensitivity = 92.6%, specificity = 87.5%, positive predictive value = 92.6%, negative predictive value = 87.5%) and the optimal cutoff value for percentages of eosinophils in polyp tissues was 26.125% (with Youden index = 0.816, sensitivity = 94.1%, specificity = 87.5%, positive predictive value = 91.3%, negative predictive value = 87.2%).

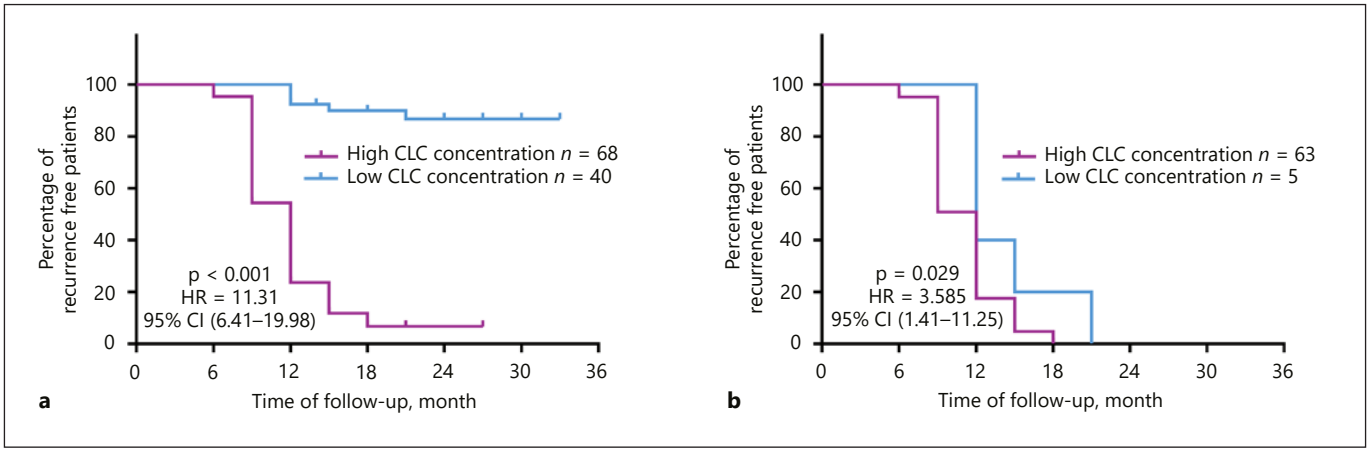

Fig. 3. Survival analysis of patients with different Charcot-Leyden crystal (CLC) protein concentrations. **a** Kaplan-Meier curve of all eligible individuals (n = 108) with CLC protein concentration higher than cutoff value (purple line, n = 68) and with CLC protein concentration lower than cutoff value (blue line, n = 40). **b** Kaplan-Meier curve of patients from recurrence group only (n = 68) with CLC protein concentration higher than cutoff value (purple line, n = 63) and with CLC protein concentration lower than cutoff value (blue line, n = 5).

Table 4. Demographic, clinical characteristics, and histological evaluations of patients with high and low CLC protein concentrations

	High CLC protein concentration (n = 68)	Low CLC protein concentration (n = 40)	p value
Sex (male/female)	40/28	29/11	0.213
Age, years (mean±SD)	43.78±12.62	45.57±11.01	0.514
Asthma (Y/N)	32/36	10/30	0.026
Allergic rhinitis (Y/N)	22/46	7/33	0.117
Atopy (Y/N)	44/24	17/23	0.029
Smoking history (Y/N)	14/54	5/35	0.433
Recurrence (Y/N)	57/11	11/29	<0.001
Nasal obstruction (median; IQR)	7.0; 6.0–8.0	7.0; 5.3–8.0	0.787
Rhinorrhea (median; IQR)	5.5; 4.0–7.0	5.0; 3.0–6.8	0.134
Facial pain/headache (median; IQR)	0; 0–3.0	0; 0–4.0	0.919
Olfactory disorder (median; IQR)	5.0; 4.0–10.0	4.0; 3.3–8.0	0.135
Lund-Mackay score (median; IQR)	19.0; 16.0–22.0	17.0; 14.0–20.8	0.055
Lund-Mackay score E/M ratio (median; IQR)	2.2; 2.0–3.5	2.0; 1.6–3.0	0.124
Nasal polyp size score (median; IQR)	5.0; 4.0–6.0	5.0; 4.0–6.0	0.897
Eosinophils% (peripheral blood) (median; IQR)	5.85; 4.00–8.38	4.55; 1.83–6.73	0.009
Neutrophils% (peripheral blood) (median; IQR)	54.85; 49.03–61.20	56.35; 49.38–59.68	0.858

IQR, interquartile range; E/M ratio, total ethmoid/maxillary sinus score ratio; CLC, Charcot-Leyden crystal.

Difference between Patients with Different CLC Protein Concentrations

Additional analysis (Table 4) of the CLC protein concentrations indicated that patients with a CLC protein concentration <34.243 ng/mL (n = 40) showed significantly greater differences in their outcome of recurrence than their counterparts with CLC protein concentrations higher than the cutoff value (n = 68; p < 0.001). Survival analysis further revealed an increasing risk of recurrence in patients with higher CLC protein concentrations both when comparing all patients (n = 108, p < 0.001, HR = 11.31, 95% CI: 6.41–19.98; Fig. 3a) and comparing only the individuals with recurrence (n = 68, p = 0.029, HR = 3.585, 95% CI: 1.14–11.25; Fig. 3b). Patients with a higher CLC protein concentration had higher rates of comorbid asthma (p = 0.026) and atopy (p = 0.029). Moreover, pa-

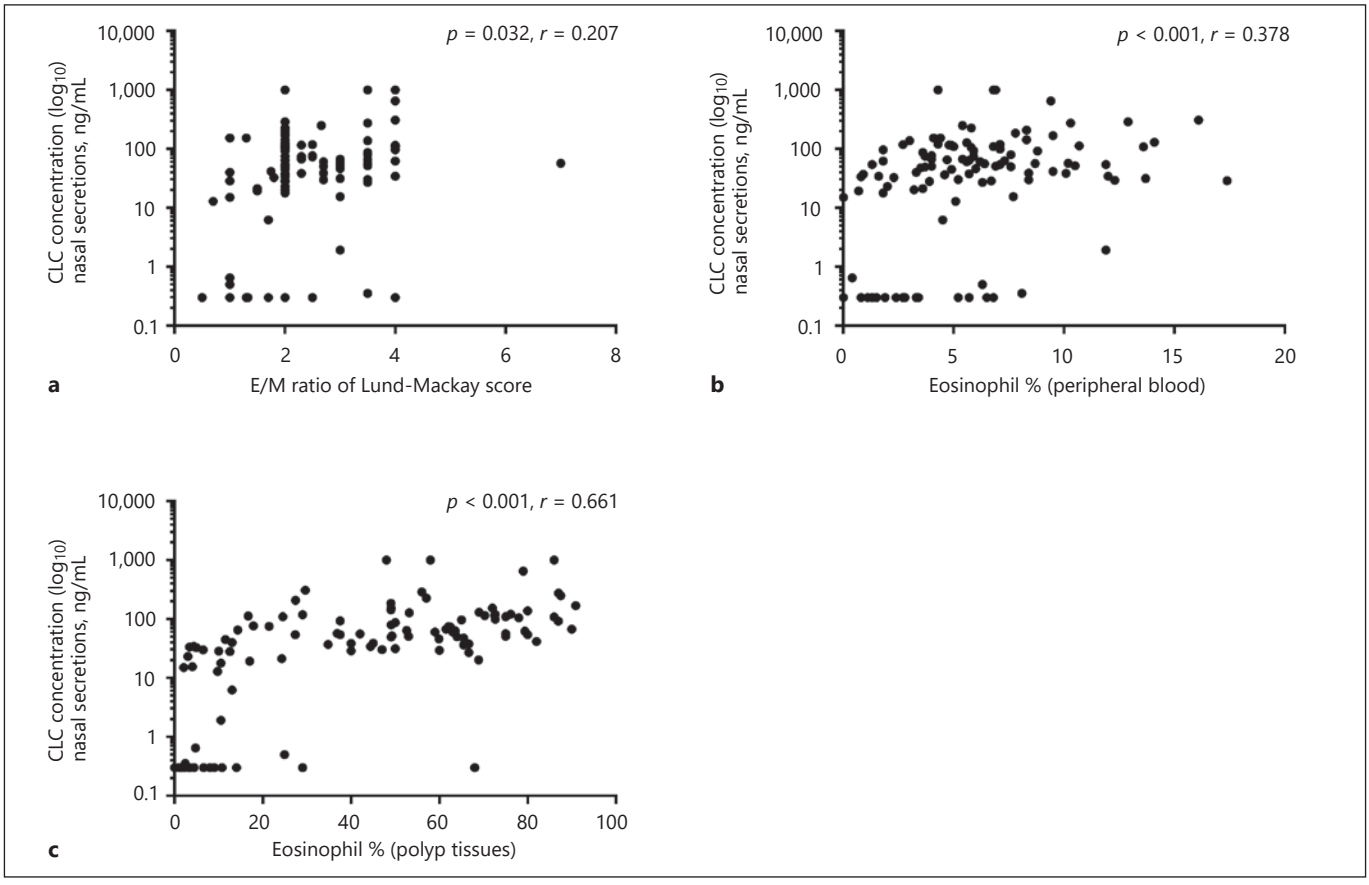

Fig. 4. Correlations between the Charcot-Leyden crystal (CLC) protein concentration in nasal secretions and other parameters. **a** CLC protein concentration in nasal secretions and the E/M ratio. **b** CLC protein concentration in nasal secretions and percentage of eosinophils in peripheral blood. **c** CLC protein concentration in nasal secretions and percentage of eosinophils in polyp tissues.

tients with a higher CLC protein concentration in nasal secretions showed higher level of eosinophils in both peripheral blood ($p = 0.009$) and polyp tissue samples ($p < 0.001$) than those with a lower CLC protein concentration. The infiltration of neutrophils ($p < 0.001$) was significantly lower in the polyp tissue samples of patients with a higher CLC protein concentration than those with a lower CLC protein concentration.

In contrast, the CLC protein concentration in nasal secretions was not significantly associated with sex, age, smoking history, or comorbid allergic rhinitis. Similarly, there was no significant association of the CLC protein concentration with any of the 4 subjective symptoms (nasal obstruction, rhinorrhea, facial pain/headache, and loss of smell), or the NPSS values, Lund-Mackay scores, or E/M ratio of Lund-Mackay scores for paranasal sinuses. In addition, there was no significant difference in the percentage of neutrophils in peripheral blood between the 2 groups.

Correlation between CLC Protein Concentrations in Nasal Secretions and Other Parameters

By generating a Spearman's rank correlation coefficient for all the eligible patients ($n = 108$), we found that the CLC protein concentration in nasal secretions was not correlated with sex, age, smoking history, comorbid allergic rhinitis, the 4 symptoms (nasal obstruction, rhinorrhea, facial pain/headache, and olfactory disorder), the CT Lund-Mackay score, NPSS value, or percentage of neutrophils in peripheral blood. However, CLC protein concentration was positively correlated with the E/M ratio ($p = 0.032$, $R = 0.207$; Fig. 4a) and the percentage of eosinophils in peripheral blood ($p < 0.001$, $R = 0.378$; Fig. 4b) and in polyp tissue samples ($p < 0.001$, $R = 0.661$; Fig. 4c). Conversely, the CLC protein concentration was negatively correlated with the percentage of neutrophils ($p < 0.001$, $R = -0.408$).

Discussion

In recent studies, CRSwNP has been further classified into eosinophilic CRSwNP and non-eosinophilic CRSwNP, according to the percentages of infiltrating eosinophils [3, 15, 17, 40]. Further investigations have revealed that a Th2-biased inflammation pattern underlying the eosinophilic infiltration is predominant among the Caucasian CRSwNP population in Western countries, whereas over 50% of CRSwNP patients in the Eastern countries present a Th1/Th17-skewed inflammation involving non-eosinophilic accumulation [20, 49–53]. CRSwNP still has a high propensity for recurrence regardless of significant improvement being facilitated by ESS intervention [13, 54–56]. Previous studies have reported that better surgical interventions or the long-term application of postoperative intranasal GCs may prevent or delay recurrence [7, 9, 13, 57]. To date, novel parameters and biomarkers, in addition to tissue and peripheral blood eosinophil counts and percentages, have been proposed as predictive factors for recurrence and are worth further investigating, including CLC [33, 34, 41, 58–61].

Previously, we have reported that CLC protein concentration in nasal secretions collected using a noninvasive approach could predict the response to oral GC therapy [35]. While the previous study investigated the association with patients' elevated concentrations of CLC protein in nasal secretion and response to GC therapy, this current study including secondary analysis of some of the same initial patient data also demonstrated an association between higher CLC protein concentration and postoperative recurrence. In this study, we have further elucidated the value of CLC protein in nasal secretions for the evaluation of CRSwNP, where CLC protein concentrations were significantly higher in patients with polyp recurrence compared with patients without recurrence. These findings were in favor of our previous publication, which was in search of the predictive value of CLC for polyp recurrence by another noninvasive approach [34]. These 2 studies demonstrated a novel capacity of CLC for recurrence prediction both in mRNA and secretive protein levels by noninvasive methods, suggesting a promising future for further research. Briefly, the studies on a protein level, including our present study and previous publication, indicate a potential application in assistance of quick screening for patients with high risk of recurrence by further developments of commercial colloidal gold kits, whereas the PCR-based genetic investigations facilitate the exploration of multiple targets especially for CRSwNP with Th1/Th2/Th17 mixed inflammation types [33–35].

Moreover, the accuracy and predictive significance of nasal CLC protein concentrations were as high as those of the percentage of eosinophils in polyp tissues. Furthermore, our findings on the predictive significance of tissue eosinophils are in accordance with previous studies that report tissue eosinophils as an important factor for refractory nasal polyps [8, 10, 12, 15]. The CLC protein concentrations in nasal secretions showed a similar capacity to the eosinophils in polyp tissues for predicting postoperative recurrence for CRSwNP. In addition to the positive correlation between CLC protein concentrations in nasal secretions and the percentage of eosinophils in polyp tissue demonstrated in our study, the detection of CLC protein concentrations in nasal secretions provides an alternative evaluative approach for postoperative outcomes with several advantages. It is a noninvasive sampling approach that can be proceeded in outpatient service during clinical practice as well as a quantitative method with an automatic readout. In this study, we used the Youden index to determine the most statistically optimal cutoff values (Table 3; Fig. 1). There were alternative methods to obtain different cutoff values to satisfy clinical practice. For instance, the cutoff value could be 24.98 ng/mL, which was determined by identifying the point on the ROC curve closest to the upper right hand corner for the highest sensitivity (with Youden index = 0.725, sensitivity = 100.0%, and specificity = 72.5%).

In our study, the associations between CLC protein concentration and demographics, as well as the clinical characteristics of CRSwNP patients were further investigated. We found that CLC protein concentrations were positively correlated with percentages of eosinophils in both polyp tissue and peripheral blood and that patients with high CLC protein concentrations had a higher state of tissue and peripheral blood eosinophilia, which was consistent with our previous study on CLC mRNA expression level in polyp tissue and tissue eosinophilia as well as a previous study about CLC proteins in sputum and its correlation with sputum eosinophilia [23, 33]. Thus, these studies indicate that CLC proteins are closely related to the eosinophilic and Th2 inflammatory processes in airway diseases [23, 30, 32]. Furthermore, previous studies including ours suggested that eosinophilic CRSwNP has a higher recurrence rate compared to CRSwNP with non-eosinophilic infiltration [7, 9, 10, 12, 22, 56]. And our study demonstrated a positive correlation between CLC protein level and eosinophilia in polyp tissue in patients with CRSwNP, supporting our hypothesis that CLC protein might be associated with polyp postoperative outcome and is likely to be a reliable predictor for the recurrence of CRSwNP.

In addition, survival analysis performed in all eligible individuals demonstrated that a higher CLC protein level in nasal secretions indicated a higher risk of recurrence when experiencing the same postoperative treating strategy. Moreover, an earlier onset of recurrence was found in the high CLC protein individuals when drafting the Kaplan-Meier curve only for patients with recurrence, suggesting that patients with high CLC protein levels needed stronger medical intervention to reverse or postpone the recurrence. According to our previous study, a more radical ESS procedure and/or additional systemic GC therapy might be a considerable option during postoperative treatment [13, 35]. However, a high CLC protein level is still an indicator of strong eosinophilic inflammation pattern, and recurrence in high CLC protein population might be inevitable in longer terms of follow-up.

The current study was slightly limited by the sample size and the relatively short follow-up period. In addition, this study was conducted in Chinese patients whose inflammation background does not represent the global population. Thus, a multicenter study with a larger sample size and an extended follow-up period is needed in the future to confirm the findings of the present study.

In summary, CLC protein concentrations in nasal secretions were higher in CRSwNP patients with postoperative recurrence and positively correlated with the percentage of eosinophils in both polyp tissue and peripheral blood. CLC protein concentrations can be collected and detected noninvasively; therefore, it is determined to be an effective predictor for polyp recurrence.

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Statement of Ethics

The study protocol was approved by the Medical Ethics Committee of Beijing TongRen Hospital, and all subjects provided written informed consent prior to participation in the study and collection of any data.

Conflict of Interest Statement

The authors declare no conflicts of interest.

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Author Contributions

All authors participated in the drafting/revision of the manuscript, final approval of the manuscript, and interpretation of findings. D.W. and B.Y. contributed to most of the experimental management, including study design, biochemical techniques, data analysis, and manuscript drafting. Y.W. participated in parts of experimental procedures. L.Z. and C.W. were involved in the design of the study, enrollment of the patients, and revision of the manuscript. All authors agree to be accountable for all aspects of the work.

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