International Archives of Allergy and Immunology

Clinical Immunology – Research Article

Int Arch Allergy Immunol 2020;181:888–896
DOI: 10.1159/000509252

Received: February 17, 2020
Accepted: June 8, 2020
Published online: July 21, 2020

Charcot-Leyden Crystal Protein in Nasal Secretions of Patients with Nonallergic Rhinitis with Eosinophilia Syndrome

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Keywords

Biomarker · Charcot-Leyden crystal protein/galectin-10 · Eosinophilia · Nasal secretions · Nonallergic rhinitis with eosinophilia syndrome · Symptoms score

Abstract

Introduction: Charcot-Leyden crystal (CLC) protein has been regarded as a hallmark of eosinophilic inflammation. ***Objective:*** The purpose of this study was to investigate the role and levels of CLC protein in patients with nonallergic rhinitis with eosinophilia syndrome (NARES). ***Methods:*** Overall, 39 NARES patients and 19 controls were recruited. The severity of nasal symptoms was measured by visual analogue scale and serum and local specific immunoglobulin E were determined in all patients. Nasal eosinophilia was assessed by semiquantitative analysis of eosinophils in nasal scrapings. Nasal secretion CLC protein concentrations were evaluated by ELISA. ***Results:*** CLC protein concentrations were significantly higher in NARES patients than in controls ($p < 0.0001$). Nasal secretion CLC protein levels were significantly correlated with the degree of eosinophilia in nasal scrapings ($r_s = 0.331$; $p = 0.04$) in NARES patients. Patients with high CLC protein concentrations displayed more severe nasal symptoms than patients with low CLC protein concentrations ($p = 0.0080$), particularly, nasal itching ($p = 0.0029$). Pilot study in 8 NARES patients demonstrated that treatment for 1 month with intranasal fluticasone propionate significantly decreased the nasal secretion CLC protein concentrations from baseline levels ($p = 0.0335$) and markedly attenuated the degree of swelling of inferior turbinate. ***Conclusions:*** CLC protein levels are significantly higher in nasal secretions of NARES patients and associated with the degree of nasal eosinophilia and the severity of nasal symptoms. Significantly, nasal secretion CLC protein levels obviously decreased after treatment with intranasal corticosteroids, suggesting its possible role in evaluating the medical treatment.

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

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Introduction

Chronic rhinitis (CR) with high morbidity is associated with a series of adverse effects such as declined quality of life, sleep disorders, psychological problems, and decreased productivity [1, 2]. Chinese chronic rhinitis patients can be clustered into different endotypes, including allergic rhinitis (AR), nonallergic rhinitis with eosinophilia syndrome (NARES), local AR, and idiopathic rhinitis, which differ in terms of inflammatory patterns [3]. NARES is classified on the basis of positive findings for local eosinophils and negative findings for both local and serum specific immunoglobulin E (sIgE) with common rhinitis symptoms [3]. Furthermore, NARES is mainly characterized by upper airway hyperresponsiveness [4].

The underlying mechanism of NARES is a hypersensitive response when the nasal mucosa is exposed to nonallergic stimuli. A chronic self-perpetuating eosinophilic infiltration with the nonspecific release of histamine has hitherto been thought to be a key component of this disease [5]. Indeed, it has been reported that NARES might be a precursor to nasal polyps, asthma, and aspirin-exacerbated respiratory disease [6]. Thus, early identification and treatment of NARES may play an important role in preventing progression and development of further respiratory disease. However, to date, the number of biomarkers for NARES is extremely limited, and identification of suitable biomarker/s for characterization of NARES is urgently warranted.

Charcot-Leyden crystal (CLC) protein, also known as galectin-10, is the 10th member of the galectin superfamily of S-type lectins. CLC protein is abundant in the cytoplasm of eosinophils [7, 8] and can be crystallized during cytolytic extracellular trap cell death [9–11], before being released by activated eosinophils. CLC protein has been described for over 150 years in tissue, body fluids, and secretions of patients with a variety of eosinophilic lesions, including asthma [12], AR [13, 14], chronic rhinosinusitis (CRS) [15, 16], and other eosinophil-associated diseases [17], and regarded as a hallmark of eosinophilic inflammation. Also, CLCs are present in CRS and asthma patients [10]. One recent study has reported that antibodies directed against key epitopes of the CLC crystallization interface rapidly dissolved pre-existing crystallized CLC protein in patient-derived mucus and also suppressed CLC-mediated airway inflammation, goblet cell metaplasia, bronchial hyperactivity, and IgE synthesis in a humanized mouse model of asthma [10].

To date, the presence and role of CLC protein in nasal secretions in patients with NARES have not been investigated. The purpose of this study was therefore to measure the levels of CLC protein in nasal secretions from patients with NARES and investigate the relationship between CLC protein, eosinophilia in nasal scrapings, and nasal symptoms. Furthermore, we compared the CLC protein concentrations in nasal secretions before and after treatment for 1 month with intranasal fluticasone propionate (FP) to investigate the potential of CLC protein as an indicator of the efficacy of intranasal corticosteroids for NARES.

Materials and Methods

Study Design and Subjects

Thirty-nine patients with NARES, defined according to Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines [18], were recruited consecutively from the Outpatient Department at Beijing TongRen Hospital, Beijing, China, from November 2018 to August 2019. In brief, these patients presented with nasal eosinophilia and persistent rhinitis symptoms, like sneezing, itching, rhinorrhoea, and occasional loss of the sense of smell, in the absence of demonstrable allergy and were diagnosed as suffering from NARES [18]. Patients were excluded from the study if they presented with CRS and/or nasal polyposis, as defined by the European Position Paper on Rhinosinusitis and Nasal Polyps [19], opacification in the nasal cavity or sinuses on the CT scan, or any chronic pulmonary disease (e.g., chronic bronchitis and chronic obstructive pulmonary disease). Similarly, patients who had used systematic corticosteroids within 3 months or nasal/inhaled corticosteroids or other medications (e.g., antibiotics, antihistamines, and antileukotrienes) within 4 weeks before sample collection, current smokers and former smokers who had ceased smoking within 1 year before study commencement, and pregnant women were also excluded. However, 19 healthy subjects with no nasal diseases and with negative allergy tests were enrolled as controls.

Demographic and clinical characteristics including age, gender, history of smoking, comorbid asthma, and nasal symptoms were recorded, and serum and local sIgE, local eosinophilia, and CLC protein concentrations in nasal secretions were determined for all subjects, as described below. The diagnosis of asthma was based on the criteria outlined in the Global Initiative for Asthma 2018 guideline [20]. Sensitization to common aeroallergens was confirmed by immunoassay of both serum and nasal secretion sIgE. Each NARES patient was treated with FP (GlaxoSmithKline, S.A., Madrid, Spain) 50 μg per spray twice daily (total 200 μg FP per day) for 1 month. In order to investigate the effect of intranasal FP treatment on CLC protein levels, nasal secretions were also collected from 8 of the 39 NARES patients before and after treatment for 1 month. 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.

Assessment of Symptoms

All subjects scored the severity of nasal symptoms, including nasal obstruction, rhinorrhoea, sneezing, and nasal itching, according to a visual analogue scale (VAS) of 10 cm. Each symptom was classified as mild (VAS: 0–3 cm), moderate (VAS: >3–7 cm), or severe (VAS: >7 cm).

Table 1. Demographic and clinical characteristics of NARES patients and control subjects

	NARES (n = 39)	Controls (n = 19)	p value
Gender (male)	20 (51.28%)	5 (26.32%)	0.0938
Age, years (mean ± SD)	34.78±10.90	33.53±7.33	0.6287
Asthma history	4 (10.26%)	0 (0.00%)	0.2921
Smoking history	7 (17.95%)	0 (0.00%)	0.0835
Serum sIgE for *Der p*, kUA/L (median ± IQR)	0.13±0.10	0.11±0.16	0.3985
Serum sIgE for *Der f*, kUA/L (median ± IQR)	0.09±0.07	0.10±0.12	0.5784
Serum sIgE for phad, kUA/L (median ± IQR)	0.08±0.06	0.06±0.12	0.3916
Local sIgE for *Der p*, kUA/L (median ± IQR)	0.10±0.09	0.09±0.13	0.1787
Local sIgE for *Der f*, kUA/L (median ± IQR)	0.11±0.13	0.08±0.15	0.3940
Local sIgE for phad, kUA/L (median ± IQR)	0.08±0.07	0.07±0.17	0.6472

NARES, nonallergic rhinitis with eosinophilia syndrome; sIgE, specific immunoglobulin E; local sIgE, nasal secretion sIgE; *Der p*, *Dermatophagoides pteronyssinus*; *Der f*, *Dermatophagoides farina*; phad, phadiatop, phadiatop contains 95% common inhaled allergens, such as cat dander, horse dander, dog dander, timothy grass, *Cladosporium herbarum,* common silver birch, olive, mugwort, wall pellitory, etc.; IQR, interquartile range.

Assessment of the Degree of Eosinophilia in Nasal Mucosa

Nasal mucosal specimens were obtained by scraping the surface in the medial aspect of the inferior turbinate of each nostril and processed for microscopic examination according to a standard procedure [21]. The degree of eosinophilia in each sample was evaluated by the same investigator, who was also blinded to the patients' details [22]. Eosinophils in each nasal sample were scored on a 5-point scale of 0 (none), 1 (few, scattered), 2 (moderate number), 3 (large clumps of cells that do not cover the entire field), and 4 (clumps of cells covering the entire field). A score ranging from 1 to 4 was classified as nasal eosinophilia [22].

sIgE Measurement

Serum and local sIgE levels to common aeroallergens were measured by a fluoroenzyme immunosorbent assay (UniCAP®, Uppsala, Sweden), using a panel of aeroallergens including house dust mites, fungi, trees, weed, and grass pollens, and animal dander. These allergens were selected on the basis of mites and mugwort being the 2 most prevalent aeroallergens in China [23], and a value of sIgE ≥0.35 kUA/L was considered as a positive result [24].

Nasal Secretion Collection

Nasal secretions were obtained bilaterally from each patient and processed as described by Watelet and colleagues [25]. Briefly, nasal secretions were collected with a sponge pack placed into each nostril for 5 min. On recovery of the sponge packs, 500 μL of 0.9% sodium chloride was added to each pack, and the packs were incubated at 4°C for 2–24 h. At the end of incubation, each pack was transferred into 5-mL BD syringe barrel (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) and centrifuged at 1,500 *g* for 15 min at 4°C. At the end of centrifugation, the supernatants were collected and stored in aliquots at −80°C until analysis for local sIgE, as for serum sIgE, and for CLC protein levels, by ELISA.

CLC Protein Concentrations in Nasal Secretions

All samples were diluted 25-fold with 0.9% normal saline before the assay. CLC protein concentrations in nasal secretions were quantified using commercial ELISA kits, with a detection range of 0.312–20 ng/mL CLC protein (Cloud-Clone Corp, Wuhan, China), following the manufacturer's instructions. This kit measures total CLC protein/galectin-10, including crystallized and soluble non-crystallized states. CLC protein concentration in each sample was normalized by total protein concentration in nasal secretions, which was measured using a commercial Enhanced BCA Protein Assay Kit (Beyotime Biotechnology, Nanjing, China). For samples with undetectable CLC protein concentrations, data were calculated as the lower limit of detection of the ELISA kit.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism 8 software (GraphPad Software, La Jolla, CA, USA) and SPSS software (Version 25.0; IBM, Armonk, NY, USA). Continuous variables were expressed as mean ± SD for normally distributed data and as the median and interquartile range (IQR) for non-normally distributed data. Comparisons of continuous variables between 2 groups were performed by Student's t test or Mann-Whitney U test according to data distribution and homogeneity of variance. The χ^2 test was used to analyse categorical variables. The linear-by-linear association test was used to analyse the linear correlation between ordinal categorical variables and binary variables. Spearman's rank correlation analysis was employed to assess the strength of the correlation between the 2 parameters. Correlation coefficient values greater than or equal to 0.70 were considered to represent a strong correlation, between 0.50 and 0.69 a moderate correlation, and between 0.30 and 0.49 a weak correlation. Matched data between baseline and after therapy were analysed by paired t test. A 2-tailed p value <0.05 was considered statistically significant.

Results

Demographic and Clinical Characteristics of Subjects

The demographic and clinical characteristics of control subjects and patients with NARES are shown in Table 1.

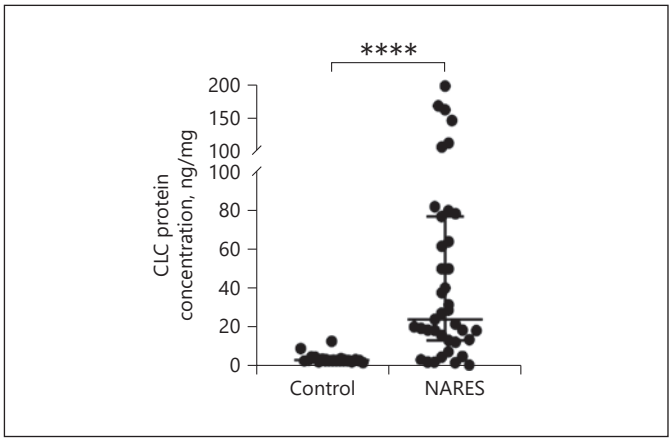

Fig. 1. CLC protein concentrations in nasal secretions of patients with NARES ($n = 39$) and control subjects ($n = 19$). CLC, Charcot-Leyden crystal; NARES, nonallergic rhinitis with eosinophilia syndrome. ****$p < 0.0001$.

There were no significant differences in the ratio of males to females (20/19 vs. 5/14, $p = 0.0938$), age distribution (mean ± SD, 34.87 ± 10.90 vs. 33.53 ± 7.33 years, $p = 0.6287$), history of asthma (4/39 vs. 0/19, $p = 0.2921$), history of smoking (7/39 vs. 0/19, $p = 0.0835$), and serum and nasal secretion sIgE for *Dermatophagoides pteronyssinus*, *Dermatophagoides farina,* and phadiatop between the 2 groups.

The Levels of CLC Protein in Nasal Secretions of NARES Patients and Controls

CLC protein concentration in nasal secretions from patients with NARES was significantly higher than that in nasal secretions from control subjects (median ± IQR, 24.10 ± 63.71 vs. 3.08 ± 1.46 ng/mg, $p < 0.0001$) (Fig. 1).

Correlation between Nasal Secretion CLC Protein Levels and Nasal Eosinophilia

CLC protein concentrations in nasal secretions were defined as high or low, when the levels exceeded or were below the median value of 24.10 ng/mg, according to the method referenced by Guéguen and colleagues [26]. Analysis of the demographic and clinical data for the patients in low- and high-CLC protein concentration groups also indicated no significant differences between these 2 groups, with respect to the ratio of males to females (8/12 vs. 11/8, $p = 0.3431$), age distribution (mean ± SD, 35.75 ± 11.93 vs. 33.95 ± 9.94 years, $p = 0.6122$), history of asthma (1/20 vs. 3/19, $p = 0.3416$), and history of smoking (2/20 vs. 5/19, $p = 0.2351$).

Linear-by-linear association test and Spearman's test were performed to analyse whether there was a linear correlation between low-high CLC protein concentration and the degree of eosinophilia in nasal scrapings, and then to assess the strength of the correlation. Linear-by-linear association test showed a significant trend in high degree of nasal eosinophilia from low- to high-CLC protein concentration group ($p = 0.035$) (Fig. 2a), and Spearman's test showed a weak correlation between low-high CLC protein concentration and the degree of nasal eosinophilia ($r_s = 0.331$, $p = 0.040$) (Fig. 2a). Comparison of the eosinophil scores between low- and high-CLC protein groups further demonstrated that the eosinophil score was higher in high-CLC protein group than in low-CLC protein group (median ± IQR, 3 ± 1 vs. 2 ± 1, $p = 0.0148$) (Fig. 2b).

Association between CLC Protein Levels in Nasal Secretions and Nasal Symptoms

We further investigated the relationship between CLC protein concentration in nasal secretions and the severity of nasal symptoms. Patients with high CLC protein concentrations displayed more severe nasal symptoms compared to patients with low CLC protein concentrations (median ± IQR, 23.00 ± 13.00 vs. 16.50 ± 4.75, $p = 0.0080$) (Fig. 3a). Assessment of the differences in individual symptoms between the low- and high-CLC protein concentration groups demonstrated that patients in high-CLC protein concentration group suffered more severe itching than in low-CLC protein concentration group (median ± IQR, 5.00 ± 3.00 vs. 2.00 ± 2.00, $p = 0.0029$) (Fig. 3b). However, no significant differences were noted in nasal obstruction (median ± IQR, 7.00 ± 3.00 vs. 6.00 ± 1.75, $p = 0.1361$) (Fig. 3c), rhinorrhoea (median ± IQR, 6.00 ± 4.00 vs. 5.00 ± 3.00, $p = 0.0529$) (Fig. 3d), or sneezing (median ± IQR, 5.00 ± 4.00 vs. 3.00 ± 2.00, $p = 0.0507$) (Fig. 3e).

Comparison of the frequency distribution of mild, moderate, or severe nasal symptoms in patients from high- and low-CLC protein concentration groups further demonstrated that there was a significant difference in frequency distribution of mild, moderate, and severe nasal itching for patients from high- and low-CLC protein concentration groups ($p = 0.018$) (Table 2). Patients in high-CLC protein concentration group had higher proportion of moderate (10/19 vs. 4/20) and severe (2/19 vs. 0/20) nasal itching and lower proportion of mild (7/19 vs. 16/20) itching than in low-CLC protein concentration group ($p = 0.018$). In contrast, no differences were found in frequency distribution of mild, moderate, and severe nasal obstruction ($p = 0.069$), rhinorrhoea ($p = 0.117$), or sneezing ($p = 0.096$) for patients from high- and low-CLC protein concentration groups (Table 2).

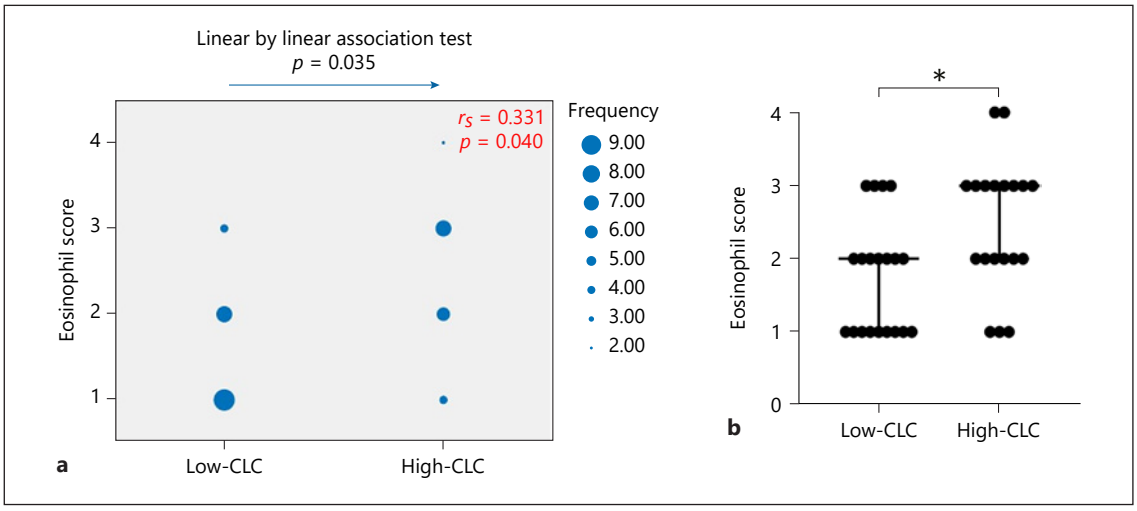

Fig. 2. a Scatter plot showing correlation between patients with low or high CLC protein concentrations and the degree of nasal eosinophilia in NARES. Linear relation was assessed using linear-by-linear association test, and correlation coefficient (r_s) was assessed by Spearman's rank correlation test. **b** Comparison of eosinophil score between patients with low ($n = 20$) and high ($n = 19$) CLC protein concentrations in nasal secretions. CLC, Charcot-Leyden crystal; High-CLC, high-CLC protein concentration group; Low-CLC, low-CLC protein concentration group; NARES, nonallergic rhinitis with eosinophilia syndrome. *$p < 0.05$.

Table 2. Comparison of the frequency distribution of mild, moderate, or severe nasal symptoms in patients with low and high concentrations of CLC protein in nasal secretions

Nasal symptom severity	Low-CLC ($n = 20$)	High-CLC ($n = 19$)	p value
Nasal obstruction			
Mild (0–3)	1	0	0.069
Moderate (>3–7)	18	13	
Severe (>7)	1	6	
Rhinorrhoea			
Mild (0–3)	7	3	0.117
Moderate (>3–7)	12	11	
Severe (>7)	1	5	
Sneezing			
Mild (0–3)	12	9	0.096
Moderate (>3–7)	8	6	
Severe (>7)	0	4	
Itching			
Mild (0–3)	16	7	**0.018***
Moderate (>3–7)	4	10	
Severe (>7)	0	2	

CLC, Charcot-Leyden crystal; High-CLC, high-CLC concentration group; Low-CLC, low-CLC concentration group; nasal symptom score (VAS 0–3 was categorized as "mild," VAS >3–7 was categorized as "moderate," and VAS >7 was categorized as "severe"). * $p < 0.05$ for a significant difference in frequency distribution of mild, moderate and severe nasal symptom for patients from high- and low-CLC protein concentration groups.

Effect of Treatment with Intranasal Corticosteroids on Nasal Secretion CLC Protein Levels

The effect of treatment with intranasal FP for 1 month on CLC protein levels in nasal secretions is demonstrated in Figure 4. Nasal secretions were obtained from 8 of the 39 patients with NARES before and after the treatment and used to assess the CLC protein. The CLC protein concentrations in nasal secretions of these patients were significantly decreased from baseline values after treatment with intranasal FP (mean ± SD, 61.09 ± 63.69 vs. 9.63 ± 10.86 ng/mg, $p = 0.0335$) (Fig. 4a). Similarly, nasal endoscopic examination of these patients demonstrated that the degree of oedema and swelling of inferior turbinate was markedly attenuated after treatment with intranasal FP for 1 month (Fig. 4b, c).

Discussion

Nonallergic rhinitis (NAR) can be subdivided into several phenotypes based on differentiating pathogenic mechanisms and clinical characteristics [27–29]. Several studies have reported that different inflammatory types affect disease severity and therapeutic reactions for patients with NAR [30–32]. NARES usually occurs perennially in middle-aged adults and may account for 13–33% of patients with NAR [6, 33]. NAR and elevated eosino-

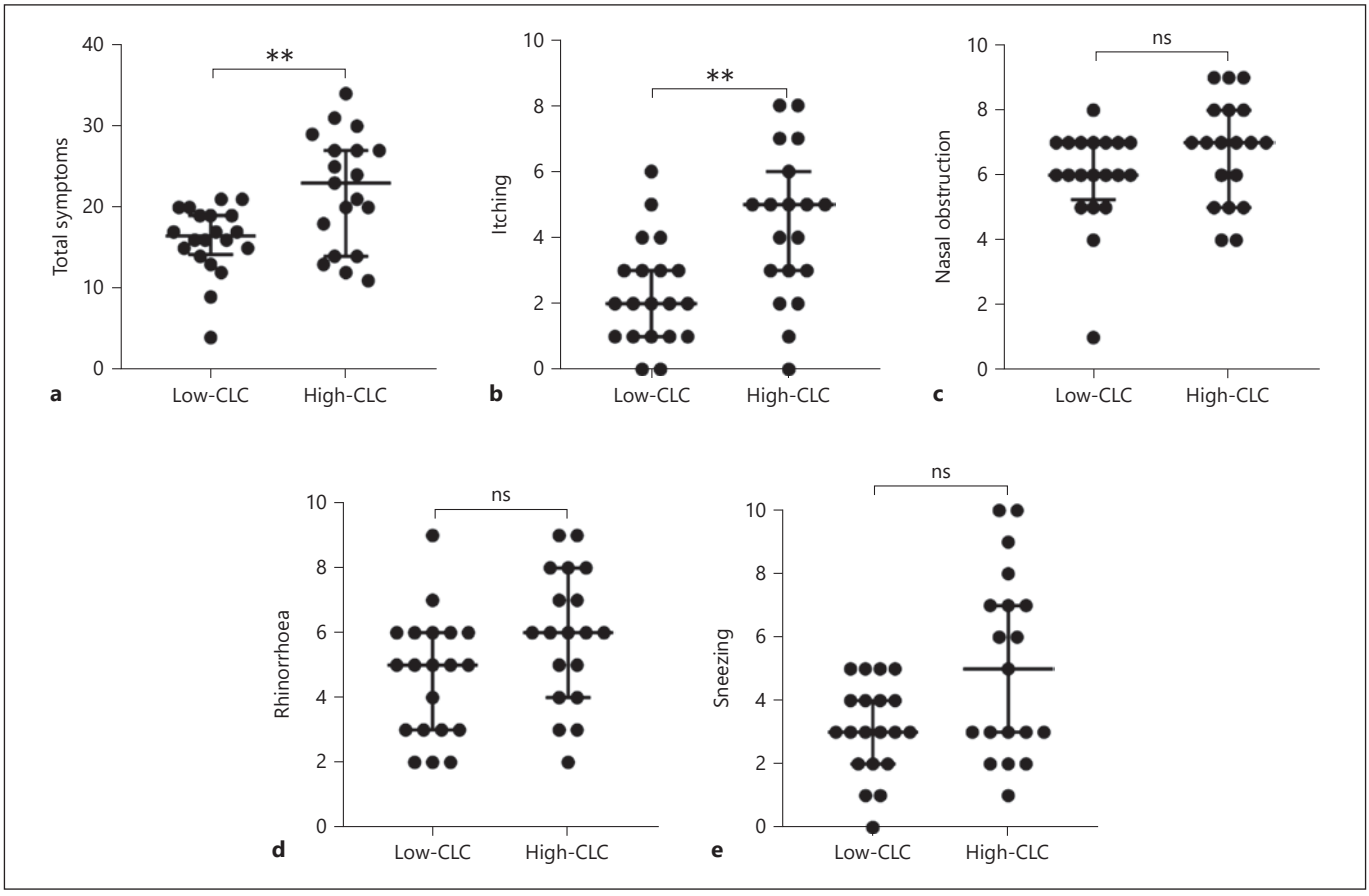

Fig. 3. Comparison of nasal symptom VAS scores between patients with low ($n = 20$) and high ($n = 19$) CLC protein concentrations in nasal secretions. Total symptom scores (**a**), itching (**b**), nasal obstruction (**c**), rhinorrhoea (**d**), and sneezing (**e**). CLC, Charcot-Leyden crystal; High-CLC, high-CLC protein concentration group; Low-CLC, low-CLC protein concentration group; VAS, visual analogue scale; ns, not significant. $^{**}p < 0.01$.

phils constituted a risk factor for chronic rhinosinusitis with nasal polyps (CRSwNP) [34]. NARES share clinical features with the ASA triad (nasal polyposis, intrinsic asthma, and intolerance to aspirin) and has been considered as an early expression of the triad [6]. In this regard, it is necessary to find a novel and reliable biomarker to identify NARES in the early phase in order to prevent disease progression.

Several studies have reported that CLC is one of the most abundant eosinophil proteins, which persists after eosinophil lysis and is thus viewed as a morphologic hallmark of eosinophilic diseases [7, 9, 10, 17]. Grozdanovic and colleagues [35] have recently reported that CLC protein interacted with both human eosinophil-derived neurotoxin and eosinophil cationic protein and served as a carrier for the sequestration and vesicular transport of the potent eosinophil granule cationic ribonucleases in the process of eosinophil granulogenesis. Previously, Calafat and colleagues [36] found that in eosinophils, CLC protein-containing granules contain also eosinophil peroxidase, suggesting a relationship between the CLC protein-containing organelle and the specific granule. What is more, it was reported that the levels of CLC protein paralleled those of eosinophil major basic proteins in tears from patients with vernal keratoconjunctivitis [37].

CLC induces the release of the proinflammatory cytokine interleukin (IL)-1β [38], involved in eosinophilic immune functions. Clinically, increased levels of CLC protein have been reported to be present in nasal secretions in AR [13, 14], sputum in asthma [12], and nasal lavage fluid in patients with the aspirin-sensitive respiratory disease [39]. More recently, we have demonstrated that CLC mRNA and protein might serve as a marker in the identification of eosinophilic CRSwNP [40] as well as

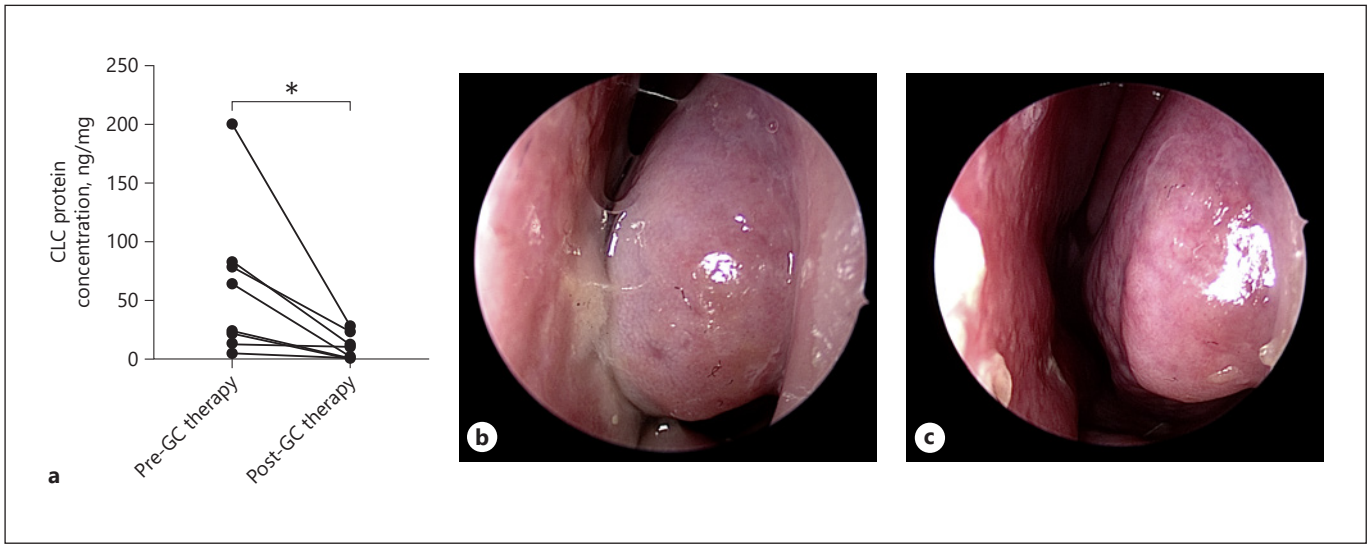

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Fig. 4. Effect of treatment for 1 month with intranasal fluticasone propionate (FP) on CLC protein concentrations in nasal secretions (**a**), and degree of oedema and swelling of inferior turbinate before (**b**) and after (**c**) treatment with intranasal FP. CLC, Charcot-Leyden crystal; GC, glucocorticoid. *$p < 0.05$.

a reliable and alternative method for the prediction of glucocorticoid response in CRSwNP patients [15] and nasal polyp recurrence [41].

Eosinophilia in nasal smears is a prominent and essential diagnostic feature of NARES in the absence of sIgE [3]. In the current study, we explored levels of CLC protein in nasal secretions and analysed their correlation with nasal eosinophilia in nasal scrapings of patients with NARES. Our study demonstrated that the levels of CLC protein were significantly increased in nasal secretions of patients with NARES compared to healthy control subjects and correlated with the degree of eosinophilia in nasal scrapings from these patients. This finding is in accordance with the findings of Nyenhuis and colleagues [12], who observed that CLC protein levels were also increased and correlated with sputum eosinophilia in asthma patients [12]. Thus, detection of CLC protein concentration in nasal secretions may serve as a useful non-invasive and surrogate method for nasal eosinophilia in NARES patients.

Our study also demonstrated that CLC protein concentrations in nasal secretions were associated with the severity of total symptoms score in patients with NARES. In particular, patients with high CLC protein concentrations suffered a more severe nasal itching problem, and there was a significant difference in the frequency distribution of mild, moderate, and severe nasal itching between patients with high and low CLC protein concentrations. In view of the findings from some studies that increased eosinophil infiltration leads to the release of neuromediators [42–44] and airway sensory nerve density [45], which result in itching [46] and scratching [45], we speculate that the eosinophil CLC protein may be involved in nasal itching in patients with NARES. However, the precise mechanism underlying eosinophil-mediated itching is still unclear and needs to be investigated in future studies.

Of note, a recent review summarized that long time and expensive eosinophil-targeted depletion therapies might lead to severe adverse effects, since distinct eosinophil subgroups were conferred different functions (immunological effector functions, tissue-protective and/or immunoregulatory functions) [47]. Thus, future studies should focus on specific predictors for the efficacy of anti-eosinophil treatments. Intranasal corticosteroids are considered as the mainstay of the medical treatment of NARES. Although we obtained nasal secretions from only 8 patients before and after treatment, the present study indicated that treatment with intranasal FP significantly decreased baseline CLC protein levels in these patients with NARES. As protein crystals, but not the soluble proteins, are powerful promoters of allergic inflammation and can be targeted with crystal-dissolving antibodies to reverse disease symptoms [10, 48], it is possible that FP might disrupt the crystalline structure of CLCs to promote its anti-inflammatory effects in NARES. Moreover, the preliminary findings for the effect of

intranasal FP on CLC protein levels in nasal secretions in the present study suggest that assessment of CLC protein in nasal secretions might be an effective and non-invasive method for assessing the effectiveness of the local response to corticosteroid treatment, similar to that assessed by measuring nasal eosinophilia. However, further clinical trials involving large populations are needed to verify these findings and the mechanisms underlying the influence of FP on CLC protein.

The main limitation of the current study is that we did not have a comparator group such as another NAR condition or even CRS, in which eosinophils are known to play a major pathophysiologic role, for estimation of CLC protein concentrations in nasal secretions from patients with these eosinophilic upper airway diseases. Similarly, the percentage and absolute numbers of peripheral blood eosinophils, which might have provided additional information on the correlation between the levels of CLC protein in nasal secretions and in peripheral blood eosinophils, were also not measured. As the sample size is relatively small, these findings also need to be confirmed in clinical trials comparing larger groups of patients, including NARES patients, other groups of NAR patients, AR patients, CRS patients, and control subjects.

In conclusion, the findings of the present study have indicated that levels of CLC protein are significantly higher in nasal secretions of patients with NARES compared to healthy control subjects. Our data also indicated that there is a significant association between the levels of CLC protein in nasal secretions and the degree of nasal eosinophilia and the severity of nasal symptoms, especially itching in patients with NARES. Furthermore, intranasal FP for 1 month decreased the levels of CLC protein in nasal secretions from these patients, suggesting a possible role for the level of nasal CLC protein in predicting the efficacy of intranasal steroid therapy in NARES.

Acknowledgement

The authors thank Na Meng for making contributions to scoring the degree of eosinophilia in nasal scrapings.

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 have no conflicts of interest to declare.

Funding Sources

This study was supported by grants from the National Natural Science Foundation of China (81870698, 81420108009, 81400444, 81470678, and 81630023), the National Key R&D Program of China (2018YFC0116801 and 2016YFC20160905200), the Program for Changjiang Scholars and Innovative Research Team (IRT13082), Beijing Municipal Administration of Hospitals' Innovation Program of Clinical Techniques (XMLX201816), the Priming Scientific Research Foundation for the Senior Researcher in Beijing TongRen Hospital, Capital Medical University (2017-YJJ-GGL-005), Beijing Municipal Administration of Hospitals Incubating Program (PX20190007), Beijing Natural Science Foundation (7194247), and Beijing Scientific and Technological Overall Plan (Z171100000117002).

Author Contributions

M.Z. and B.Y. performed assays, analysed the data, and prepared the manuscript. C.W. and L.Z. designed the study, recruited subjects, collected the samples, and revised the manuscript. All authors contributed to analysis of data and agreed with the final version of the article.

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