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ORIGINAL ARTICLE

Correlation between inflammatory biomarkers and disease control in chronic rhinosinusitis with nasal polyps

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Abstract

Background: Chronic rhinosinusitis with nasal polyps (CRSwNP) may have a heterogeneous response to medical/surgical treatments based on endotypes. Data correlating biomarkers and severity of the disease are lacking. We aimed to determine if IL-5 and calprotectin may be useful in defining severity of disease and identifying uncontrolled patients.

Methods: This was a case–control study including 81 patients with diffuse CRSwNP who underwent at least one previous surgery and treated with intranasal steroids. We enrolled 39 uncontrolled patients (SNOT-22 $\geq$ 40 and two or more cycles of systemic corticosteroids in last year) (Group A) and 42 controlled one (SNOT-22 < 40 and less than two cycles of systemic corticosteroids in last year) (Group B). We analyzed IL-5 and calprotectin in both nasal secretions and nasal polyp tissue.

Results: Calprotectin and IL-5 were significantly higher in Group A in both secretions and tissue, and the higher the number of previous surgeries, the higher the levels detected in nasal secretions. At univariate analyses, smoking, asthma, non-steroidal anti-inflammatory drugs-exacerbated respiratory disease (NSAID-ERD), blood eosinophilia, neutrophils, and eosinophils at nasal cytology were significantly associated with uncontrolled disease. Multivariate analyses showed that asthma, NSAID-ERD, and IL-5 in nasal secretion/polyp tissue were significantly related to the risk of uncontrolled disease.

Conclusions: Our data suggest that asthma, NSAID-ERD, and IL-5 in nasal secretions/tissue may be helpful to identify more severe patients, as they are related to the risk of uncontrolled disease. Nonetheless, high levels of

calprotectin and neutrophilia were also observed in uncontrolled patients, especially after multiple surgeries.

KEYWORDS
biomarker, chronic rhinosinusitis, endoscopic sinus surgery, eosinophilic rhinitis and nasal polyposis, SNOT-22

1 | INTRODUCTION

Chronic rhinosinusitis with nasal polyposis (CRSwNP) has complex pathophysiologic mechanisms that lead to heterogeneity in terms of severity of disease and response to treatments. For this reason, the spectrum of severity of the disease can range from easy-to-control forms to difficult-to-manage CRSwNP, in which despite appropriate medical and surgical treatment, patients cannot achieve adequate control of symptoms. Although the majority of CRSwNP patients can achieve control, those affected by severe disease will not, even with maximal systemic steroids and surgery. This group of patients has the poorest outcomes and quality of life, requiring high costs for management.[1,2] Functional endoscopic sinus surgery (FESS) is generally reported to be a safe and effective treatment for CRSwNP,[3] improving symptoms and quality of life. However, in severe cases patients may experience recurrence of symptoms and return to a state of uncontrolled disease, despite complete and appropriate surgical treatment and adequate postoperative medical therapy with steroids. In the past, patients affected by the most severe forms needed multiple revision surgeries in the attempt to offer relief of symptoms for at least a few years, which was related to the lack of an alternative medical treatment, such as biologics, which have been entering routine clinical practice in the last couple of years.[4,5]

Research efforts have expanded our understanding of the pathophysiological mechanisms of chronic rhinosinusitis. Different endotypes have been described according to the specific imbalance in immune cell response. In type 2 CRSwNP, there is very high expression of inflammatory mediators involved in the Th2 response, such as IL-5, IL-4, and IL-13, which have been associated with pathognomonic eosinophilia. On the other hand, in types 1 and 3 CRSwNP, an increased level of TH1 cytokines (INF-G, TNF-alpha, IL-17, etc.)[6] has been documented together with peculiar neutrophilia. In previous studies, we examined potential biomarkers for specific endotypes and obtained interesting results with IL-5 for type 2 disease[7,8] and with calprotectin for nontype 2 disease[9] in nasal secretions. More specifically, we demonstrated a potential role of type 2 biomarkers, and in particular IL-5, to identify patients with a more severe phenotype based on inflammatory load. Similar results have been described by authors investigating the role of IL-5 as a clinical biomarker of type 2 inflammation in CRSwNP.[10,11] Furthermore, we recently demonstrated that calprotectin in nasal secretions may be considered a biomarker of nontype 2 inflammation with a good correlation with neutrophilia; we also observed that calprotectin levels significantly correlate with the number of previous surgeries.[9] Although as per the observations of our previous study, the role of calprotectin in uncontrolled type-2 CRSwNP patients remains poorly investigated in the literature.

Current scientific interest is focused on the identification of biomarkers that can better characterize the spectrum of severity of CRSwNP. In fact, biomarkers may be helpful to define the severity of disease and the risk of poor control with treatments. The primary aim of the present study was to determine if IL-5 and calprotectin in nasal secretion and nasal polyps may be useful as clinical biomarkers to define the severity of disease and to identify difficult-to-treat patients with CRSwNP.

2 | MATERIALS AND METHODS

2.1 | Study population

This is a monocentric observational case–control study including 81 patients affected by CRSwNP and followed at "A. Gemelli" Hospital Foundation-IRCCS, Catholic University of Sacred Heart, Rhinology Unit, in Rome, Italy. We enrolled patients diagnosed with diffuse bilateral CRSwNP according to EPOS2020 criteria[12] on regular treatment with intranasal corticosteroids (INCS) and had been submitted to at least one previous surgery. Patients referring to our rhinology unit were screened and enrolled in the period between October 2021 and December 2022. The study was approved by the local ethics committee (protocol number: 36127/19; ID:2758).

Inclusion criteria were: male or female, 18–65 years old, willing and able to provide written informed consent; primary diffuse bilateral CRSwNP diagnosed according to EPOS 2020 criteria[12]; regular treatment with intranasal corticosteroids; underwent at least one previous endoscopic sinus surgery (any kind); underwent an additional

workup in the last 3 months including evaluation by a pulmonologist or allergologist, maxillofacial computed tomography (CT) scan, and eosinophil blood count; and/or approval by the investigators as suitable for participation in the study without risk to the patient.

Exclusion criteria were: primary localized CRS (unilateral involvement of paranasal sinuses or involvement of only one sinus for each side); CRSwNP previously treated or undergoing treatment with biologics; ongoing respiratory tract infection including nasal cavity at inclusion and acute exacerbation of chronic rhinosinusitis as defined in the EPOS 2020 guidelines[12]; secondary diffuse or localized CRS (cystic fibrosis, sino-nasal tumor, primary ciliary dyskinesia, autoimmune disease); sino-nasal granulomatous disease and sino-nasal tumor; continuous systemic steroid treatment; pregnancy; and/or previous radiotherapy for head or neck cancer.

We enrolled 39 severe uncontrolled patients who were defined according to most recent literature[13] as those who underwent at least one previous surgery who have a SNOT-22 $\geq$ 40 and two or more brief cycles of systemic corticosteroids ($\geq$5 and $\leq$21 days) in the last year (Group A). Patients with a SNOT-22 < 40 who underwent at least one previous surgery and less than two brief cycles of systemic corticosteroids in the last year were considered as "controlled" ($n = 42$) and were used as a control group (Group B).

2.2 | Study design and procedures

Patients were evaluated at the screening visit (V0) and 1 month later (V1). At V0, all patients underwent a baseline interview to obtain all clinical history and information from additional workup, such as blood eosinophilia, asthma, smoke, allergy, non-steroidal anti-inflammatory drugs-exacerbated respiratory disease (NSAID-ERD), Lund–Mackay score (LMS), previous surgeries, and therapies. Blood eosinophilia was defined as a peripheral absolute eosinophil count greater than 250/μL.[14] We collected information about disease control taking into account previous surgeries and previous medical treatment, including brief cycles of steroids (cycles with $\geq$5 and <21 days of systemic corticosteroids in the last year). Patients willing to participate in the study were required to stop intranasal steroids (INCS) for 4 weeks before V1.

At V1, all patients underwent:

- *Evaluation of quality of life by SNOT-22*: We used the validated Italian version of SNOT-22.[15] The SNOT-22 questionnaire assesses the quality of life using 22 items validated for CRS, using a scale of values in which 0 corresponds to "I never have this symptom," 1 "rarely and mildly present symptom," 2 "mild-moderate symptom," 3 "moderate," 4 "frequent and severe symptom," and 5 "severe and ever-present symptom, which affects the quality of life." The range of SNOT-22 varies from 0 to 110. According to literature data, we defined severe disease for a SNOT-22 $\geq$ 40.[13]
- *Nasal endoscopic evaluation and polyp biopsy*: Rigid nasal endoscopy was performed in all subjects under local anesthesia with topical application of 2% xylocaine and using 0° and 30°, 4 mm diameter rigid nasal endoscopes (Karl Storz). Nasal endoscopy was done by using the standard three pass technique as described by Kennedy. Nasal endoscopy findings are noted such as nasal mucosa edema, presence of secretion, and presence of polyps.[16] In patients who gave consent, a biopsy of polypoid tissue at the level of the ostium-meatal complex was performed for tissue assays. The materials were collected and then stored at −80°C.
- *Nasal cytology* to determine the presence and type of sino-nasal inflammation. The examination was carried out on the material taken from the lower and middle turbinate bilaterally by "scraping" the mucosa with a rhinoprobe (Farmark).[14,17,18] The sample was gently spread on glass slides and immediately fixed in 95% ethanol and stained with May–Grunwald–Giemsa. Eosinophil and neutrophil counts were expressed as mean count per high power field (hpf) on at least three of the richest observed fields at high magnification (×400). Slides were examined under oil immersion by light microscopy at a magnification of 1000×.[16,17,19]
- *Nasal secretions* were collected bilaterally from each patient. A sinus sponge pack Merocel (Medtronic Xomed) was introduced parallel to the sagittal plane into the middle meatus of each nostril. Thereafter, 1 mL of 0.9% normal saline was added to the sponge to extract the secretion. Each sponge was maintained for 5 min, transferred to a 5-mL syringe (BD), and centrifuged at 1500 × g for 15 min at 24°C. The supernatants were divided in aliquots and stored at −80°C until further analysis.[7,8,19–21]

2.3 | Biochemical assays

We measured biomarkers of inflammation in nasal secretions: IL-5 as a marker of eosinophilic inflammation[8]; calprotectin as marker of neutrophilic inflammation.[9] IL-5 concentration in the cell-free unconcentrated NAL fluids was assayed in duplicate by ELISA "Quantikine" Human IL Immunoassay (R&D Systems) during a single session and the mean values were calculated. The coefficient of variation (CV) of duplicates was always less than 10%;

the limit of detection of IL-5 (LOD) was 0.13 ng/L. The determination of calprotectin was performed using the DiaSorin "LIAISON" calprotectin kit (DiaSorin Inc.), an in vitro diagnostic chemiluminescent immunoassay (CLIA) for the quantitative measurement of fecal calprotectin. We report the calprotectin value in mg/L.[9,22]

For a subgroup of patients ($n = 49$; 21 in Group A and 28 in Group B), we also performed biochemical assays in tissue homogenates obtained with biopsy of nasal polys and healthy mucosa (floor of the nasal cavity). Pretreatment was performed for the polyp and healthy nasal mucosa. For extraction of proteins from tissue, we cut a portion with a diameter of 3 mm, which was washed in 200 μL of pure H_2O to eliminate any external contaminants. Cell Lysis Buffer 2 (R&D Systems Bio-Techne) and protease inhibitor cocktail were added to each sample. The mixture was then homogenized and centrifuged. For each sample, the total protein content was determined by the Bradford method. All samples (NAL fluids and tissue homogenates) were stored at −80°C and brought to room temperature before starting pretreatment and assays.[23]

TABLE 1 Clinical characteristics of the entire population.

Epidemiology	
Age (mean ± SD; range)	46.2 ± 11.34; 23–64
Male (*n*/total; %)	50/81; 61.7%
Phenotyping	
Concomitant allergy (*n*/total; %)	48/81; 59.2%
Number of previous sino-nasal surgeries	
1 surgery	46/81 (56.7%)
2 surgeries	8/81 (9.8%)
3 surgeries	8/81 (9,8%)
4 surgeries	9/81 (11.1%)
5 or more surgeries	8/81 (9.8%)
Concomitant asthma (*n*/total; %)	45/81; 55.5%
Peripheral blood hyper-eosinophilia (*n*/total; %)	49/81; 60.5%
NSAID-ERD (*n*/total; %)	11/81; 13.6%
Smoking (*n*/total; %)	24/81; 29.6%
SNOT-22 (mean ± SD)	38.5 ± 10.64
CT Lund Mackay score (mean ± SD)	19.0 ± 3.69

2.4 | Statistical analysis

Statistical analysis was performed using SPSS package version 25.0 (IBM SPSS Statistics for Windows, Version 25.0, IBM Corp.). Continuous values such as levels of IL-5 and calprotectin, symptom scores, endoscopic scores, cell count were expressed as mean ± standard deviation (SD). Continuous variables were tested for normality using Kolmogorov–Smirnoff test. For univariate analysis, we used the following tests: chi-square test for dichotomous variables; Student's *t*-test for independent samples for variables normally distributed; log-rank test and Turkey post hoc test for non-normally distributed variables. When comparing more than two groups, we used one-way ANOVA and Bonferroni post hoc analysis. The strength of the correlation between the two parameters was obtained by Spearman's rank correlation test. Multivariate analysis was performed with binary logistic regression. All significance levels were Bonferroni corrected. A corrected threshold of 0.05 was considered significant. In logistic regression, we considered significance and expected variation, considering variation of risk for dependent variable at one single unit or one category variation of the independent variables in equation.

3 | RESULTS

Based on inclusion and exclusion criteria, we included 81 patients affected by CRSwNP with at least one previous surgery (50 men and 31 women [M:F = 1.6:1] and a mean age of 46.2 years [range: 23−64 years]). Baseline characteristics are reported in Table 1. Cases were further divided into Group A including 39 patients affected by uncontrolled CRSwNP (27/39 males; M:F = 2.2:1; mean age 45.8 years), and Group B including the remaining 42 patients who were well controlled (23/42 males; M:F = 1.2:1; mean age 47.1 years). The differences in age and gender between groups were not statistically significant ($p > 0.05$). We collected nasal secretions in all patients. Finally, 21 patients in Group A and 28 patients in Group B agreed to nasal biopsy.

Patients with uncontrolled CRSwNP complained of worse nasal symptoms with a higher average value of SNOT-22 compared to the control group (respectively, 49.4 vs. 30.5; $p = 0.02$). A significantly higher prevalence of NSAID-ERD ($p = 0.001$) and blood eosinophilia ($p = 0.003$) was observed in Group A. Furthermore, cigarette smoking habit was significantly higher in Group A compared to Group B ($p = 0.01$). Differences between the two groups are summarized in Table 2.

The mean IL-5 levels in nasal secretion of all 81 CRSwNP patients was 3.1 ± 2.2 ng/L, and was significantly higher in Group A compared to Group B (3.9 ± 2.3 vs. 2.2 ± 1.4 ng/L; $p = 0.04$) (Figure 1A). The mean calprotectin levels in nasal lavage of all CRSwNP patients was 15.6 ± 5.9 mg/L, and was significantly higher in Group A compared to Group B (19.1 ± 13.8 vs. 10.7 ± 6.4 mg/L; $p = 0.001$) (Figure 2A). The statistical analysis of cytologic cell count at hpf revealed that neutrophil and eosinophil counts were significantly higher in Group A. The median eosinophil count at nasal cytology was 23 (confidence interval [CI]: 14–50) in Group

TABLE 2 Characteristics of the population and distribution of biomarkers in the two groups along with the results of the univariate and multivariate analysis.

Biomarkers	Group A Uncontrolled	Group B Controlled	Univariate *p*-Values	Multivariate Risk variation of uncontrolled CRSwNP (expected)	Multivariate *p*-Value
Clinical factors					
Age (mean)	45.8	47.1	N.S.		N.S.
Sex (males; %)	27/39 (69.2%)	23/42 (54.8%)	N.S.		N.S.
Smoking habit	18/39 (46.1%)	6/42 (14.3%)	<0.05		N.S
Asthma	25/39 (64.1%)	20/42 (47.0%)	<0.01	1.881	<0.05
Allergy	22/39 (56.4%)	26/42 (61.9%)	N.S.		N.S.
Blood eosinophilia (>250 cells/μL)	27/39 (69.2%)	22/42 (52.4%)	<0.05		N.S.
NSAID-ERD	9/39 (23.1%)	2/42 (4.8%)	<0.01	2.25	<0.05
Mean SNOT-22	49.4	30.5	N.S.		N.S.
Mean number of systemic corticosteroids in the last 2 years	2.9 + 1.56	1 + 1.1	N.S.		N.S.
Biomarkers analyzed					
Calprotectin: nasal lavage (mg/L)[a]	19.1 ± 13.8	10.7 ± 6.4	0.001		N.S.
Calprotectin: polyp tissue (mg/L)[a]	174.5 ± 141.8	63.4 ± 50.1	0.007		N.S.
Interleukin-5: nasal lavage (ng/L)[a]	3.9 ± 2.3	2.2 ± 1.4	0.04	2.64	<0.01
Interleukin-5: polyp tissue (ng/L)[a]	21.6 ± 13.1	16.4 ± 10.1	0.04	1.16	<0.05
Eosinophil count at nasal cytology[b]	23 [14–50]	11 [7–30]	0.04		N.S.
Neutrophil count at nasal cytology[b]	37 [10–50]	18 [12–40]	0.008		N.S.

Abbreviations: CRSwNP, chronic rhinosinusitis with nasal polyps; NSAID-ERD, non-steroidal anti-inflammatory drugs-exacerbated respiratory disease.

[a]Mean values ± DS. Student's *t*-test for continuous parameters.

[b]Median values. Cytology cell count at high power field: Kruskal–Wallis test for nonparametric variables.

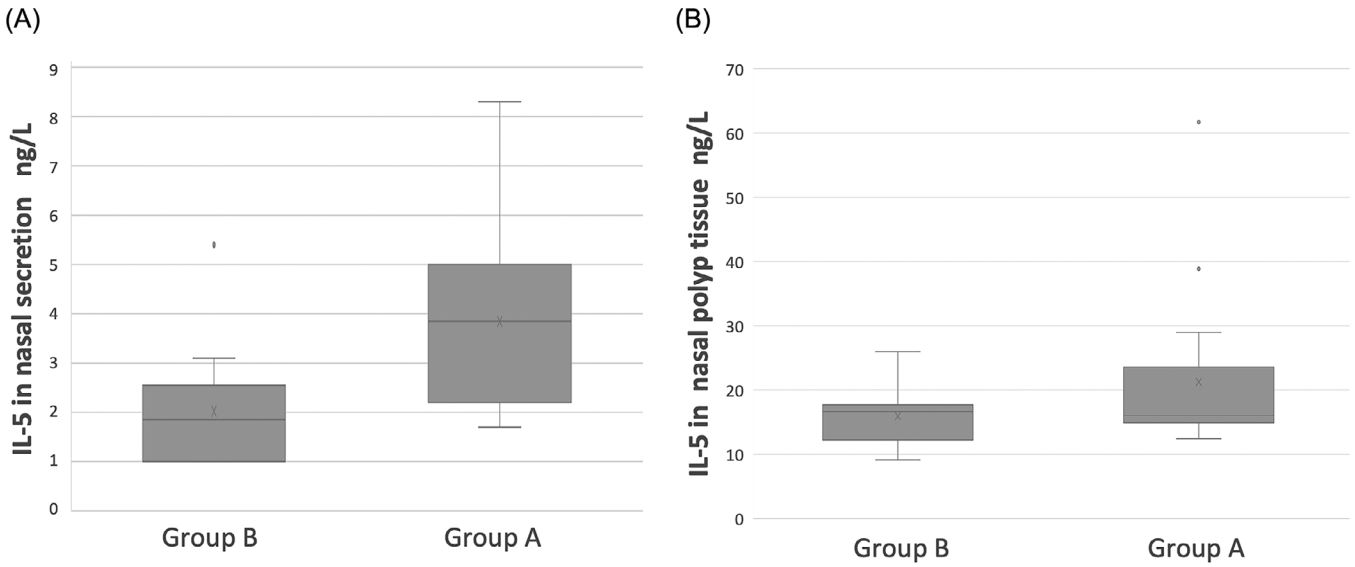

FIGURE 1 IL-5 levels in nasal secretions (A) and nasal polyp tissue (B).

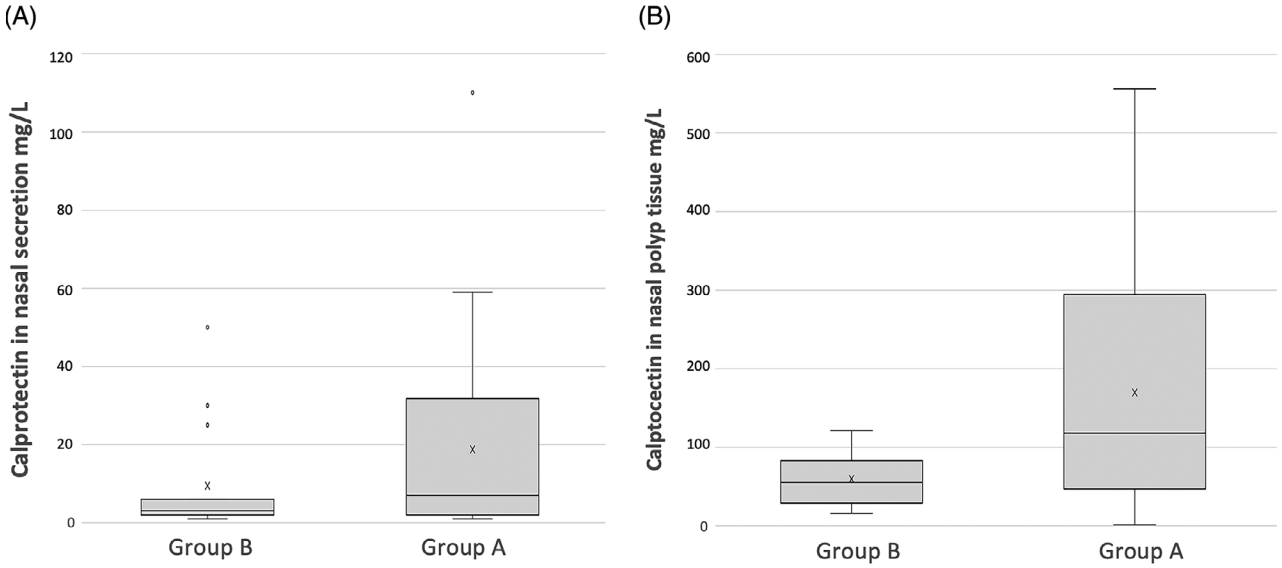

FIGURE 2 Calprotectin levels in nasal secretions (A) and nasal polyp tissue (B).

A versus 11 (CI: 7–30) in Group B ($p = 0.04$). Finally, the median neutrophilic count at nasal cytology was 37 (CI: 10–50) in Group A and 18 (CI: 12–40) in Group B ($p = 0.008$).

We measured the levels of IL-5 and calprotectin in tissue homogenates obtained from nasal polyps and healthy mucosa of all patients. Considering the entire population, we detected significantly higher levels of biomarkers in nasal polyp tissue compared to healthy mucosa for both IL-5 (19.2 ± 11.8 vs. 5.7 ± 3.1 ng/L; $p = 0.002$) and calprotectin (123.5 ± 98.8 vs. 2.3 ± 1.1 mg/L; $p = 0.006$).

Regarding IL-5, we observed that its levels in nasal polyp tissue were significantly higher compared to nasal secretions (19.2 ± 11.8 vs. 3.1 ± 2.2 ng/L; $p = 0.003$). We detected a trend of correlation between levels of IL-5 in polyp tissue and that in nasal secretions by Spearman's linear regression that did not reach significance ($p = 0.06$). Finally, we observed a significant difference between Group A and Group B for IL-5 in nasal polyp tissue (21.6 ± 13.1 vs. 16.4 ± 10.1 ng/L; $p = 0.04$) (Figure 1B).

Considering calprotectin, its levels in tissue were significantly higher compared to that in nasal secretions (123.5 ± 98.8 vs. 15.6 ± 5.9 mg/L; $p = 0.005$). We found a significant correlation between calprotectin in polyp tissue and in nasal secretions at Spearman's linear regression with a regression coefficient r of 0.35 ($p = 0.03$). Finally, the levels of calprotectin were significantly higher in Group A compared to Group B in nasal tissue (174.5 ± 141.8 vs. 63.4 ± 50.1 mg/L; $p = 0.007$) (Figure 2B).

Interesting results were obtained by correlating the values of biomarkers in secretions based on number of surgeries. Calprotectin levels, in fact, increased exponentially with an increase in the number of surgeries. The mean value reached was less than 200 mg/L until the second intervention and between 200 and 300 mg/L in patients who underwent more than three FESS. Figure 3 shows the stratification of calprotectin levels by number of interventions. The same trend was observed for IL-5 values in nasal secretions and number of surgical interventions. Figure 4 shows the progressive increase in IL-5 levels based on the number of surgeries. IL-5 levels are less than 3 ng/L until three FESS, and increased to more than 3 ng/L in patients who had underwent more than three surgeries. These data confirm that calprotectin and IL-5 can be considered as markers that correlate with disease severity.

We performed both univariate and multivariate analysis to assess the risk of uncontrolled CRSwNP (Table 2). In univariate analysis, the following factors were significantly correlated with the risk of uncontrolled CRSwNP: cigarette smoking, the presence of asthma, NSAID-ERD, blood eosinophilia (>250), high levels of calprotectin and IL-5 in tissue and polyps, and high eosinophil and neutrophil cell counts at nasal cytology. However, on multivariate analysis, only the presence of asthma, NSAID-ERD, and levels of IL-5 in tissue and blood were confirmed as independent risk factors for uncontrolled CRSwNP. The risk variation (expected) of uncontrolled CRSwNP was 1.88 ($p < 0.05$) for asthma, 2.25 for NSAID-ERD ($p < 0.05$), 2.64 ($p < 0.01$) for IL-5 in nasal lavage, and 1.16 ($p < 0.05$) for IL-5 in nasal polypoid tissue.

4 | DISCUSSION

A comprehensive approach has been adopted over the years to manage CRSwNP patients, including long-term

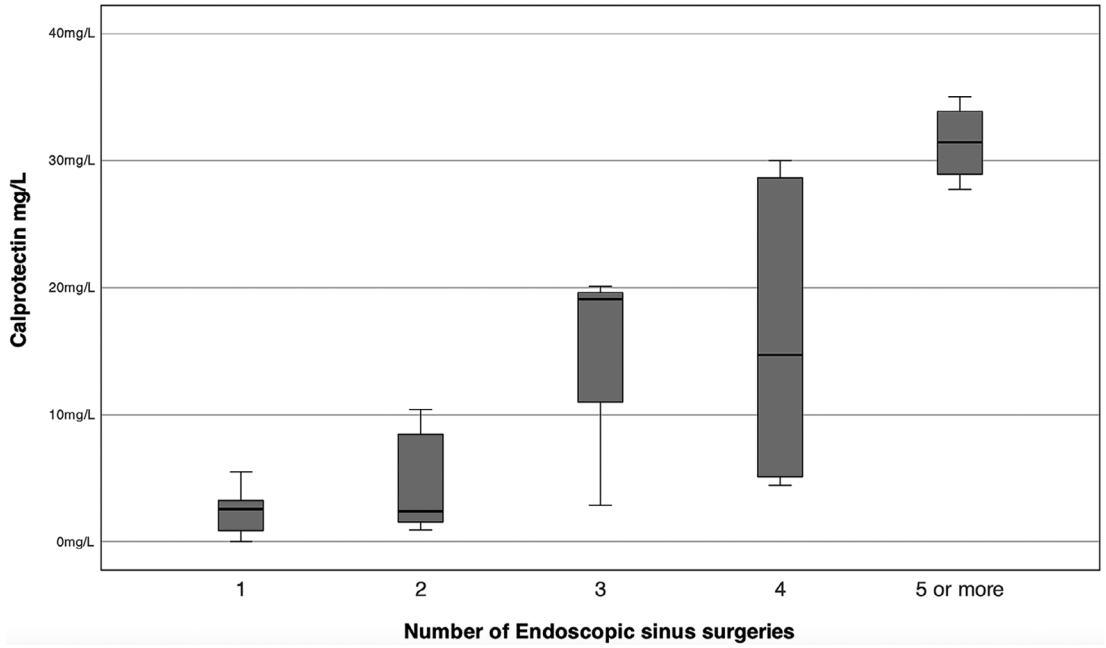

FIGURE 3 Calprotectin levels in nasal secretions according to the number of previous sinus surgeries.

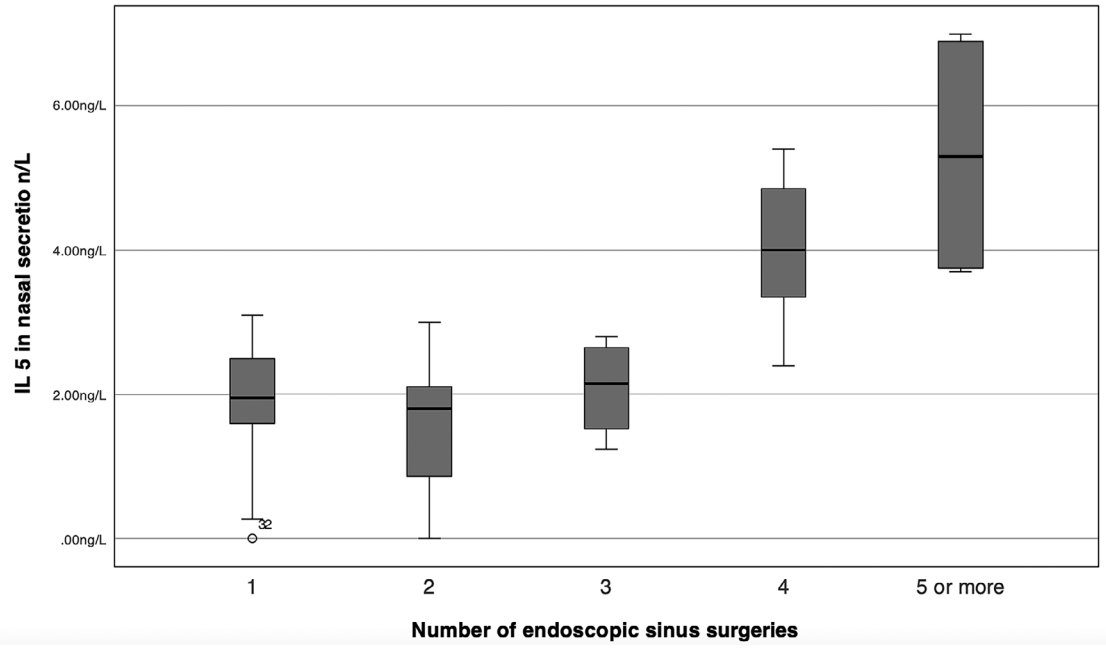

FIGURE 4 IL-5 levels in nasal secretions according to the number of previous sinus surgeries.

treatment with INCS, brief cycles of antibiotics/systemic steroids and FESS in the case of failure of adequate medical treatment. Nevertheless, poor control of disease was common in the past until biologics were introduced. These drugs interfere with the underlying type-2 inflammation and improve disease control, both in terms of symptoms and endoscopic findings.[24–27] The EPOS steering group[12] defined "difficult-to-treat patients" as those who do not reach an acceptable level of control despite continuous treatment with INCS, up to two short courses of antibiotics or systemic corticosteroids in the last year, and FESS.[28,29]

In the era of biologic drugs, it is very important to identify the most severe cases with a high risk of noncontrol to first-line treatments and therefore eligible for targeted therapy with biologics. Given this premise, identification of predictors of noncontrol may be helpful to identify severe cases. Several clinical factors have been

considered as potentially influencing the rate of good control, such as asthma, NSAID-ERD, occupational dust exposure, allergy, extension of disease at the time of first surgery, type and extension of surgery, and history of multiple surgeries.[16,30–32] Finally, the need for revision surgery is a risk factor for subsequent surgery, because such an "aggressive" phenotype may be related to the underlying endotype of the disease.[32] In agreement with literature data, patients affected by difficult-to-treat CRSwNP in our series more frequently had comorbid asthma, blood eosinophilia, NSAID intolerance, and cigarette smoke exposure.[30] In our series, multivariate logistic regression analyses showed that asthma and NSAID intolerance were the only factors that were independently and significantly related to uncontrolled CRSwNP after surgery despite continuous INCS and brief cycles of systemic corticosteroids.

Biomarkers have been suggested by some authors[7–9] to be factors that are predictive of severity. Indeed, biomarkers are measurable in nasal secretions and nasal tissue, reflecting the underlying pathophysiology of CRSwNP and may be helpful to identify and characterize endotypes that are more frequently associated with more severe disease.[7,33] In this regard, it has been demonstrated that type 2 inflammation is more frequently associated to severe phenotypes of CRSwNP, and IL-5 has been suggested to be implicated in its pathogenesis[34] due to its role in the activation and survival of eosinophils once they have been recruited in the inflammatory process. It has been demonstrated that high eosinophilic infiltration and high IL-5 expression in CRSwNP correlates with a higher rate of polyp recurrence and that IL-5 in nasal secretions is a reliable biomarker that can differentiate clusters with high and low inflammatory load.[7] In this series, we confirmed our previous observations and found a high level of IL-5 in nasal secretions and in nasal polyp tissue of patients with uncontrolled disease compared to controlled disease. Furthermore, we observed that the higher the number of previous surgeries, the higher the level of IL-5 in nasal secretions. The multivariate logistic regression analyses confirmed that IL-5 in nasal polyp tissue and in nasal secretions was an independent factor that was significantly related to the risk of uncontrolled disease.

Although considerable importance has been given to type 2 inflammation and to tissue eosinophilia over the years, the relevance of an associated high neutrophilic infiltrate should not be overlooked. According to previous literature data,[35] neutrophils seem to be involved in the activation, regulation, and effector functions of innate and adaptive immune cells with a crucial role in the pathogenesis of chronic inflammation. The presence of neutrophils in the subepithelial layer of nasal polyps has been associated with refractory CRSwNP and poor surgical outcomes.[36–38] Finally, neutrophilia is associated with a lower response to corticosteroids, and some authors have demonstrated that patients with a neutrophilic phenotype had less response to oral corticosteroid therapy.[39]

In addition, enhanced neutrophil activation may be observed contextually in type 2 CRSwNP (mixed phenotypes) with release of the neutrophilic proteases elastase and cathepsin G. These molecules may increase the secretion of IL-1 and IL-33[40] and may serve as chemoattractants for Th2 cells and promote the production of type 2 cytokines in nasal polyps.[41] Additionally, because it promotes goblet cell hyperplasia and mucus production, neutrophil elastase has a significant impact on airway inflammation.[42] This may show that neutrophils may play a role in a type 2 environment and have a greater capacity to influence local inflammation through increased proteolytic activity. Furthermore, neutrophil migration may be enhanced by *Staphylococcus aureus* colonization, which is frequently associated with severe CRSwNP.[43] Variations in sinus bacterial microbiota after surgery may also play a role in neutrophilic activation.[44]

Several reports in the literature have analyzed the role of calprotectin in the pathogenesis of CRS. In fact, it has been shown that the expression of calprotectin correlated well with levels of neutrophils and was significantly increased in polyp tissue compared to healthy mucosa.[45,46] In this series, we confirmed our preliminary results, showing that the levels of calprotectin were significantly higher compared to controls in nasal secretions of all CRSwNP endotypes associated with significant neutrophilia.[9] Herein, we further demonstrated that its levels were significantly higher not only in nasal secretions, but also in nasal polyp tissue of uncontrolled cases compared to controlled patients. Our results suggest that neutrophilic inflammation should not be disregarded, because it may contribute to the state of uncontrolled disease. Bearing this in mind, neutrophilia should be always suspected in the case of poor response to biologics targeting Th2 inflammation, although future studies are required to confirm this hypothesis.

The identification of biomarkers that can outperform or add clinical insight in CRSwNP is an area of active investigation, although additional work is needed to introduce them in routine clinical practice. In this series, we observed that the levels of IL-5 and calprotectin were significantly higher in both nasal secretions and nasal polyps from patients with severe uncontrolled disease (failing after at least one previous surgery and two brief cycles of systemic steroids in the last year) compared to well-controlled patients. The results of multivariate logistic regression analyses confirmed that among the biomarkers that we

investigated, IL-5 was the only significant independent factor related to the risk of uncontrolled disease. Interestingly, both biomarkers (IL-5 and calprotectin) showed an increase that goes hand-in-hand with the number of previous surgeries. Based on these results, we can hypothesize that multiple surgical damage may lead to a greater permeability of the mucosa toward external pathogens (allergens, microorganisms, etc.) that amplifies the processes of chronic inflammation and interferes with scarring and re-epithelialization, along with worsening of symptoms that require an increase in topical and systemic steroids and a consequent vicious circle of hyperneutrophilia. This would explain why patients with mixed eosinophilic and neutrophilic infiltrates have a more aggressive disease phenotype and a higher risk of poor outcomes after surgery.[47]

In conclusion, our data confirm that some clinical factors (smoking, asthma, NSAID intolerance, and blood eosinophils > 250 cells/μL) and high levels of some biomarkers (IL-5 and calprotectin in nasal secretion/tissue; eosinophil and neutrophil count at nasal cytology) were more frequently observed in uncontrolled CRSwNP compared to controlled disease. Nevertheless, multivariate logistic regression analysis showed that asthma, NSAID intolerance, and IL-5 in nasal secretion/polyps tissue were independently and significantly related to an increased risk of uncontrolled disease. Our data seem to confirm that on the one hand, IL-5 (both in nasal secretion and tissue) represents a reliable and effective biomarker of type 2 inflammation and of the severity of disease, on the other hand, calprotectin and neutrophilia are frequently associated with uncontrolled disease, especially after multiple surgeries. Despite the encouraging results of our study, we are aware that while significant progress has been made in characterizing endotypes, phenotypes, and biomarkers in CRS, additional studies are needed to determine if and how biomarkers can assist physicians in providing more individualized clinical care. Indeed, the applicability in clinical practice is very complex; first of all, it is necessary to standardize the methodology and then identify a cut-off indicative of severity and noncontrol. We have already addressed this issue in the past by identifying a cut-off for IL-5 that corresponds to a group of patients with high inflammatory load that we have termed type 2 high-risk patients.[7] However, additional studies with appropriate design, possibly prospective, and on a larger number of patients are needed to establish clinical cut-offs that are predictive of noncontrol.

CONFLICT OF INTEREST STATEMENT

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