

Clinical and Biological Markers Predict the Efficacy of Glucocorticoid- and Macrolide-Based Postoperative Therapy in Patients With Chronic Rhinosinusitis

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Abstract

Background: Although subsequent anti-inflammatory treatments are indispensable for patients with chronic rhinosinusitis (CRS) undergoing sinus surgery, few studies have explored the factors influencing the efficacy of postoperative anti-inflammatory treatment.

Objective: We aimed to develop prediction models for the response to glucocorticoid- and macrolide-based postoperative therapy in CRS patients.

Methods: We performed a post-hoc analysis of our previous study comparing the efficacy of fluticasone propionate and clarithromycin in the postoperative treatment of CRS patients. Clinical characteristics and treatment outcome information were collected. In addition, diseased sinonasal mucosal tissues obtained during surgery were processed for Bio-Plex analysis of protein levels of 34 biomarkers. Classification trees were built to predict refractory CRS based on clinical characteristics and biological markers for patients treated with fluticasone propionate or clarithromycin. A random forest algorithm was used to confirm the discriminating factors that formed the classification trees.

Results: One year after surgery, 22.7% of the patients (17/75) treated with fluticasone propionate, and 24.3% of those (18/74) treated with clarithromycin were diagnosed with refractory CRS. Nasal tissue IL-8 and IgG3 levels and headache VAS scores in the fluticasone propionate group, and nasal tissue IgG4 levels and overall burden of symptoms VAS scores in the clarithromycin group, were identified as discriminating factors forming the classification tree to predict refractory CRS. The overall predictive accuracy of the model was 89.3% and 87.8% for fluticasone propionate- and clarithromycin-based post-surgical treatment, respectively.

Conclusions: Classification trees built using clinical and biological parameters could be helpful in identifying patients with poor response to fluticasone propionate- and clarithromycin-based postoperative treatment.

Keywords

chronic rhinosinusitis, clarithromycin, fluticasone propionate, prediction, postoperative treatment

Introduction

Chronic rhinosinusitis (CRS) is a disease characterized by chronic sinonasal inflammation with or without the formation of edematous polyp tissues.^{1–4} Medical treatment options for patients with CRS remain limited, and endoscopic sinus surgery (ESS) plays a prominent role in the treatment of CRS. Nevertheless, even with sufficient surgical approaches, followed-up postoperative medical treatments such as topical glucocorticoids and oral

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macrolide antibiotics are indispensable.^{2,4} However, a considerable number of patients do not respond well to these comprehensive treatments, and these patients are identified as difficult-to-treat or refractory cases.^{2,4-7} Hence, a better understanding of the mechanisms underlying the heterogeneity of treatment response in patients with CRS is critical to the development of 'personalized medicine'. New approaches aiming to tailor treatment to individual patients could be facilitated by the identification of markers that predict responses to different therapies.^{4,8,9}

The treatment outcome estimates of CRS are usually based on clinical parameters that include symptoms, nasal endoscopy, and radiological findings.⁴ Some studies have demonstrated that clinical factors such as computerized tomography (CT) and symptom scores may have a certain value in predicting the response to medical and surgical treatments in patients with CRS.^{7,8,10-12} In addition, increasing evidence suggests that the inflammatory endotypes of CRS may have a more profound impact on the CRS treatment response.¹³⁻¹⁵ Several biomarkers, such as eosinophils, neutrophils, IL-25, and Charcot-Leyden crystals, have been found associated with the response to systemic glucocorticoid treatment in patients with CRSwNP.¹⁶⁻¹⁸ Meanwhile, serum IgE (sIgE) has been associated with poor response to macrolide treatment in patients with CRSsNP.³ However, most of those previous studies focused on the prediction of response to preoperative treatments. Few studies have explored the clinical and biological markers associated with the efficacy of postoperative anti-inflammatory drugs in patients with CRS. Previously, we divided the CRS patients into CRS without nasal polyps (CRSsNP), eosinophilic CRS with nasal polyps (CRSwNP), and non-eosinophilic CRSwNP subgroups, and compared the efficacy of fluticasone propionate versus clarithromycin based postoperative treatment.¹⁹ However, we did not find a significant difference between these two anti-inflammatory medicines in any of the subgroups of CRS, indicating that eosinophilia and the presence of nasal polyps might not hold sufficient power to determine the efficacy of postoperative fluticasone propionate and clarithromycin treatment.¹⁹ Therefore, identifying factors associated with the efficacy of postoperative anti-inflammatory treatments in CRS patients is still quite challenging.

In this post-hoc analysis of our previous prospective randomized controlled trial comparing the efficacy of postoperative fluticasone propionate and clarithromycin treatment in patients with CRS,¹⁹ we studied a large number of clinical characteristics and tissue biological markers. We aimed to develop prediction models integrating clinical and tissue biological markers through classification tree analysis (a machine-learning method) to identify patients eligible for postoperative treatment of fluticasone propionate and clarithromycin. Classification tree analysis usually yields better

predictive accuracy than the multivariate logistic regression model and forms a flow-chart-like structure that can be easily implemented and interpreted without requiring numeric calculations.²⁰

Methods

Subjects

The original study was registered as a prospective two-arm, randomized, and controlled trial that aimed to compare the benefits of fluticasone propionate nasal spray and clarithromycin tablets following ESS in different phenotypic CRS defined by the presence or absence of nasal polyps and eosinophilic inflammation; that is, CRSsNP, eosinophilic CRSwNP, and non-eosinophilic CRSwNP (NCT02182492). Full details of the protocol are available in the published study.¹⁹ The inclusion and exclusion criteria of the original study are shown in Table E1 in this article's Online Repository. In brief, randomization was carried out according to CRS phenotypes. One week after surgery, patients were randomly assigned to receive either fluticasone propionate nasal spray (Glaxo Wellcome, S.A., Burgos, Spain), 200 µg once daily, or a 250-mg tablet of clarithromycin (Abbot China, Shanghai, China) once daily for 3 months. Additionally, all patients underwent nasal saline irrigation (250 mL twice a day) for 1 year. If polyps and/or the moderate-to-severe clinical symptoms of CRS recurred and/or were not controlled after stopping the study medication, rescue therapy with oral prednisone tablets (30 mg daily on days 1-5, 20 mg daily on days 6-10, 10 mg daily on days 11-15, and 5 mg daily on days 16-20) was prescribed.¹⁹ Patients still having bothersome symptoms, although these treatments were defined as refractory cases at the 12-month post-dosing follow-up visit.¹⁹ Diseased ethmoid sinus mucosal tissue and polyp tissue samples were obtained from CRSsNP and CRSwNP patients, respectively, during surgery.^{9,21} The subjects without enough tissue for analysis of biological markers were excluded from this post-hoc study. Since the numbers of finally-analyzed refractory cases were limited in every phenotypic CRS group, we pooled all phenotypic CRS patients together as a single group in this post-hoc study. This study was approved by the Ethics Committee of the Tongji Hospital, and written informed consent was obtained from all participants.

Demographic Characteristic and Clinical Measurement

As previously mentioned,^{7-11,19} descriptive data, including age, sex, atopy, asthma and allergic rhinitis comorbidity, current smoking, and prior sinus surgery history,

Table 1. The Demographic and Clinical Characteristics of CRS Patients in Fluticasone Propionate Group.

Characteristic	Non-refractory	Refractory	P-value
Subject, n (%)	58 (77.3)	17 (22.7)	—
Sex, male	35 (60.3)	12 (70.6)	0.443
Age (years)	43 (33–51)	38 (24.5–45.5)	0.071
Patients with NPs	38 (65.5)	14 (82.4)	0.186
Patients with eosinophilic NPs	20 (34.5)	6 (35.3)	0.951
Smoking*	21 (36.2)	3 (17.7)	0.149
Patients with atopy	13 (22.4)	5 (29.4)	0.552
Patients with AR	8 (13.8)	3 (17.7)	0.693
Patients with asthma	1 (1.7)	1 (5.9)	0.349
Patients with prior sinus surgery	11 (19)	8 (47.1)	0.019
slgE level (kU/L)	55.9 (29–168.1)	78.6 (57.6–168.1)	0.311
Blood eosinophil absolute count ($\times 10^9/L$)	0.26 (0.15–0.38)	0.2 (0.14–0.47)	0.490
Blood eosinophil percent (%)	3 (1.9–5.6)	2.3 (1.6–4.6)	0.288
Bilateral CT score	14 (9–18.3)	13 (11–15.5)	0.955
Nasal obstruction VAS score	6.5 (5–8)	7 (6–8)	0.309
Rhinorrhea VAS score	5 (4–6)	7 (4–8)	0.107
Loss of sense of smell VAS score	3 (1–7.3)	8 (2–8.5)	0.102
Facial pain or pressure VAS score	0 (0–0)	0 (0–1.5)	0.054
Headache VAS score	3 (1.8–4.3)	5 (2.5–6)	0.048
Total VAS score	18.5 (13.8–24)	21 (19.5–33)	0.017
Overall burden of symptoms VAS score	7 (5–8)	7 (5.5–8)	0.825
Polyp endoscopic score	2.5 (0–4)	4 (2–4)	0.177
Discharge endoscopic score	2 (2–4)	2 (2–4)	0.408
Edema endoscopic score	2 (2–3)	2 (2–4)	0.909
Crusting endoscopic score	0 (0–0)	0 (0–1)	0.047
Scarring endoscopic score	0 (0–0)	0 (0–1)	0.337
Total endoscopic score	8 (6–10)	10 (8–10)	0.088

For continuous variables, the results are expressed as medians and interquartile ranges. Categorical variables are summarized using frequency and percentage. For continuous variables, the 2-tailed Mann-Whitney *U* test was used for between-group comparison. For categorical variables, the Chi-squared test was performed to determine the difference between groups. $P < 0.05$ was considered statistically significant. The *P* values in bold are less than 0.05 and indicate a statistical significance.

CRS, chronic rhinosinusitis; NPs, nasal polyps; AR, allergic rhinitis; slgE, serum IgE; CT, computer tomography; VAS, visual analog scale.

*Active smoking history >1 year.

were obtained. Two to three days before surgery, total serum IgE levels were determined by ImmunoCAP (Phadia, Uppsala, Sweden). A complete peripheral blood cell count with differential was performed through automated analysis, and the percentage and absolute count of eosinophils were determined.^{19,22} Radiologic disease severity was assessed using the Lund-Mackay computed tomography (CT) scoring system, and total bilateral CT scores were analyzed.¹⁹ Symptoms were scored by patients on a visual analog scale (VAS) of 0–10, with 0 being “no complaint whatsoever” and 10 being “the worst imaginable complaint.” Five major symptoms were focused on: nasal obstruction, rhinorrhea, loss of sense of smell, facial pain or pressure, and headache.¹⁹ A total VAS symptom score was calculated based on the sum of VAS scores of these five symptom domains.¹⁹ In addition, every patient was asked to rate his/her overall burden of CRS symptoms.¹⁹ Nasal endoscopic evaluation was performed by a senior investigator (Z.L.) who remained blinded to the treatment. Nasal

endoscopic findings were recorded using the Lanza-Kennedy scoring system.^{9,19}

Measurement of Protein Levels of Biological Markers in Nasal Tissues

The protein levels of a panel of 34 biological markers in diseased sinus mucosal tissue and polyp samples were detected using the Bio-Plex suspension chip method (Bio-Rad, Hercules, CA, USA).^{9,21} Tissue processing and the lower detection limit of each biological marker were provided in this article’s online repository. The protein concentrations of the detected biomarkers were normalized to the total tissue protein levels determined by a BCA protein detection kit (Guge Biotechnology, Wuhan, China).²¹

Statistical Analysis

For continuous variables, the 2-tailed Mann-Whitney *U* test was used for between-group comparisons.

The chi-square test was used to compare the difference in proportions between groups. Differences were considered statistically significant if the *P-value* was less than 0.05. These statistical analyses were performed using IBM SPSS 22.0 package (SPSS Inc., Chicago, IL).

The classification trees were built and further supported by random forest analysis.²³ The variables that were significantly different between the refractory and non-refractory groups were included in the classification tree analysis. The classification tree algorithms allow the selection of variables based on the order of predictive powers, and the most important variables are included first as the root of the classification tree; the searching process would end when additional variables cannot contribute enough to the predictive model. Random forests were constructed to support the classification trees, allowing for the investigation of consistency in results. The importance of the variables in the dataset can be measured by using importance scores.²³ Here, we used the mean decrease in the Gini index. The ranking of an index indicates its importance. The classification tree and random forest analysis were performed in R (R Development Core Team).

Results

Patient's Demographic and Clinical Characteristics

One hundred and seventy-two CRS patients completed the clinical trial with the required follow-up in the original study.¹⁹ In this post-hoc analysis, 11 subjects in the nasal fluticasone propionate group and 12 subjects in the clarithromycin group were excluded because of insufficient tissue for Bio-Plex analysis. Therefore, 75 patients treated with fluticasone propionate and 74 patients treated with clarithromycin were finally analyzed. One year after surgery, 17 subjects in the fluticasone propionate group (17/75, 22.7%) and 18 subjects in the clarithromycin group (18/74, 24.3%) were identified as refractory cases. No significant difference in the ratio of refractory cases was found between the two treatment arms.

The demographic and preoperative clinical characteristics of refractory and non-refractory patients in the fluticasone propionate and clarithromycin treatment groups are shown in Tables 1 and 2, respectively. The proportion of patients with prior sinus surgery was significantly higher among the refractory subjects than among the non-refractory subjects in both treatment groups. Headache VAS scores, total VAS scores, and endoscopic scores of crusting were found to be significantly higher in the refractory subjects than in non-refractory subjects in the fluticasone propionate group (Table 1). As for the loss of sense of smell and overall burden of symptoms, VAS scores were significantly

higher among the refractory subjects than among the non-refractory subjects in the clarithromycin group (Table 2).

Difference in Biological Markers between Refractory and Non-Refractory CRS

We compared the difference in protein levels of biomarkers in sinonasal mucosa samples between refractory and non-refractory patients with CRS. In the fluticasone propionate group, the levels of IL-8, granulocyte-colony stimulating factor (G-CSF), and IgG3 were significantly higher in refractory subjects than in non-refractory subjects (Table 3). In the clarithromycin group, we found that the levels of IgG4, IgM, and IgE were significantly higher in refractory subjects than in non-refractory subjects (Table 4).

Creation of Prediction Models for Refractory CRS in Different Treatment Groups

We designed a classification tree analysis based on the clinical and biological markers that exhibited significant differences between refractory and non-refractory subjects and searched for variables that would be predictive for refractory CRS in the fluticasone propionate and clarithromycin groups, respectively. First, we identified IL-8, headache VAS score, and IgG3 as the main determinants in the classification tree for predicting refractory CRS in patients with fluticasone propionate-based postoperative treatment. The created classification tree formed 4 groups after three splits: (a) Split No.1: all 75 enrolled patients were divided based on IL-8 <731 pg/mg (*n* = 47) or ≥731 pg/mg (*n* = 28), with refractory ratios of 9% and 46%, respectively; (b) Split No.2: patients with IL-8 ≥731 pg/mg were divided based on headache VAS score <2 (*n* = 11) or ≥2 (*n* = 17), with a refractory ratio of 9% and 71%, respectively; (c) Split No.3: patients with IL-8 ≥731 pg/mg and headache VAS score ≥2 were divided based on IgG3 <18 ng/mg (*n* = 8) or ≥18 ng/mg (*n* = 9), with a refractory ratio of 37% and 100%, respectively (Figure 1(A)). The high-risk group was associated with an odds ratio (OR) of 29.9 (95% confidence interval, CI: 1.29-692) (Figure 1(A)). The overall accuracy of this prediction model was 89.3%. The random forest analysis confirmed the classification of tree data (Figure 1(B)). In the fluticasone propionate group data, IL-8, headache VAS score, and IgG3 were the first 3 parameters with a large mean decrease in the Gini index (Figure 1(B)). This supports the presence of IL-8 in the root of the tree and headache VAS score and IgG3 as the subsequent splits in the classification tree (Figure 1(A)).

For patients treated with clarithromycin after surgery, IgG4 and overall burden of symptom VAS score

Table 2. The Demographic and Clinical Characteristics of CRS Patients in Clarithromycin Group.

Characteristic	Non-refractory	Refractory	P-value
Subject, n (%)	56 (75.7)	18 (24.3)	—
Sex, male	35 (62.5)	11 (61.1)	0.916
Age (years)	39 (27.5–53)	36.5 (21.8–53.5)	0.406
Patients with NPs	37 (66.1)	12 (66.7)	0.963
Patients with eosinophilic NPs	17 (30.4)	7 (38.9)	0.501
Smoking*	15 (26.8)	4 (22.2)	0.699
Patients with atopy	17 (30.4)	6 (33.3)	0.812
Patients with AR	15 (26.8)	5 (27.8)	0.934
Patients with asthma	2 (3.6)	0 (0)	0.416
Patients with prior sinus surgery	8 (14.3)	10 (55.6)	<0.001
slgE level (kU/L)	63.4 (28.3–153.5)	56.7 (29–87.2)	0.584
Blood eosinophil absolute count ($\times 10^9/L$)	0.17 (0.11–0.37)	0.27 (0.12–0.52)	0.371
Blood eosinophil percent (%)	2.3 (1.6–4.1)	3.3 (1.2–6.8)	0.348
Bilateral CT score	13.5 (9.3–17.8)	15 (9–17)	0.810
Nasal obstruction VAS score	6 (5–8)	7 (5.8–8.3)	0.505
Rhinorrhea VAS score	6 (4–7)	6.5 (4–8)	0.625
Loss of sense of smell VAS score	3.5 (0–8)	7 (1.5–10)	0.045
Facial pain or pressure VAS score	0 (0–1)	0 (0–0)	0.293
Headache VAS score	3 (2–5)	2 (2–3.3)	0.440
Total VAS score	18 (14–26)	23.5 (13.5–28.3)	0.340
Overall burden of symptoms VAS score	7 (5–8)	8 (7–8)	0.049
Polyp endoscopic score	2 (0–4)	3 (0–4)	0.393
Discharge endoscopic score	2 (2–4)	2 (2–4)	0.380
Edema endoscopic score	2 (2–3.8)	2 (2–3.3)	0.679
Crusting endoscopic score	0 (0–0)	0 (0–0)	0.641
Scarring endoscopic score	0 (0–0)	0 (0–0.25)	0.634
Total endoscopic score	7.5 (6–9.8)	8 (5.8–9.3)	0.437

For continuous variables, the results are expressed as medians and interquartile ranges. Categorical variables are summarized using frequency and percentage. For continuous variables, the 2-tailed Mann-Whitney *U* test was used for between-group comparison. For categorical variables, the Chi-squared test was performed to determine the difference between groups. $P < 0.05$ was considered as statistical significance. The *P* values in bold are less than 0.05 and indicate a statistical significance.

CRS, chronic rhinosinusitis; NPs, nasal polyps; AR, allergic rhinitis; slgE, serum IgE; CT, computer tomography; VAS, visual analog scale.

* Active smoking history > 1 year.

were identified as the main determinants in the classification tree for predicting refractory CRS. The developed classification tree formed 3 groups after two splits: (a) Split No.1: all 74 enrolled patients were divided based on IgG4 < 8.1 ng/mg ($n = 58$) or ≥ 8.1 ng/mg ($n = 16$) with refractory ratios of 14% and 62%, respectively; (b) Split No.2: patients were divided based on IgG4 ≥ 8.1 ng/mg with overall burden of symptom VAS score < 7 ($n = 7$) or ≥ 7 ($n = 9$), with a refractory ratio of 14% and 100%, respectively (Figure 2(A)). The high-risk group had an OR of 82.3 (95% CI: 2.88–2353) (Figure 2(A)). The overall accuracy of this prediction model was 87.8%. Using the random forest analysis, IgG4 and overall burden of symptoms VAS score were identified as the two parameters with the largest mean decrease in the Gini index (Figure 2(B)). This supported the presence of IgG4 in the root of the tree and overall burden of symptoms VAS score as the subsequent split in the classification tree (Figure 2(A)).

Discussion

Postoperative anti-inflammatory therapies are indispensable for CRS patients after ESS.^{4,24} Conventional postoperative anti-inflammatory treatments are usually based on either topical steroids or short-term oral steroids.^{1,4} In addition, macrolides are recommended as an option of anti-inflammatory therapy based on their immunomodulatory effect, particularly for neutrophil-dominated CRS.^{1,3,4} However, the rate of refractory CRS could be up to 30%, regardless of which postoperative anti-inflammation treatment option was chosen.^{4,9} We found that the frequency of refractory cases was 22.7% (17 of 75) in patients with nasal fluticasone propionate-based postoperative treatment and 24.3% (18 of 74) in patients with clarithromycin-based postoperative treatment,^{4,9,25} respectively, highlighting the need for selecting the right patients prior to the initiation of therapy.

In this post-hoc study, we analyzed a number of clinical parameters and CRS-relevant molecules in tissues in

Table 3. The Difference in Biological Markers Between Refractory and Non-Refractory CRS in Fluticasone Propionate Group.

Mediator	Non-Refractory (n = 58)	Refractory (n = 17)	P-value
IL-1 β (pg/mg)	13 (8.7–21.1)	11.6 (7–19.7)	0.519
IL-1Ra (ng/mg)	2.1 (1.3–3.5)	1.9 (0.8–2.8)	0.239
IL-2 (pg/mg)	2.3 (1.5–3.7)	2.1 (1.2–2.8)	0.199
IL-4 (pg/mg)	119.9 (78.4–187.3)	125.2 (93–181.9)	0.854
IL-5 (pg/mg)	36.1 (17.5–63.3)	19 (9.5–49)	0.082
IL-6 (pg/mg)	95 (50.7–193.5)	108.6 (61.8–226.7)	0.404
IL-7 (pg/mg)	396.9 (241.7–632.1)	311 (218.4–448.1)	0.117
IL-8 (pg/mg)	537.8 (364.6–775.8)	887.8 (697.5–1815)	<0.001
IL-9 (pg/mg)	71.7 (52.5–175.3)	76.5 (54.8–131.7)	0.786
IL-10 (pg/mg)	66 (32.7–143.4)	41.3 (11.7–92.9)	0.126
IL-12 (pg/mg)	62 (39–105.7)	51.5 (31.9–72.4)	0.255
IL-13 (pg/mg)	53.1 (27.5–106.8)	42.2 (24.6–63.8)	0.271
IL-15 (pg/mg)	13.3 (8–20.3)	17.3 (9.1–23.4)	0.242
IL-17A (pg/mg)	268.5 (173.6–456.7)	274.3 (193.1–474.7)	0.723
Eotaxin (pg/mg)	259.7 (169.6–526)	231 (137.9–517.5)	0.479
bFGF (pg/mg)	1167 (689.8–2252)	991.4 (495.9–1573)	0.277
G-CSF (pg/mg)	406.5 (229.6–880)	1167 (322.6–2712)	0.033
IFN- γ (pg/mg)	346.5 (214.5–637.3)	248.6 (167.8–448.9)	0.180
IP-10 (ng/mg)	4.7 (2.6–7.9)	3.2 (1.5–6)	0.160
MCP-1 (pg/mg)	613.8 (345.4–885.2)	600.1 (332.1–880.2)	0.761
MIP-1 α (pg/mg)	25.8 (15.8–60.1)	25.5 (16.2–42.8)	0.573
PDGF-BB (pg/mg)	307.6 (161.5–516.4)	269.6 (155.3–491.1)	0.519
MIP-1 β (pg/mg)	416 (269.3–613.7)	428.1 (215.1–679.3)	0.830
TNF- α (pg/mg)	157.3 (106.7–313.5)	161.2 (100.3–232.4)	0.411
VEGF (ng/mg)	1.6 (0.7–2.9)	1.2 (0.7–3.3)	0.631
IL-25 (pg/mg)	4.1 (1.9–7.6)	2.4 (1.4–4.9)	0.124
IL-33 (ng/mg)	6.5 (3.4–10.5)	7.5 (3.4–10.8)	0.617
IL-22 (pg/mg)	15.8 (6.6–29.9)	9.5 (4.8–19.2)	0.151
IgG1 (ng/mg)	51.2 (25.2–72.6)	62.5 (32.2–83.7)	0.463
IgG2 (ng/mg)	24.6 (17.8–35.7)	24.1 (16.7–52.4)	0.742
IgG3 (ng/mg)	13.6 (7–17.6)	25.3 (10.1–44.6)	0.020
IgG4 (ng/mg)	3.2 (1.4–5.6)	4.8 (2–9.4)	0.123
IgM (ng/mg)	5.1 (3.3–6.6)	5.5 (2.3–8.6)	0.859
IgE (pg/mg)	20.5 (8.9–43.5)	22.9 (7.2–34.3)	0.742

For continuous variables, results are expressed as medians and interquartile ranges, and the 2-tailed Mann-Whitney U test was used for between-group comparison. $P < 0.05$ was considered statistically significant. The P values in bold are less than 0.05 and indicate a statistical significance.

CRS, chronic rhinosinusitis; IL, interleukin; bFGF, basic fibroblast growth factor; G-CSF, granulocyte-colony stimulating factor; IFN- γ , interferon- γ ; IP-10, interferon-inducible protein-10; MCP-1, monocyte chemoattractant protein 1; MIP-1 α , macrophage inflammatory protein-1 α ; PDGF-BB, platelet-derived growth factor-BB; MIP-1 β , macrophage inflammatory protein-1 β ; TNF- α , tumor necrosis factor; VEGF, vascular endothelial growth factor; Ig, immunoglobulin.

order to identify the markers helpful in determining the effect of postoperative glucocorticoid and macrolide treatment. As a result, there were differences in several clinical parameters between refractory and non-refractory patients, including prior sinus surgery in both groups, headache VAS scores, total VAS scores, and endoscopic scores of crusting in the fluticasone propionate group, and loss of sense of smell VAS scores and overall burden of symptom VAS scores in the clarithromycin group. However, only headache VAS and overall burden of symptoms VAS scores were included in the classification trees and confirmed as secondary discriminative factors by the random forest model in the

fluticasone propionate and clarithromycin groups, respectively. No clinical parameters were present in the root of the classification trees, indicating that biomarkers might hold more remarkable power for predicting refractory CRS. Of note, several candidate clinical predictors were not present in the classification tree, although they showed significant differences between refractory and non-refractory patients, suggesting that they are less important in distinguishing between refractory and non-refractory patients. Consequently, they were absent from the classification tree.^{20,23} In this study, we included peripheral blood eosinophil absolute count and percentage as the clinical candidate

Table 4. The Difference in Biological Markers Between Refractory and Non-Refractory CRS in Clarithromycin Group.

Mediator	Non-refractory (n = 58)	Refractory (n = 17)	P-value
IL-1 β (pg/mg)	18.5 (13.7–37.3)	18.6 (8.4–28.5)	0.374
IL-1Ra (ng/mg)	2.4 (1.4–3.5)	1.6 (1.1–3)	0.137
IL-2 (pg/mg)	2.5 (1.5–3.6)	2.8 (1.3–5.4)	0.645
IL-4 (pg/mg)	153.9 (108.3–245.2)	129 (69.3–294.5)	0.632
IL-5 (pg/mg)	25 (13.5–50.3)	31.8 (14.9–56.4)	0.406
IL-6 (pg/mg)	89 (53.9–170.3)	83.9 (47.7–169.3)	0.767
IL-7 (pg/mg)	341.8 (234.4–498.3)	320.6 (215.4–443.7)	0.406
IL-8 (pg/mg)	700.7 (368.9–1355)	525.6 (278.4–835.3)	0.279
IL-9 (pg/mg)	117.8 (69.9–154.2)	90.4 (62.4–151.5)	0.562
IL-10 (pg/mg)	81.3 (44.3–161.3)	78.5 (24.1–124.9)	0.500
IL-12 (pg/mg)	69.5 (46.4–107.7)	75.5 (44.8–102)	0.729
IL-13 (pg/mg)	63.1 (30.9–84.3)	58.6 (33.7–89.5)	0.743
IL-15 (pg/mg)	17.4 (11.7–28.2)	20.8 (13.1–39.3)	0.424
IL-17A (pg/mg)	265.4 (190.5–440.8)	300.5 (181.4–430)	0.899
Eotaxin (pg/mg)	334.8 (174.7–603)	263.3 (138.4–440.6)	0.302
bFGF (pg/mg)	1391 (664.9–2461)	922.3 (670.8–2081)	0.296
G-CSF (pg/mg)	488.4 (249.3–1511)	270.4 (152.1–745.6)	0.127
IFN- γ (pg/mg)	324.4 (215–511.9)	255.6 (178.6–396.7)	0.442
IP-10 (ng/mg)	3.5 (2.2–9.2)	3.5 (1.7–8.9)	0.860
MCP-1 (pg/mg)	634.1 (426.8–1008)	625.7 (415.2–998.5)	0.950
MIP-1 α (pg/mg)	36.2 (21.8–62)	34.3 (13.6–55.4)	0.399
PDGF-BB (pg/mg)	317.3 (185.9–514)	286.6 (113.1–627.4)	0.687
MIP-1 β (pg/mg)	591.9 (355.9–873.7)	625.6 (352.1–781)	0.710
TNF- α (pg/mg)	172.7 (119.3–294.3)	130.3 (81.8–215.1)	0.131
VEGF (ng/mg)	1.4 (0.8–2.4)	1.0 (0.7–2.5)	0.562
IL-25 (pg/mg)	4.2 (2.5–6.6)	3.6 (2.4–7.7)	0.682
IL-33 (ng/mg)	6.9 (4–10)	6.1 (4.3–7.5)	0.293
IL-22 (pg/mg)	16.6 (9.4–25.7)	19.9 (14.3–47.2)	0.305
IgG1 (ng/mg)	62.6 (47–78.7)	70 (35.5–81.5)	0.960
IgG2 (ng/mg)	29.8 (18.3–48.4)	33.9 (14–53.9)	0.990
IgG3 (ng/mg)	14.4 (7.4–26.8)	17.3 (6.8–45.4.2)	0.420
IgG4 (ng/mg)	4.3 (1.7–6.7)	8.4 (4.5–13.8)	0.004
IgM (ng/mg)	4.3 (2.5–7.2)	7.8 (3.2–10.6)	0.022
IgE (pg/mg)	25.9 (12.7–60.6)	68.7 (17–111.9)	0.011

For continuous variables, results are expressed as medians and interquartile ranges, and the 2-tailed Mann-Whitney U test was used for between-group comparison. $P < 0.05$ was considered statistically significant. The P values in bold are less than 0.05 and indicate a statistical significance.

CRS, chronic rhinosinusitis; IL, interleukin; bFGF, basic fibroblast growth factor; G-CSF, granulocyte-colony stimulating factor; IFN- γ , interferon- γ ; IP-10, interferon-inducible protein-10; MCP-1, monocyte chemoattractant protein 1; MIP-1 α , macrophage inflammatory protein-1 α ; PDGF-BB, platelet-derived growth factor-BB; MIP-1 β , macrophage inflammatory protein-1 β ; TNF- α , tumor necrosis factor; VEGF, vascular endothelial growth factor; Ig, immunoglobulin.

predictors. Consistent with our previous studies,⁹ we could not identify the peripheral blood eosinophil count as a key determinative marker to predict the response to both postoperative glucocorticoid- and macrolide-based treatment. This is also in line with the result that no significant difference in the ratio of patients with eosinophilic CRSwNP was found between refractory and non-refractory patients in both treatments (Tables 1 and 2), confirming the limited predictive value of eosinophil for the postoperative treatments.

We found that refractory CRS subjects in the fluticasone propionate group demonstrated high levels of IL-8, G-CSF, and IgG3 in diseased nasal tissues. Interestingly, IL-8 was present in the root of the classification tree as

an important discriminating factor for predicting refractory CRS in the fluticasone propionate group. Notably, high levels of IL-8 usually represent neutrophilic inflammation. Therefore, this finding is consistent with those of our previous study showing that increased neutrophilia in nasal polyps reduced the response to oral corticosteroid therapy.¹⁶ Previous studies found that IL-25 and Charcot-Leyden crystals might predict the pre-operative efficacy of glucocorticoids in CRS patients,^{17,18} and IL-5 was elevated in recurrent CRSwNP.^{26,27} However, here, we did not find significant differences in the baseline IL-5, IL-4, eotaxin, or IL-25 levels between refractory and non-refractory cases in the fluticasone propionate group. Compared

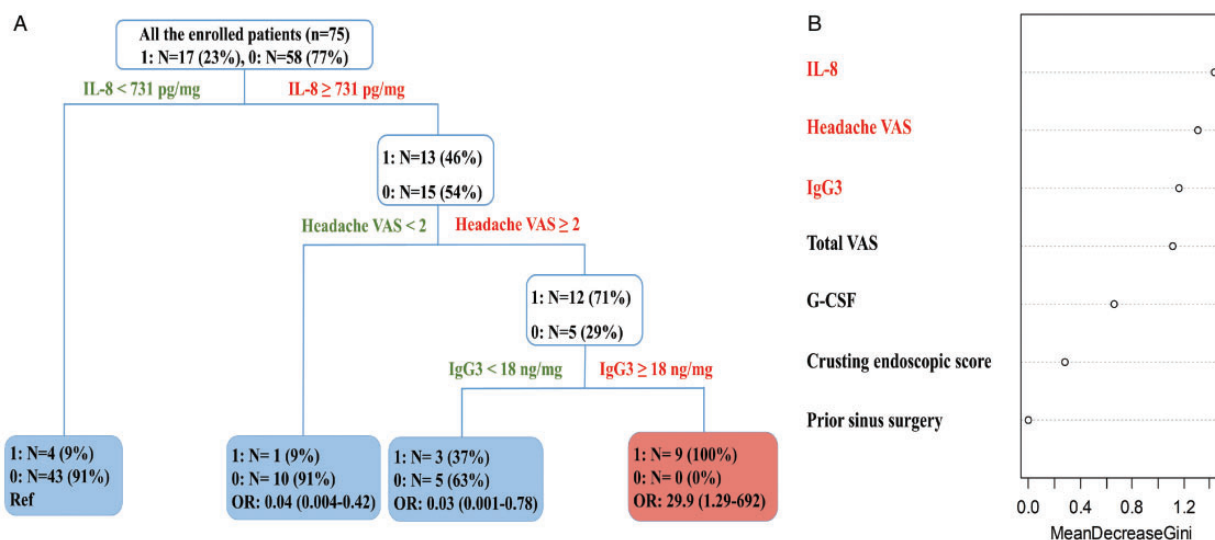


Figure 1. Classification tree and random forest analysis of predictors for refractory chronic rhinosinusitis (CRS) in patients with nasal fluticasone propionate based postoperative treatment. A, The classification tree was developed by tissue expression level of IL-8 and IgG3, and headache VAS score. B, Mean Decrease Gini represents the relative predictive importance of each variable. Presence or absence of refractory chronic rhinosinusitis = “1” or “0”. The Odds ratios (OR) was given with 95% confidence interval (CI) in comparison with the IL-8 < 731 pg/mg group, which was set as a reference (Ref). IL, interleukin; VAS, visual analog scale; Ig, immunoglobulin; G-CSF, granulocyte-stimulating factor.

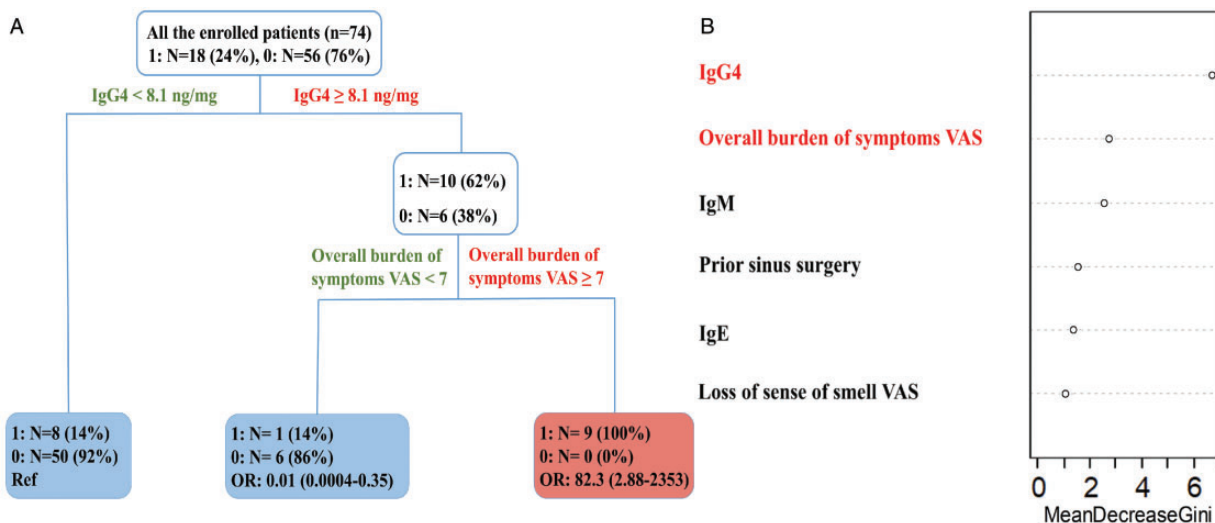


Figure 2. Classification tree and random forest analysis of predictors for refractory chronic rhinosinusitis (CRS) in patients with clarithromycin based postoperative treatment. A, The classification tree was developed by tissue expression level of IgG4 and overall burden of symptoms VAS score. B, Mean Decrease Gini represents the relative predictive importance of each variable. Presence or absence of refractory chronic rhinosinusitis = “1” or “0”. The Odds ratios (OR) was given with 95% confidence interval (CI) in comparison with the IgG4 < 8.1 ng/mg group, which was set as a reference (Ref). Ig, immunoglobulin; VAS, visual analog scale.

to preoperative treatment, the response to postoperative treatment may be modified by the alteration of inflammation pattern and intensity, and anatomical structures after surgery.^{25,28,29} Nevertheless, further studies are needed to evaluate the value of other eosinophilic inflammation-relevant biomarkers such as Charcot-Leyden crystals and eosinophil cationic proteins which

were not included in the current study in predicting postoperative glucocorticoid treatment. Our results highlight the fact that the CRS patients with relatively high levels of IL-8, headache VAS scores, and high levels of IgG3 bear a significant risk of being refractory to glucocorticoid-based postoperative treatment with an OR of up to 29.9. As a predictive tool, the classification

tree provides a good approach to predict refractory CRS in patients with nasal fluticasone propionate-based postoperative treatment, attaining an overall predictive accuracy of 89.3%.

Regarding patients with clarithromycin-based postoperative treatment, upregulated levels of IgG4, IgM, and IgE were found to be associated with refractory disease. In the following classification tree and random forest analyses, only IgG4 was proven to be an important discriminating factor and was present in the root of the tree. IgG4 is generally considered to be a non-inflammatory immunoglobulin due to its limited ability to fix complement and bind to Fc receptors.³⁰ IgG4 might act as a neutralizing antibody that competes with IgE for allergen binding and prevents the activation and degranulation of effector cells.^{31–33} Recently, it has been shown that IgE and IgG4 levels may be closely correlated in nasal polyps,^{9,34} and they may both represent a type-2 skewed immune microenvironment. In fact, the expression level of IgG4 is much higher than that of IgE in local tissues,⁹ making it easier to detect and more sensitive to reflect local inflammation. This might be the reason that IgE had a much smaller mean decrease in Gini index and was not included in the prediction model. Therefore, our results indicated that the response of CRS patients to clarithromycin might be impaired by the skewed type-2 inflammatory microenvironment. Coincidentally, Wallwork et al. found that long-term low-dose macrolide treatment was only efficient for CRSsNP patients without elevated serum IgE levels, suggesting a better efficacy of macrolides for patients with non-type-2 inflammation.³ The classification tree of the clarithromycin group achieved an overall predictive accuracy of 87.8% and identified a group of CRS patients with relatively high levels of IgG4 and overall burden of symptom VAS score to have a high risk of developing refractory disease (odds ratio of 82.3). Based on the predictive tools, further efforts to detect effective treatments for patients in the high-risk groups in both postoperative treatment groups are needed.

With regard to refractory CRS, the previously-reported prediction models were mostly developed by multivariate logistic regression analysis and were difficult to implement in clinical practice, especially for non-statisticians.^{7,8} For the first time, the present study used a classification tree analysis to comprehensively evaluate the hierarchy of clinical and biological predictors associated with CRS outcomes after different postoperative anti-inflammatory treatments. A classification tree with visualizable risk factors was generated, and the results could be easily interpreted for clinical application. The overall accuracy of this prediction model was 89.3% and 87.8% in the fluticasone propionate and clarithromycin groups, respectively, which were higher than those of the prediction models generated by multivariate logistic

regression analysis (data not shown). In addition, we performed a random forest analysis to verify the predictive importance of variables and confirm the results of the classification tree analysis.

Our present study demonstrated that IL-8 and IgG4 were important discriminating factors for predicting refractory CRS in the fluticasone propionate and clarithromycin groups, respectively. Thus, in line with the findings of preoperative treatment, our study suggests that postoperative nasal steroids may be ineffective for CRS patients with neutrophilic inflammation, and macrolides are unlikely to work on patients with type 2 inflammation.^{3,16} However, it should be noted that by using either IL-8 or IgG4 alone, we failed to develop a model with acceptable predictive accuracy. Therefore, the classification trees built by the combination of clinical and biological parameters are more precise tools for identifying patients eligible for postoperative fluticasone propionate or clarithromycin treatment.

We acknowledge that there are several limitations to our current study. First, the statistical power might not be sufficient for this post-hoc study. Second, the subjects with different phenotypic CRS were pooled together in this post-hoc analysis because of the limited number of refractory cases. We were unable to detect the potential predictors of CRSsNP and CRSwNP separately. However, recent studies have demonstrated that a type 2 immune response was also observed in a number of patients with CRSsNP, which was previously considered a type 1 disease.³⁵ Therefore, the analysis of pooled CRS regardless of phenotype might not limit the efficacy of the prediction model but instead be beneficial for detecting the predictors that are closely related to the intrinsic inflammatory patterns of CRS. Third, although the predictive accuracy is high for patients classified under high risk, the predictive accuracy for other patients needs to be improved. Fourth, the efficacy of our predictive models needs to be confirmed by further studies. Fifth, our models rely on the measurement of tissue cytokine and immunoglobulin levels, which might not be feasible in some clinical settings. Sixth, the standard operating procedure for the measurement of molecular markers in nasal tissue should be established before the generalization of our models.

Conclusion

Classification trees built by clinical and biological parameters provide new visualized and simplified tools to predict the response to fluticasone propionate- and clarithromycin-based treatments after ESS. These models will be helpful in guiding the clinical choice of postoperative therapy for patients with CRS.

Author Contributions

ZL designed the study, performed endoscopic examination and surgery, and prepared the manuscript. MZ designed the study, analyzed the data, and prepared the manuscript. H.W. participated in subject enrollment. BL, HW, and X-BL collected surgical samples and performed the Bio-Plex assay. JM and J-XL performed the symptom severity evaluation.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was supported by grants from the Natural Science Foundation of Hubei Province of China [2017CFA016 (Z.L.) and 2018CFB602 (M.Z.)], National Key R&D Program of China (2018YFC0116800), and National Natural Science Foundation of China (NSFC) [81630024 and 81920108011 (Z.L.), and 81873694 (H.W.)].

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