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Original Article

Serum IgG4 as a biomarker reflecting pathophysiology and post-operative recurrence in chronic rhinosinusitis

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Abbreviations:
AERD, aspirin-exacerbated respiratory disease; AUC, area under the curve; CRS, chronic rhinosinusitis; CRSsNP, chronic rhinosinusitis without nasal polyps; CRSwNP, chronic rhinosinusitis with nasal polyps; CT, computed tomography; FEV_1/FVC, 1-s forced expiratory volume/forced vital capacity; ECRS, eosinophilic chronic rhinosinusitis; IgG4-RD, IgG4-related

ABSTRACT

Background: Type 2 chronic rhinosinusitis (CRS), especially eosinophilic CRS (ECRS), is an intractable upper airway inflammatory disease. Establishment of serum biomarkers reflecting the pathophysiology of CRS is desirable in a clinical setting. As IgG4 production is regulated by type 2 cytokines, we sought to determine whether serum IgG4 levels can be used as a biomarker for CRS.
Methods: Association between the serum IgG4 levels and clinicopathological factors was analyzed in 336 CRS patients. Receiver operating characteristics (ROC) analysis was performed to determine the cut-off value of serum IgG4 levels that can be used to predict the post-operative recurrence.
Results: Serum IgG4 levels were significantly higher in patients with moderate to severe ECRS versus those with non to mild ECRS. The levels were also significantly higher in asthmatic patients and patients exhibiting recurrence after surgery compared to controls. ROC analysis determined that the best cut-off value for the serum IgG4 level to predict the post-operative recurrence was 95 mg/dL. The corresponding sensitivity and specificity were 39.7% and 80.5%, respectively. When we combined the two cut-off values for the serum IgG4 and periostin, patients with high serum levels of either IgG4 or periostin exhibited a high post-operative recurrence (OR: 3.95) as compared to patients having low serum levels of both IgG4 and periostin.
Conclusions: The present results demonstrate that the serum IgG4 level is associated with disease severity and post-operative course in CRS. In particular, the combination of serum IgG4 and periostin could be a novel biomarker that predicts post-operative recurrence.
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diseases; JESREC, Japanese epidemiological survey of refractory eosinophilic chronic rhinosinusitis; NP, nasal polyps; NSAIDs, non-steroidal anti-inflammatory drugs; ROC, receiver operating characteristics

Introduction

Chronic rhinosinusitis (CRS) is a prevalent inflammatory disease in the upper airway. It has been shown that some phenotypes/endotypes, especially moderate to severe eosinophilic chronic rhinosinusitis (ECRS), are comorbid with asthma and refractory to pharmacotherapy and/or surgery.[1,2] Although the precise etiology and pathophysiology underlying intractable CRS remain poorly understood, altered humoral immunity, including IgG, IgA, IgD and IgE, appears to be involved.[3–8] For example, we have recently reported that the number of IgG4-positive cells was significantly higher in nasal polyp (NP), especially in severe ECRS patients, as compared to that in uncinate tissue. The expression was significantly higher in asthmatic patients, especially those with aspirin-exacerbated respiratory diseases (AERD), versus non-asthmatic patients. In addition, the number of infiltrating IgG4-positive cells was significantly higher in patients with a poor post-operative course (sustained sinus shadow 6 months after surgery) as compared to patients with a good post-operative course. This suggests that local expression of IgG4 on cells is associated with the pathophysiology of CRS, including eosinophilia, asthma comorbidity and post-operative course.[8]

IgG4 is the least abundant IgG subclass in human serum, comprising approximately 5% of the total IgG.[9] The production of IgG4 is regulated by IL-10 together with a co-stimulatory signal through CD40/CD40L and type 2 cytokines of IL-4 and IL-13, which are crucial factors for IgE production.[10] It has been reported that either non-specific or antigen-specific serum IgG4 could potentially be a biomarker for type 2 inflammatory diseases.[8,9] It has also been established that serum antigen-specific IgG4 is elevated after allergen immunotherapy.[11] Elevation of serum IgG4 has additionally been seen in patients with AERD, nasal polyposis, eosinophilia and celiac disease.[12] Elevated serum total IgG4 has also been reported in patients with IgG4-related diseases (IgG4-RD).[13]

As previously discussed above, our recent report presented preliminary results on 17 serum samples that showed a significant and positive correlation between the numbers of infiltrating IgG4-positive cells in the tissues and serum levels of IgG4, which suggests that the serum IgG4 level is associated with the severity in ECRS.[8] In addition, we have recently showed that serum level of periostin, a matricellular protein induced by type 2 cytokines such as IL-4 and IL-13, increased significantly along with the severity of ECRS. Post-operative recurrence rate was significantly higher in patients with high serum periostin level.[14,15] In our present study, we expanded the preliminary study and further analyzed a larger number of serum samples (n = 336) in order to determine whether the level of serum IgG4, alone or in combination with periostin, can be used for a biomarker that reflects the pathophysiology of CRS, including the prediction of the post-operative recurrence.

Methods

Patients

Patients with CRS (n = 336) having an indication for and undergoing endoscopic sinus surgery (ESS) were enrolled from 5 university hospitals as per our previously described method.[14] Thirteen patients received oral glucocorticoid at serum collection. CRS and its phenotypes, CRS without nasal polyps (CRSsNP: n = 58) and CRS with nasal polyps (CRSwNP: n = 278) were diagnosed based on the clinical definition reported by the European Position Paper on Rhinosinusitis and Nasal Polyps 2012.[16] None of the patients met the diagnostic criteria for IgG4-RD.[13] Patients were divided into non-ECRS (n = 119), mild ECRS (n = 57), moderate ECRS (n = 94) and severe ECRS (n = 66) groups based on the Japanese Epidemiological Survey of Refractory Eosinophilic Chronic Rhinosinusitis (JESREC) criteria.[1] The characteristics of the subjects in each group are shown in Table 1. Serum samples were collected prior to the surgery, and stored at −20 °C until use. Post-operative recurrence was defined by an otorhinolaryngologist as the occurrence of the condition with NPs or purulent discharge in the middle meatus for more than 28 days after the surgery as shown by a nasal endoscope.[14] Since oral glucocorticoid treatment is effective for ECRS and may decrease the rate of recurrence, a substantial number of subjects received short-course oral glucocorticoid treatment after surgery.[15] For example, 76 out of 85 patients in one institution (89.4%) received the treatment subsequent to surgery for preventing post-operative inflammation or when edema formation in paranasal sinus was occurred during the post-operative period. Informed consent was obtained from each of the patients. The ethical committees of the Department of Otorhinolaryngology Head & Neck Surgery, University of Fukui; Department of Otolaryngology - Head & Neck Surgery, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences; Department of Otolaryngology, Jichi Medical University Saitama Medical Center; Department of Otorhinolaryngology, Head & Neck Surgery, Dokkyo Medical University and Department of

Table 1
Subject characteristics.

Groups	Non-ECRS	Mild ECRS	Moderate ECRS	Severe ECRS
Number	119	57	94	66
Age (years old)	54.3 (13–86)	53.8 (22–75)	52.9 (20–78)	54.0 (26–82)
Sex (female/male)	48/71	12/45	29/65	35/31
Blood eosinophil rate (%)	2.6 (0.2–15.8)	4.7 (2.2–15.3)	6.6 (2.1–15.1)	11.0 (5.1–26.0)
Serum total IgE (IU/mL)	274.9 (5–2385)	295 (20–1326)	398 (11–2580)	516 (43–4266)
FEV_1/FVC ratio (%)	97.0 (53.6–140)	93.1 (67.2–135)	98.6 (68.0–129.9)	82.1 (51.7–101.3)
Comorbidity of Asthma (n)	23	0	39	63
aspirin-exacerbated respiratory diseases (n)	4	0	10	15

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Otorhinolaryngology, Yokohama City Medical Center approved this research and study protocol.

Determination of serum IgG4 and periostin

Levels of serum IgG4 were determined using IgG4 Human ELISA Kit (Thermo Fisher Scientific, Waltham, MA) according to the manufacturer's instructions. Determination of serum levels of periostin was performed as per a previously reported method.[14]

Statistical analysis

Values are given as the median. The nonparametric Mann–Whitney U test was used to compare data between the groups, and Wilcoxon's signed rank test was used to analyze data within each group. A Kruskal–Wallis test followed by a Dunn's test was used for multiple comparisons. Correlation analyses were performed using Spearman's rank correlation. Statistical analyses were performed with GraphPad Prism 6 software (GraphPad Software, La Jolla, CA). Receiver operating characteristics (ROC) analyses were performed to determine the cut-off values for the serum IgG4 and *P*-values for sensitivity and specificity. Recurrence-free curves after surgery were drawn using the Kaplan–Meier method where recurrence-free rate was calculated a percentage of patients without recurrence in each time point. All values were calculated using JMP Pro 14.0 (SAS Institute, Cary, NC), while logistic regression analyses were conducted by STATA 12.1 (StataCorp, College Station, TX). *P*-values less than 0.05 (two-tailed) were considered to be statistically significant.

Results

Comparison of serum IgG4 levels among the phenotypes of CRS

Levels of serum IgG4 were significantly higher in CRSwNP patients (median: 45.0 mg/dL, n = 278) as compared to the CRSsNP patients (median: 28.5 mg/dL, n = 58) (P = 0.001; Mann–Whitney U test, Fig. 1A). When we divided patients into non-ECRS and ECRS groups according to the JESREC criteria, the levels were significantly higher in the ECRS patients (median: 52.0 mg/dL, n = 217) versus the non-ECRS patients (median: 31.0 mg/dL, n = 119) (P < 0.001, Fig. 1B). Similar results were seen when CRSwNP patients were selected and divided into non-ECRS (median: 34.5 mg/dL, n = 72) and ECRS (median: 52.0 mg/dL, n = 206) groups (P = 0.029). When we precisely divided CRS patients into four groups (non, mild, moderate and severe ECRS), the Kruskal–Wallis test revealed a significant difference in the level of the serum IgG4 among these groups (P < 0.001). Dunn's test further showed that the level was significantly higher in the moderate (median: 52.5 mg/dL, P = 0.016) and severe (median: 52.5 mg/dL, P < 0.001) but not the mild (median: 42.0 mg/dL, P = 0.887) ECRS patients as compared to the non-ECRS (median: 31.0 mg/dL) patients (Fig. 1C). The level was similar between patients with and without pre-operative oral glucocorticoid treatment (median: 54.0 vs 42.0 mg/dL, P = 0.415).

Pathophysiological significance of serum IgG4 level in CRS

Subsequently, we then pathophysiologically characterized the level of serum IgG4 in CRS. There was a significant positive and weak correlation between the level of serum IgG4 and eosinophils in the sinonasal tissues (n = 193, r = 0.258, *P* < 0.001; Fig. 2A). The level was also weakly albeit significantly and positively correlated with the peripheral blood eosinophilia (n = 336, r = 0.202, *P* < 0.001; Fig. 2B). No statistically significant correlations were seen between the serum IgG4 level and the serum IgE level (n = 96, r = 0.177, *P* = 0.084; Fig. 2C) or the FEV_1/FVC ratio (n = 101, r = −0.056, *P* = 0.580; Fig. 2D). Since these data were collected from independent 5 university hospitals, some were missing, leading to unequal numbers of patients. The patients were divided into non-asthmatic (n = 211) and asthmatic (n = 125) groups. Levels of serum IgG4 were significantly higher in the asthmatic patients (median: 57.0 mg/dL) versus the non-asthmatic (median: 37.0 mg/dL) patients (P < 0.001; Fig. 2E). AERD was seen in 34 cases. Serum

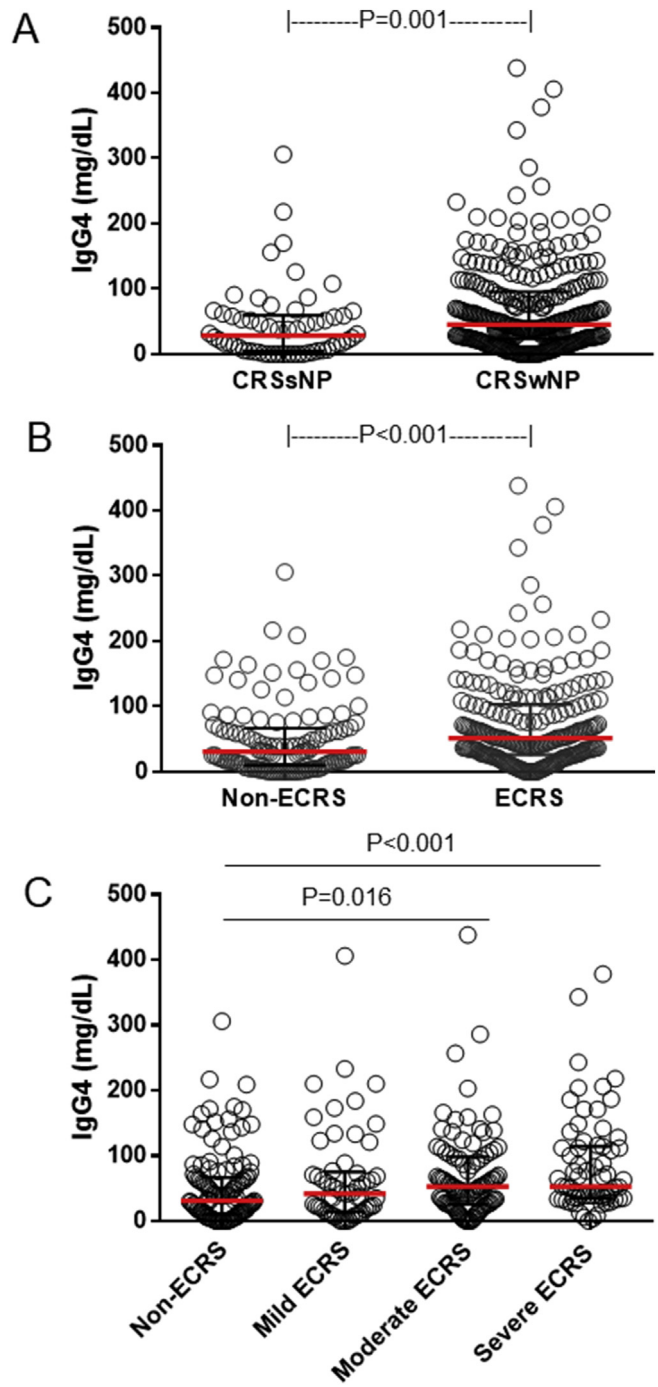

Fig. 1. Comparison of serum IgG4 levels among the phenotypes of CRS. **A:** Comparison between CRSsNP and CRSwNP patients. **B:** Comparison between non-ECRS and ECRS patients. **C:** Comparison among four groups of the non, mild, moderate and severe ECRS patients. P value was determined by a Mann–Whitney U test (A and B) and Dunn's test (C).

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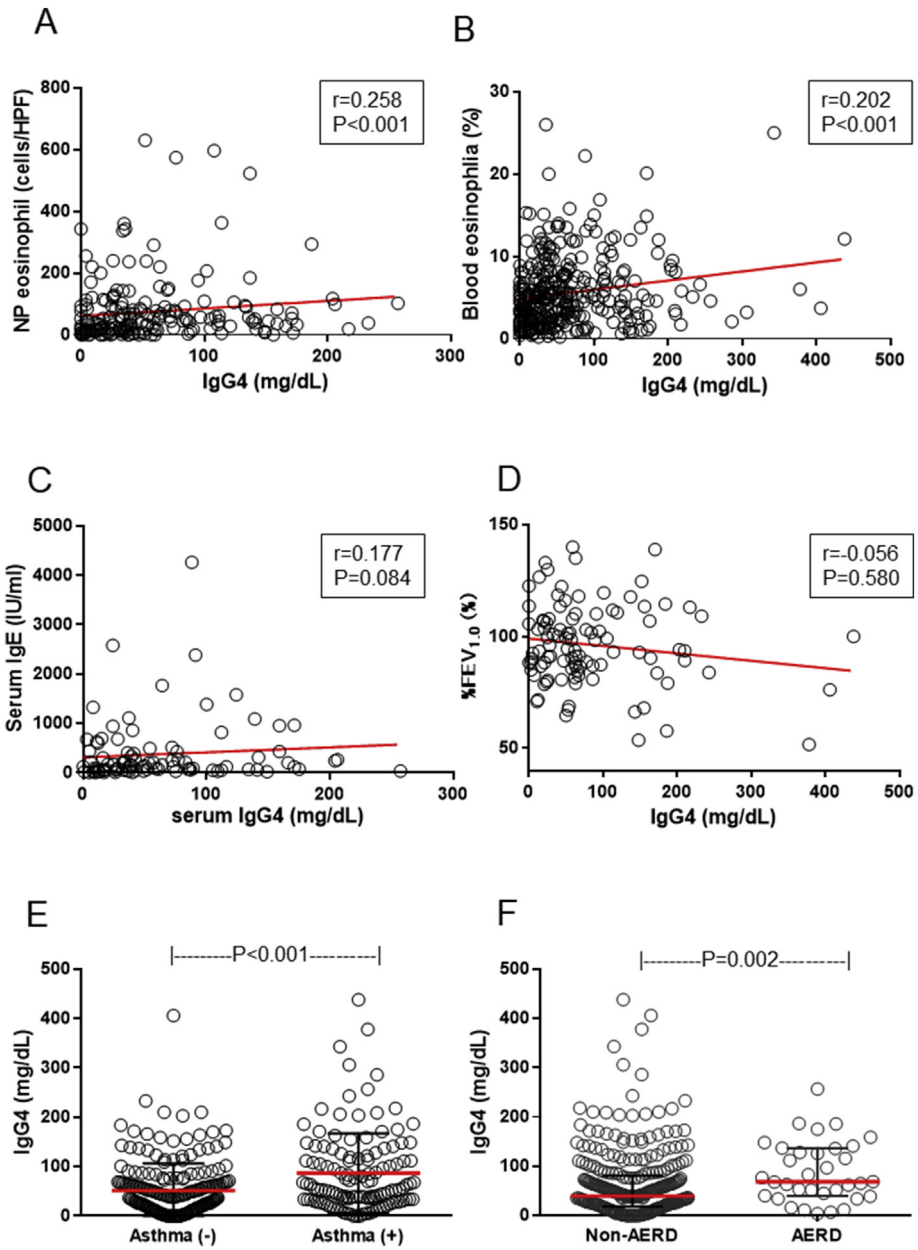

Fig. 2. Relationship between the serum IgG4 levels and pathophysiological characterizations of CRS including eosinophilia in the sinonasal tissues **(A)**, peripheral blood eosinophilia **(B)**, serum IgE level **(C)** and FEV_1/FVC ratio **(D)**. Comparison of serum IgG4 level between non-asthmatic and asthmatic patients **(E)**, and non-ARED and AERD patients **(F)**. P value was determined by a Mann–Whitney U test **(E, F).**

IgG4 levels were also significantly higher in the AERD (median: 68.5 mg/dL) patients versus the non-AERD (median: 39.5 mg/dL) patients (P = 0.002; Fig. 2F).

Significance of serum IgG4 level on the post-operative recurrence in CRS

There were 253 patients who could be followed throughout the post-operative course. There were 58 patients (22.9%) who showed post-operative recurrence. Serum IgG4 levels were significantly higher in patients exhibiting recurrence (median: 52.5 mg/dL, median follow-up period: 40.1 months) as compared to those without recurrence (median: 37.0 mg/dL, median follow-up period: 12.5 months) (P = 0.011; Fig. 3A).

Next, we used the ROC analysis to assess the possibility of using serum IgG4 levels as a biomarker for predicting the post-operative course in patients with CRS. The area under the curve (AUC) was 0.610 (95% CI: 0.528–0.693) (Fig. 3B). This analysis indicated that the best cut-off for serum IgG4 was 95 mg/dL based on the best positive likelihood ratio of 2.05, with the corresponding sensitivity and specificity to predict the recurrence at 39.7% (95% CI: 27.1–53.4) and 80.5% (95% CI: 74.3–85.8), respectively. With this cut-off value, the Kaplan–Meier plot of the post-operative recurrence showed a significant difference between the low serum IgG4 (less than 95 mg/dL, n = 192) and high serum IgG4 (equal to or more than 95 mg/dL, n = 61) groups (P = 0.004; log-rank test). In high IgG4 group, a patient with the longest follow-up period (4.65 years) showed a recurrence, whereas such a patient in low IgG4 group (follow-up period: 8.08 years) kept a recurrence-free status (Fig. 3C).

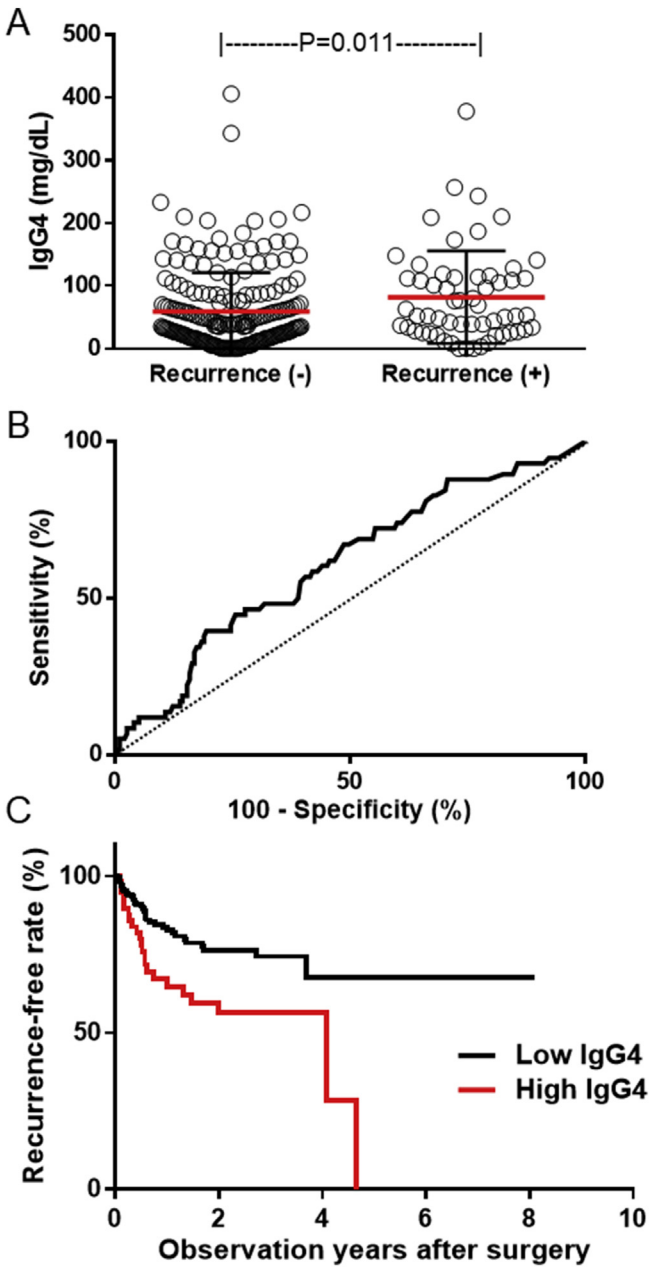

Fig. 3. Significance of the serum IgG4 level on the post-operative recurrence in CRS. **A:** Comparison of serum IgG4 levels between patients with recurrence and those without recurrence. **B:** ROC curve for the serum IgG4 levels that were able to predict the post-operative course. **C:** Kaplan–Meier plot of post-operative recurrence between the groups of low (<95 mg/dL: black line) and high (≥95 mg/dL: red line) serum IgG4. P value was determined by a Mann–Whitney U test.

Use of serum IgG4 and periostin level for the prediction of post-operative recurrence of CRSwNP

We have recently reported that when the cut-off value is set at 115.5 ng/ml, serum periostin could be used as a biomarker for the prediction of post-operative recurrence of CRSwNP.[14] Therefore, we sought to determine whether the simultaneous use of serum IgG4 and periostin levels would improve the prediction. There was few correlation noted between the serum periostin and IgG4 levels ($r = 0.149$, 95% CI: 0.039–0.255, $P = 0.006$; Fig. 4A). When using these two cut-off values, the Kaplan–Meier plot of the post-operative recurrence showed there was a significant difference between the four groups (low periostin/low IgG4: $n = 111$, low periostin/high IgG4: $n = 81$, high periostin/low IgG4: $n = 34$, high periostin/high IgG4: $n = 27$, $P < 0.001$; log-rank test, Fig. 4B). Logistic regression analysis showed that the odds ratio (OR) for a poor post-operative course was 3.95 (95% CI: 1.97–7.92) in patients having a high serum level of either periostin or IgG4 as compared to patients who had a low serum level of both periostin and IgG4 ($P < 0.001$; Fig. 4C). This relationship essentially remained the same even after we adjusted for the age and sex (OR = 3.85: 95% CI: 1.92–7.69, $P < 0.001$), presence of NP and ECRS in addition to age and sex (OR = 3.57: 95% CI: 1.69–7.14, $P < 0.001$), and institution where the ESS was performed in addition to the age, sex, presence of NP and ECRS (OR = 3.33: 95% CI: 1.56–7.14, $P = 0.002$).

Discussion

The present study examined the serum IgG4 levels in CRS, especially ECRS. We found that moderate to severe ECRS exhibited higher serum IgG4 levels. The level was significantly and positively correlated with the degree of local and blood eosinophilia. In addition, the serum IgG4 level was associated with post-operative recurrence. Furthermore, patients having either a high serum IgG4 or high serum periostin also exhibited a high post-operative recurrence as compared to those patients having low serum levels of both IgG4 and periostin. These results may provide a basis for the diagnostic use of serum IgG4 together with periostin as a biomarker for not only confirming the severity of ECRS, but also for predicting the outcome after surgery.

Serum IgG4 was significantly higher in ECRS patients as compared with non-ECRS patients. IgG4 production is regulated by type 2 (IL-4 and IL-13) and regulatory (IL-10) cytokines, all of which are known to be induced in ECRS.[17–20] The precise regulation of IgG4 production remains unclear. We have recently reported that the production of IL-10 in response to *Staphylococcus aureus* enterotoxin, which is a candidate thought to be involved in the pathogenesis of ECRS, is lower in patients with ECRS as compared to those with non-ECRS.[20] Thus, the presence of IL-10 but not the amount of IL-10 may play an important role in the production of IgG4 in ECRS.

Moderate to severe ECRS patients, as determined by the JESREC criterion, showed a significant elevation of the serum IgG4 as compared with the non to mild ECRS patients. This result was similar to our previous finding that severe ECRS was associated with an augmented infiltration of IgG4-positive cells into the NP.[8] A positive correlation with the blood/tissue eosinophilia was also found for both the level of the serum IgG4 and the number of tissue IgG4-positive cells. Moderate to severe ECRS is categorized as a designated intractable disease in Japan and thus, patients are able to use the medical expense support system to cover the cost of treatments. Although the role of IgG4 in the pathogenesis of CRS remains unclear, the serum IgG4 level could potentially become a biomarker that can be used to distinguish the intractable phenotype of CRS, which may also be useful as a certification that can be utilized for determining eligibility for the medical support system. The possibility that comorbid asthma is associated with the refractory disease in CRS was supported by our finding that asthmatic CRS patients exhibited a significant elevation of the serum IgG4 as compared to the non-asthmatic CRS patients.[2]

The serum IgG4 level was also correlated with the post-operative recurrence. After we set 95 mg/dL as a cut-off level, we were able to predict the post-operative recurrence at 39.7% sensitivity and 80.5% specificity. 39.7% of sensitivity may be a low value. However, we selected this cut-off point based on best positive likelihood ratio of 2.05. If we increase the sensitivity, specificity must be decreased. For example, when we set the cut-off point as

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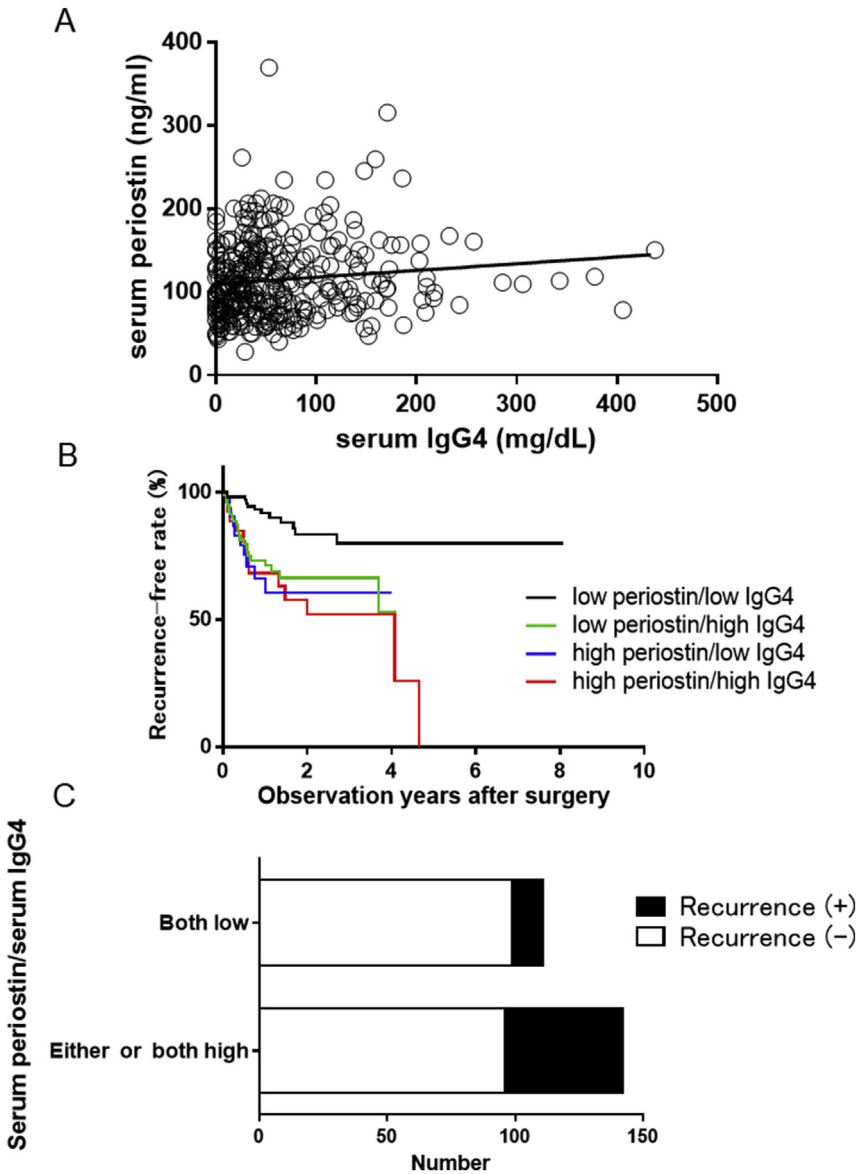

Fig. 4. Use of serum IgG4 and periostin levels for the prediction of the post-operative recurrence of CRSwNP. **A:** Correlation between the serum periostin and IgG4 levels. **B:** Kaplan−Meier plot for post-operative recurrence of the four groups based on the cut-off values of periostin and IgG4, which included low periostin/low IgG4 (black line), low periostin/high IgG4 (green line), high periostin/low IgG4 (cyan line) and high periostin/high IgG4 (red line). **C:** Comparison of the number of patients with or without recurrence after surgery between patients showing both low serum periostin and IgG4 and those showing high periostin and/or high IgG4.

25.0 mg/dL, sensitivity and specificity is 81.0 and 33.9%, respectively, where the positive likelihood ratio becomes 1.22. This result was also similar to our previous finding that the number of IgG4-positive cells in the NP was correlated with improvement of the Lund−Mackay CT grading score after surgery, where the residual sinus shadow in CT could be predicted at 73.3% sensitivity and 82.5% specificity.[8] One of the reasons for the difference in the sensitivity is related to the difference of the endpoints after the surgery. The primary endpoint after surgery in the present study was the post-operative recurrence, which was based on the endoscopic results of our previous study that found there was no NP formation or purulent discharge for more than 28 days. On the other hand, the post-operative course was defined as poor when the post-operative CT score was equal to more than half of the pre-operative scores in the previous study. Although the local expression of IgG4-positive cells seems to be more sensitive due to a closer reflection of the local inflammation, serum samples were more suitable as a clinical biomarker due to the ease of collecting samples. Measurement of serum IgG4 is available in clinical settings in Japan and used to diagnose IgG4-RD.[21]

In order to predict the post-operative recurrence of CRSwNP, we have recently reported that serum periostin can be utilized as a biomarker, when the cut-off value is set to 115.5 ng/ml with 60.7% sensitivity and 61.9% specificity.[14] When we combined the two serum cut-off values for IgG4 and periostin, patients with high serum levels of either periostin or IgG4 exhibited a high post-operative recurrence (OR: 3.95) as compared to those patients who showed low serum levels of both periostin and IgG4. Furthermore, the OR was higher than that for the single cut-off value in IgG4 OR: 2.72 (95% CI: 1.44−5.12) and in periostin OR: 2.80 (95% CI: 1.53−5.12). These results suggest that the combination use of serum IgG4 and serum periostin might be a more valuable biomarker for predicting the post-operative course as compared to when using a single estimation. To avoid any

postoperative recurrence, more precise follow-ups and intensive post-operative treatments such as biologics may be required in cases having either a high serum IgG4 or high serum periostin. On the other hand, the present stud.

In conclusion, our results demonstrated that the serum IgG4 level is associated with disease severity and the post-operative course in CRS, especially for moderate to severe ECRS. In particular, the combination of serum IgG4 and periostin might be a potential novel biomarker that can be used to predict the post-operative course. However, the role of IgG4 in the pathogenesis of CRS remains unclear and a further study will need to be undertaken. Since refractory CRS is resistant to conventional pharmacological treatment and surgery, new treatments that are currently under development including the use of biologics such as anti-IL-4/IL-13 receptor antibody may become a treatment option in the future. Additional studies to determine whether these serum markers, either alone or in combination, are useful in predicting the efficacy of new treatments will need to be undertaken.

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Conflict of interest

The authors have no conflict of interest to declare.

Authors' contributions

AO, TN, KN, SF and MO designed the study and wrote the manuscript. SH, NY, YSak, THar, THig, SK, TK, TT, YI, MS and MK contributed to collection of serum samples and patients' information. TF, YSat and YG contributed to determination of serum IgG4 level. AM and ST performed the statistical analysis. KI and JO contributed to interpretation of the results regarding periostin. MT contributed to interpretation of the results regarding asthma and AERD.

References

1. Tokunaga T, Sakashita M, Haruna T, Asaka D, Takeno S, Ikeda H, et al. Novel scoring system and algorithm for classifying chronic rhinosinusitis: the JESREC study. *Allergy* 2015;**70**:995–1003.
2. Okano M, Kariya S, Ohta N, Imoto Y, Fujieda S, Nishizaki K. Association and management of eosinophilic inflammation in upper and lower airways. *Allergol Int* 2015;**64**:131–8.
3. Baba S, Kondo K, Toma-Hirano K, Kanaya K, Suzukawa K, Ushio M, et al. Local increase in IgE and class switch recombination to IgE in nasal polyps in chronic rhinosinusitis. *Clin Exp Allergy* 2014;**44**:701–12.
4. Gevaert P, Nouri-Aria KT, Wu H, Harper C, Takhar P, Fear DJ, et al. Local receptor revision and class switching to IgE in chronic rhinosinusitis with nasal polyps. *Allergy* 2013;**68**:55–63.
5. Tan BK, Li Q, Suh L, Kato A, Conley DB, Chandra RK, et al. Evidence for intranasal antinuclear autoantibodies in patients with chronic rhinosinusitis with nasal polyps. *J Allergy Clin Immunol* 2011;**128**:1198–206.
6. Min JY, Nayak JY, Hulse KE, Stevens WW, Raju PA, Huang JH, et al. Evidence for altered levels of IgD in the nasal airway mucosa of patients with chronic rhinosinusitis. *J Allergy Clin Immunol* 2017;**140**:1562–71.
7. Zhai GT, Wang H, Li JX, Cao PP, Jiang WX, Song J, et al. IgD-activated mast cells induce IgE synthesis in B cells in nasal polyps. *J Allergy Clin Immunol* 2018;**142**:1489–99.
8. Koyama T, Kariya S, Sato Y, Gion Y, Higaki T, Haruna T, et al. Significance of IgG4-positive cells in severe eosinophilic chronic rhinosinusitis. *Allergol Int* 2019;**68**:216–24.
9. Davies AM, Sutton B. Human IgG4: a structural perspective. *Immunol Rev* 2015;**268**:139–59.
10. James LK, Till SJ. Potential mechanisms for IgG4 inhibition of immediate hypersensitivity reactions. *Curr Allergy Asthma Rep* 2016;**16**:23.
11. Berings M, Karaaasian C, Altunbulaki C, Gevaert P, Akdis M, Bachert C, et al. Advances and highlights in allergen immunotherapy: on the way to sustained clinical and immunological tolerance. *J Allergy Clin Immunol* 2017;**140**:1250–67.
12. Engelhart S, Glynn RJ, Schur PH. Disease association with isolated elevations of each of the four IgG subclass. *Semin Arthritis Rheum* 2017;**47**:276–80.
13. Sato Y, Notohara K, Kojima M, Takata K, Masaki Y, Yoshino T. IgG4-related disease: histological overview and pathology of hematological disorders. *Path Int* 2010;**60**:247–58.
14. Ninomiya T, Noguchi E, Haruna T, Hasegawa M, Yoshida T, Yamashita T, et al. Periostin as a novel biomarker for postoperative recurrence of chronic rhinosinusitis with nasal polyps. *Sci Rep* 2018;**8**:11450.
15. Fujieda S, Imoto Y, Kato Y, Ninomiya T, Tokunaga T, Tsutsumiuchi T, et al. Eosinophilic chronic rhinosinusitis. *Allergol Int* 2019;**68**:408–12.
16. Fokkens WJ, Lund VJ, Mullol J, Bachert C, Alobid I, Baroody F, et al. Epos 2012: European position paper on rhinosinusitis and nasal polyps 2012. A summary for otorhinolaryngologists. *Rhiology* 2012;**50**:1–12.
17. Tomassen P, Vandeplas G, Van Zele T, Cardell LO, Arebro J, Olze H, et al. Inflammatory endotypes of chronic rhinosinusitis based on cluster analysis of biomarkers. *J Allergy Clin Immunol* 2016;**137**:1449–56.
18. Wang X, Zhang N, Bo M, Holtappels G, Zheng M, Lou H, et al. Diversity of Th cytokine profiles in patients with chronic rhinosinusitis: a multicenter study in Europe, Asia, and Oceania. *J Allergy Clin Immunol* 2016;**138**:1344–53.
19. De Grave G, Helling PW, Fokkens WJ, Pugin B, Steelant B, Seys SF. Endotype-driven treatment in chronic upper airway diseases. *Clin Trans Allergy* 2017;**7**:22.
20. Haruna T, Kariya S, Fujiwara T, Higaki T, Makihara S, Kanai K, et al. Association between impaired IL-10 production following exposure to Staphylococcus aureus enterotoxin B and disease severity in eosinophilic chronic rhinosinusitis. *Allergol Int* 2018;**67**:392–8.
21. Shimosegawa T, Kanno A. Autoimmune pancreatitis in Japan: overview and perspective. *J Gastroenterol* 2009;**44**:503–17.

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