Title page

Title:

A decreased prevalence of group 2 innate lymphoid cells in blood is associated with good postoperative outcomes in patients with chronic rhinosinusitis

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Key words:

Chronic rhinosinusitis, sinonasal tissues, blood, group 2 innate lymphoid cells, Lund-

Mackay computed tomography scan score, postoperative outcome

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Disclosure Statement:

The authors declare that they have no relevant conflicts of interest.

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Title:

A decreased prevalence of group 2 innate lymphoid cells in blood is associated with good postoperative outcomes in patients with chronic rhinosinusitis

Abstract

Objective: Due to the high postoperative recurrence rate in eosinophilic chronic rhinosinusitis (eCRS) patients, there is a need for an index to predict the postoperative outcomes. Group 2 innate lymphoid cells (ILC2s) are important effector cells for type 2 immune responses in eosinophilic airway inflammation. The aim of this study was to investigate whether the prevalence of ILC2s in sinonasal tissues or in peripheral blood is associated with the postoperative outcome in CRS patients.

Methods: Twelve patients with eCRS and ten patients with non-eCRS were recruited. We examined the ILC2 prevalence in sinonasal tissues and in peripheral blood before and after endoscopic sinus surgery (ESS). Pre- and postoperative blood eosinophil counts were also examined. Lund-Mackay computed tomography (LMK-CT) scores were used to evaluate the disease severities and the postoperative outcomes; cases with more than 50% improvement were categorized into the good outcome group, and cases with less than 50% improvement were categorized into the poor outcome group.

Results: The ILC2 prevalence in sinonasal tissues was correlated with that in preoperative blood in eCRS and non-eCRS patients. The ILC2 prevalence in sinonasal tissues and in preoperative blood was not correlated with the pre- or postoperative

LMK-CT scores. Postoperatively, the ILC2 prevalence in blood was decreased in eCRS and non-eCRS patients, and blood eosinophil count was also decreased in eCRS patients but not in non-eCRS patients. The ILC2 prevalence in postoperative blood was decreased in the good outcome group but not in the poor outcome group. Blood eosinophil counts were not decreased postoperatively in both good and poor outcome groups.

Conclusion: The decreased ILC2 prevalence in postoperative blood may be a predictive biomarker for evaluating postoperative outcomes in eCRS and non-eCRS patients.

Key words:

Chronic rhinosinusitis, sinonasal tissues, blood, group 2 innate lymphoid cells, Lund-Mackay computed tomography scan score, postoperative outcome

Main body

1. INTRODUCTION

Chronic rhinosinusitis (CRS) is characterized by persistent symptomatic inflammation of the nasal and paranasal mucosa that lasts longer than 12 weeks[1]. CRS can be further classified into two major subtypes: CRS without nasal polyps (CRSsNP) and CRS with nasal polyps (CRSwNP)[1]. In the United States and European countries, CRSsNP presents as the predominant infiltration of neutrophils and type 1 or type 3 cytokines, whereas CRSwNP is characterized by eosinophilic infiltration and type 2 cytokines. However, the phenotypes of inflammation in CRSwNP differ between European and East Asian countries. Half of CRSwNP cases in Japan exhibit neutrophilic inflammation[2], and CRSwNP patients in other East Asian countries also represent both eosinophilic and neutrophilic phenotypes[3,4]. For the diagnosis of eosinophilic CRS (eCRS), a novel scoring system and algorithm were established based on the assessment of bilateral disease, nasal polyp formation, ethmoid sinus-dominant computed tomography (CT) shadows, and blood eosinophilia in the Japanese Epidemiological Survey of Refractory Eosinophilic CRS (JESREC) study. eCRS is characterized by marked eosinophilia in nasal polyps, and is associated with greater clinical and radiological severity, higher morbidity with bronchial asthma, and a higher

59 risk of polyp recurrence when compared to non-eCRS[5].

60 The Lund-Mackay CT scan (LMK-CT) score[6] is the most commonly used metric for
61 evaluating the radiological severity of CRS[1]. Preoperative LMK-CT scores have been
62 shown to be positively correlated with a nasal component of the 22-Item Sino-Nasal
63 Outcomes Test (SNOT-22), a validated disease-specific survey for the quality of life[7].
64 For the treatment of CRS, intranasal corticosteroids (INCS) and nasal saline irrigation
65 are the mainstays[1], and low-dose and long-term 14- and 15-membered macrolides
66 (macrolide therapy) are widely used in Japan[8]. When such conservative treatments fail,
67 endoscopic sinus surgery (ESS) is performed. Due to the high postoperative recurrence
68 rate in eCRS patients, there is a need for an index to predict the postoperative outcomes.

69 Group 2 innate lymphoid cells (ILC2s) are important effector cells for type 2
70 immune responses in eosinophilic airway inflammation, such as eCRS, allergic rhinitis
71 (AR), and bronchial asthma[9]. Environmental allergens induce the rapid release of
72 epithelial-derived cytokines, such as interleukin (IL)-33, IL-25, and thymic stromal
73 lymphopoietin; in response to these cytokines and to lipid mediators, such as
74 prostaglandin D_2 and cysteinyl leukotrienes, ILC2s in mucosal tissues quickly produce
75 large amounts of IL-5 and IL-13, leading to airway eosinophilia, mucus production, and
76 tissue repair[10]. It previously reported that the ILC2 prevalence in sinonasal tissues is

77 increased in eCRS patients, and is positively correlated with the number of infiltrating
78 eosinophils, but that the ILC2 prevalence is not increased in the peripheral blood of
79 eCRS patients[11]. Although the importance of ILC2s in sinonasal tissues is well-known,
80 the impact of ILC2s in peripheral blood has not yet been thoroughly investigated in
81 CRS patients.
82 This study aimed to determine whether the ILC2 prevalence in sinonasal tissues
83 and in peripheral blood is associated with the postoperative outcomes in CRS. The CRS
84 cases were classified as eCRS or non-eCRS according to the JESREC study. The
85 disease severity and postoperative outcome were evaluated using the LMK-CT scores at
86 an average of 14 months after ESS. The ILC2 prevalence and eosinophil count in
87 postoperative blood was examined at an average of 6 months after ESS to determine
88 whether the changes in the ILC2 prevalence or eosinophil count in blood are associated
89 with the postoperative outcome.
90

2. METHODS

2.1 Study participants

A cross-sectional study of CRS patients undergoing ESS was conducted. CRS patients were recruited from the department of Otorhinolarynogology-Head and Neck Surgery, Shiga University of Medical Science according to a protocol approved by the Institutional Review Board of Shiga University of Medical Science (ethics approval number 25-36). Informed consent was obtained from all participants. All experiments were conducted according to the Declaration of Helsinki on biomedical research involving human subjects. Sinonasal tissues, such as uncinate tissues and nasal polyps, were obtained during ESS, and peripheral blood samples were collected preoperatively (the day before ESS) and postoperatively. The diagnosis of CRS was made based on clinical, endoscopic, and radiographic criteria as described in the European Position Paper on Rhinosinusitis and Nasal Polyps (EPOS) 2020[1]. The CRS cases were classified as eCRS or non-eCRS according to a published clinical scoring system (JESREC score)[5], and ten patients with eCRS and twelve patients with non-eCRS were recruited. A detailed list of the patient characteristics is presented in Table 1. The JESREC score was calculated with the following point breakdown: bilateral disease sites (3 points); nasal polyps (2 points); CT shadow, ethmoid > maxillary sinus (2 points); and blood

eosinophilia, 2% to 5% (4 points), 5% to 10% (8 points), or more than 10% (10 points). Patients with scores higher than 11 points and mucosal eosinophilia higher than 70 per high-power field were defined as eCRS patients. Conservative medical treatments and nasal saline irrigations had previously failed in all CRS patients. The patients were not treated with systemic corticosteroids (SCS) within 4 weeks before the surgery. Patients for whom postoperative blood samples were unavailable were excluded from the study. All of the ESS were performed by a single skilled surgeon, with little difference in surgical quality.

2.2 Postoperative treatments

The postoperative treatments are shown in Table 2. All participants were treated with low-dose and long-term clarithromycin (200 mg/day, macrolide therapy), muco-active drug (S-carboxymethylcysteine, 1500 mg/day), and nasal saline irrigation for at least 3 months after ESS. All patients with eCRS used INCS, and 90% (9/10) of them used INCS for more than 12 months after ESS. Four patients with non-eCRS used INCS, but three patients stopped by 6 months after ESS. In addition, 60% (6/10) of eCRS patients used SCS; three patients used betamethasone starting at 0.5 mg with tapering from 2 to 12 months after ESS, and three patients used prednisolone starting at

15 mg with tapering from 7 days to 2 months after ESS. Only one patient with eCRS was taking low-dose SCS (betamethasone, 0.25 mg/day) at the time of postoperative blood collection. 60% (6/10) of eCRS patients used anti-leukotriene drugs, but only one patient with non-eCRS used it. None of the patients received biologic therapies, such as dupilumab, omalizumab, mepolizumab, or benralizumab.

2.3 Identification of ILC2s in sinonasal tissues and peripheral blood

The identification of ILC2s in sinonasal tissues and blood was performed as described previously[11,12]. Briefly, sinonasal tissues were cut into fine pieces, and digested for 30 to 45 min at 37°C with Liberase TM (125 μg/mL) and DNase I (200 μg/mL; both from Roche Diagnostics GmbH, Mannheim, Germany). Alternatively, cells were isolated by the mechanical disruption of tissues with the gentleMACS Dissociator and Tumor Dissociation Kit (Miltenyi Biotec Inc., Auburn, CA) according to the procedure recommended by the manufacturer. The cell suspensions were filtered through a 70-μm nylon mesh. The remaining cells were treated with ACK lysing buffer (Lonza Corp., Walkersville, MD) to lyse the red blood cells. Peripheral blood mononuclear cells (PBMCs) were isolated with Histopaque 1083 (Sigma-Aldrich, St. Louis, MO).

The total cell suspensions from sinonasal tissues or PBMCs were treated (30 minutes, 4 °C, in the dark) with Red LIVE/DEAD fixable dead cell-staining reagent (Invitrogen, Carlsbad, CA) as a live/dead discriminator, and then washed. Cells were blocked (10 minutes, 4 °C, in the dark) with Fc Blocking Reagent (Miltenyi Biotec, Auburn, CA). Cells then were incubated (30 minutes, 4 °C, in the dark) with the following antibody cocktail: fluorescein isothiocyanate-labeled antibodies to lineage markers (CD3, CD11b, CD11c, CD14, CD16, CD19, CD20, CD56, CD123, TCRαβ, TCRγδ, and FceR1α (Lineage)), phycoerythrin-cyanine 7-labeled antibody to CD45, phycoerythrin-labeled antibody to CD127, and Alexa Fluor®-labeled antibody to CRTH2 (BioLegend, San Diego, CA, or eBioscience, San Diego, CA). As shown in supplementary Figure 1, ILC2s were identified as Lineage$^-$ CD45$^+$ CD127$^+$ CRTH2$^+$ cells using FACSAria (BD Biosciences, San Jose, CA). All analysis was performed with FlowJo software, version 9.3 (TreeStar, Ashland, OR). The ILC2 prevalence was calculated as the number of ILC2s divided by the total number of Lineage$^-$ CD45$^+$ cells. The purity of blood ILC2s was greater than 98% (data not shown).

2.4 Main outcome measures

Postoperative blood collection for the measurement of blood ILC2s and

eosinophils was performed at 6.2 ± 1.2 months after ESS in non-eCRS patients, and at 5.8 ± 1.2 months after ESS in eCRS patients. The pre- and postoperative blood collections were made out of the Japanese cedar pollen season (February to April). The radiographic severity of CRS was assessed using the LMK-CT score[6], and postoperative CT imaging was performed at 13.8 ± 4.3 months after ESS in non-eCRS patients, and at 14.2 ± 4.3 months after ESS in eCRS patients. We calculated the postoperative LMK-CT score improvement rate by dividing the preoperative score minus postoperative score by the preoperative score (pre score – post score/pre score x 100, e.g., pre score "8" – post score "2"/pre score "8" x 100 = postoperative improvement rate 75%). In addition, using the postoperative LMK-CT score improvement rate, cases with more than 50% (≥ 50%) improvement were classified into the good outcome group (13 cases), and cases with less than 50% (< 50%) improvement were classified into the poor outcome group (9 cases). There was no difference in the postoperative treatment with SCS. SCS was used in 2 of 13 patients in the good outcome group and 4 of 9 patients in the poor outcome group shortly after the ESS (the average duration of SCS was 28.6 days in the good outcome group and 26.7 days in the poor outcome group). Only one patient with eCRS in the good outcome group was taking low-dose SCS (betamethasone, 0.25 mg/day) at the time of postoperative blood collection.

182

183 **2.5 Statistical analyses**

184 Data are presented as the means ± standard error of the mean (SEM) for the

185 number of subjects or experiments indicated. Statistical analysis was performed using

186 the Mann-Whitney *U* test. Correlations were assessed using Spearman's rank

187 correlation. A Student's two-tailed *t* test was used to determine the level of significance

188 for differences between two groups. $P < 0.05$ was considered to be statistically

189 significant.

190

3. RESULTS

3.1 Correlation analysis of the ILC2 prevalence in sinonasal tissues and in preoperative blood with the LMK-CT scores before and after ESS

There was a positive correlation between the ILC2 prevalence in sinonasal tissues and that in preoperative peripheral blood ($p=0.049$, $R=0.47$, Figure 1A). The prevalence of ILC2s in sinonasal tissues and that in preoperative blood were not correlated with the preoperative LMK-CT score (Figure 1B and C) or the postoperative LMK-CT score (Figure 1D and E).

3.2 Correlation analysis of the ILC2 prevalence in sinonasal tissues and in preoperative blood with the postoperative LMK-CT score improvement rate

The ILC2 prevalence in sinonasal tissues was not correlated with the postoperative LMK-CT score improvement rate in eCRS and non-eCRS patients (Figure 2A). The ILC2 prevalence in preoperative blood was not correlated with the postoperative LMK-CT score improvement rate in eCRS and non-eCRS patients (Figure 2B).

3.3 Changes in the blood ILC2 prevalence, the blood eosinophil count, and the LMK-CT score before and after ESS

The ILC2 prevalence and eosinophil count in postoperative blood was examined at an average of 6 months after ESS, and the postoperative LMK-CT score was examined at an average of 14 months after ESS. The ILC2 prevalence in blood and the LMK-CT score were significantly decreased after ESS in eCRS and non-eCRS patients (Figure 3A and C). Blood eosinophil counts were also decreased after ESS in eCRS patients but not in non-eCRS patients (Figure 3B).

The ILC2 prevalence in postoperative blood was significantly decreased in the good outcome group but not in the poor outcome group (Figure 4A). Blood eosinophil counts were not changed after ESS in both good and poor outcome groups (Figure 4B).

4. DISCUSSION

ILC2s in sinonasal tissues play critical roles in eosinophilic inflammation by producing type 2 cytokines in eCRS patients. Our previous study revealed that the ILC2 prevalence in sinonasal tissues is positively correlated with the number of tissue-infiltrating eosinophils[11]. It also has been reported that the ILC2 prevalence in sinonasal tissues is positively correlated with nasal symptom scores in CRS patients[13], and that SCS reduce the ILC2 prevalence in eosinophilic nasal polyps[14]. In the present study, all patients were not treated with SCS at least four weeks before ESS, and the ILC2 prevalence in sinonasal tissues was correlated with that in preoperative blood in eCRS and non-eCRS patients, but was not correlated with the pre- or postoperative LMK-CT scores. Postoperatively, the ILC2 prevalence in blood was decreased, and it was associated with a good outcome (LMK-CT score improvement rate ≥50%) after ESS. This is the first report to show a correlation between the ILC2 prevalence in tissue and in blood and the clinical outcome after ESS in eCRS and non-eCRS patients, and to show that a decreased ILC2 prevalence in postoperative blood is associated with a good postoperative outcome after ESS.

Tissue ILC2s are important in eosinophilic inflammation; however, the role of ILC2s in blood is not well understood. In patients with nonsteroidal anti-inflammatory

239 drug-exacerbated respiratory disease (N-ERD), the ILC2 prevalence is increased in
240 nasal scraping samples, but decreased in blood at the time of cyclooxygenase-1 inhibitor
241 reactions[15]. eCRS is often comorbid with N-ERD. Similarly, segmental allergen
242 challenge in patients with allergic asthma results in increased numbers of ILC2s in
243 bronchoalveolar fluids and decreased numbers of ILC2s in blood[16]. These results
244 suggest that ILC2s may be recruited from blood to the airway in response to allergen
245 exposure. In the present study, the ILC2 prevalence in blood did not differ between the
246 eCRS and non-eCRS patients before and after ESS, whereas the ILC2 prevalence in
247 sinonasal tissues was increased in eCRS patients when compared to non-eCRS patients
248 (Table 1), and was positively correlated with the ILC2 prevalence in preoperative blood.
249 We previously revealed that the ILC2 prevalence in blood is increased in
250 patients with AR[11], and it also has been reported that the ILC2 prevalence is positively
251 correlated with the serum IL-13 levels[17]. The ILC2 prevalence in blood is increased
252 during pollen season in patients with grass pollen-induced AR, and it is decreased after
253 subcutaneous allergen-specific immunotherapy[18]. Increased levels of blood ILC2s have
254 also been reported in asthmatic patients[19,20.21], and the increased levels of IL-13$^+$ ILC2s
255 in the blood of uncontrolled asthmatic patients decrease when the asthma is well-
256 controlled[20]. These results suggest that effective therapeutic interventions result in a

257 decrease of blood ILC2s in patients with AR and bronchial asthma. In the present study,
258 blood ILC2s were decreased after the ESS in eCRS and non-eCRS patients. The
259 mechanism by which effective therapy such as ESS induced a decrease in blood ILC2s
260 is unclear. Intranasal challenge with *Alternaria* induced the ILC2 release from the bone
261 marrow to blood and lung in the allergic mouse model[22]. Blood ILC2s may be increased
262 by the bone marrow-derived ILC2s in eosinophilic inflammation. ESS attenuates
263 sinonasal inflammation and may inhibit the induction of bone marrow-derived ILC2s by
264 suppressing the local production of inflammatory mediators such as cytokines and
265 chemokines.

266 Similar to previous studies that reported no increase in the ILC2 prevalence in
267 the blood of eCRS patients[11,23], the present study showed that the ILC2 prevalence in
268 preoperative blood was not increased in eCRS patients when compared to non-eCRS
269 patients. All patients received macrolide therapy, muco-active drug, and nasal saline
270 irrigation, and there were apparent differences in the postoperative treatments between
271 eCRS and non-eCRS patients. All eCRS patients used INCS for a long time, and more
272 than half of them used anti-leukotrienes and SCS. Despite these differences, the ILC2
273 prevalence in blood and the LMK-CT score were significantly decreased after ESS in
274 both eCRS and non-eCRS patients. Although these postoperative therapies in eCRS

patients may affect the postoperative decrease in the ILC2 prevalence in blood, it is interesting to note that the ILC2 prevalence in blood was also decreased in non-eCRS patients who were not treated with such eosinophilic inflammation-suppressing therapy. It has been reported that type 2 inflammation is partially involved in non-eCRS[24], and a recent study revealed that a monoclonal antibody treatment for CRS patients using dupilumab, a potent anti-IL-4 and IL-13 antibody, significantly improved total nasal symptom scores, SNOT-22, and visual analog scale even for non-eCRS patients[25]. Our results suggest that surgical intervention attenuates type 2 inflammation even in non-eCRS patients.

We evaluated the postoperative outcomes of CRS patients using the LMK-CT score at an average of 14 months after ESS, and the CRS patients were divided into a good outcome group and a poor outcome group. The ILC2 prevalence in blood at an average of 6 months after ESS was decreased in the good outcome group, but not in the poor outcome group. Blood eosinophil count was not changed in both good and poor outcome groups. These results indicate that a decreased ILC2 prevalence in postoperative blood may be a predictive biomarker for evaluating postoperative outcomes. Further studies using symptom scores, such as SNOT-22, will be needed to evaluate the clinical efficacy after ESS.

5. CONCLUSIONS

The ILC2 prevalence in sinonasal tissues was correlated with that in preoperative blood in eCRS and non-eCRS patients. The ILC2 prevalence in blood was decreased after ESS, and it was associated with a good outcome as evaluated by the LMK-CT scores. Blood ILC2s may play important roles in the pathophysiology of CRS and be a useful biomarker for predicting the postoperative outcomes after ESS.

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DISCLOSURE STATEMENT

The authors declare that they have no conflicts of interest.

REFERENCES

1. Fokkens WJ, Lund VJ, Hopkins C, Hellings PW, Kern R, Reitsma S, et al. European Position Paper on Rhinosinusitis and Nasal Polyps 2020. Rhinology 2020;58(Suppl S29):1-464.
2. Fujieda S, Imoto Y, Kato Y, Ninomiya T, Tokunaga T, Tsutsumiuchi T, et al. Eosinophilic chronic rhinosinusitis. Allergol Int 2019;68(4):403-412.
3. Zhang N, Van Zele T, Perez-Novo V, Van Bruaene N, Holtappels G, DeRuyck N, et al. Different types of T-effector cells orchestrate mucosal inflammation in chronic sinus disease. J Allergy Clin Immunol 2008;122(5):961-968.
4. Hulse KE, Stevens WW, Tan BK, Schleimer RP. Pathogenesis of nasal polyposis. Clin Exp Allergy 2015;45(2):328-346.
5. 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(8):995-1003.
6. Lund VJ, Mackay IS. Staging in rhinosinusitus. Rhinology 1993;31(4):183–184
7. Sedaghat AR, Gray ST, Caradonna SD, Caradonna DS. Clustering of chronic rhinosinusitis symptomatology reveals novel associations with objective clinical and demographic characteristics. Am J Rhinol Allergy 2015;29(2):100-105.

8. Shimizu T, Suzaki H. Past, present and future of macrolide thrapy for chronic rhinosinusitis in Japan. Auris Nasus Larynx 2016:43(2):131-136.
9. Schleimer RP. Immunopathogenesis of chronic rhinosinusitis and nasal polyposis. Annu Rev Pathol 2017;12:331-357.
10. Tojima I, Shimizu T. Group 2 innate lymphoid cells and eosinophilic chronic rhinosinusitis. Curr Opin Allergy Clin Immunol 2019;19(1):18-25.
11. Tojima I, Kouzaki H, Shimizu S, Ogawa T, Arikata M, Kita H, et al. Group 2 innate lymphoid cells are increased in nasal polyps in patients with eosinophilic chronic rhinosinusitis. Clin Immunol 2016;170:1-8.
12. Tojima I, Matsumoto K, Kikuoka H, Hara S, Yamamoto S, Shimizu S, et al. Evidence for the induction of Th2 inflammation by group 2 innate lymphoid cells in response to prostaglandin D2 and cysteinyl leukotrienes in allergic rhinisits. Allergy 2019;74(12):2417-2426.
13. Ho J, Bailey M, Zaunders J, Mrad N, Sacks R, Sewell W, et al. Group 2 innate lymphoid cells (ILC2s) are increased in chronic rhinosinusitis with nasal polyps or eosinophilia. Clin Exp Allergy 2015;45(2):394-403.
14. Walford HH, Lund SJ, Baum RE, White AA, Bergeron CM, Husseman J, et al. Increased ILC2s in the eosinophilic nasal polyp endotype are associated with

corticosteroid responsiveness. Clin Immunol 2014;155(1):126-135.

15. Eastman JJ, Cavagnero KJ, Deconde AS, Kim AS, Karta MR, Broide DH, et al. Group 2 innate lymphoid cells are recruited to the nasal mucosa in patients with aspirin-exacerbated respiratory disease. J Allergy Clin Immunol 2017;140(1):101-108.e3.

16. Winkler C, Hochdörfer T, Israelsson E, Hasselberg A, Cavallin A, Thörn K, et al. Activation of group 2 innate lymphoid cells after allergen challenge in asthmatic patients. J Allergy Clin Immunol 2019;144(1):61-69.

17. Zhong H, Fan XL, Yu QN, Qin ZL, Chen D, Xu R, et al. Increased innate type 2 immune response in house dust mite-allergic patients with allergic rhinitis. Clinical Immunol 2017;183:293-299.

18. Lao-Araya M, Steveling E, Scadding GW, Durham SR, Shamji MH. Seasonal increases in peripheral innate lymphoid type 2 cells are inhibited by subcutaneous grass pollen immunotherapy. J Allergy Clin Immunol 2014;134(5):1193-1195.e4.

19. Bartemes KR, Kephart GM, Fox SJ, Kita H. Enhanced innate type 2 immune response in peripheral blood from patients with asthma. J Allergy Clin Immunol 2014; 134(3):671-678.

20. Jia Y, Fang X, Zhu X, Bai C, Zhu L, Jin M, et al. IL-13^{+} Type 2 Innate Lymphoid

Cells Correlate with Asthma Control Status and Treatment Response. Am J Respir Cell Mol Biol 2016;55(5):675-683.

21. Liu T, Wu J, Zhao J, Wang J, Zhang Y, Liu L, et al. Type 2 innate lymphoid cells: A novel biomarker of eosinophilic airway inflammation in patients with mild to moderate asthma. Respir Med 2015;109(11):1391-1396.

22. Karta MR, Rosenthal PS, Beppu A, Vuong CY, Miller M, Das S, et al. β2 integrins rather than β1 integrins mediate Alternaria-induced group 2 innate lymphoid cell trafficking to the lung. J Allergy Clin Immunol 2018;141(1):329-338.

23. Poposki JA, Klingler AI, Tan BK, Soroosh P, Banie H, Lewis G, et al. Group 2 innate lymphoid cells are elevated and activated in chronic rhinosinusitis with nasal polyps. Immun Inflamm Dis 2017;5(3):233-243.

24. Bachert C, Marple B, Schlosser RJ, Hopkins C, Schleimer RP, Lambrecht BN, et al. Adult chronic rhinosinusitis. Nat Rev Dis Primers 2020;6(1):86.

25. Fujieda S, Matsune S, Takeno S, Ohta N, Asako M, Bachert C, et al. Dupilumab efficacy in chronic rhinosinusitis with nasal polyps from SINUS-52 is unaffected by eosinophilic status. Allergy 2022;77(1):186-196.

Table 1. Patient characteristics

Note: Data are presented as the mean ± SEM.

Abbreviations: eCRS, eosinophilic chronic rhinosinusitis; N-ERD, nonsteroidal anti-inflammatory drug-exacerbated respiratory disease; AR, allergic rhinitis; IgE, immunoglobulin E; JESREC, Japanese Epidemiological Survey of Refractory Eosinophilic Rhinosinusitis; pre-op, preoperative; LMK-CT score, Lund-Mackay computed tomography scan score; ILC2s, group 2 innate lymphoid cells; Lin, lineage; NPs, nasal polyps; PBMCs, peripheral blood mononuclear cells; post-op, postoperative; pre-op, preoperative; M, month.

Table 2. Postoperative treatments

Abbreviations: INCS, intranasal corticosteroid; SCS, systemic corticosteroid; NS, not significant.

FIGURE LEGENDS

Figure 1. Correlation analysis of the ILC2 prevalence in sinonasal tissues and in preoperative (pre-op) blood. Correlations between the ILC2 prevalence in sinonasal tissues and that in pre-op blood (A), the ILC2 prevalence in sinonasal tissues and the

pre-op Lund-Mackay CT (LMK-CT) score (B), the ILC2 prevalence in pre-op blood and the pre-op LMK-CT score (C), the ILC2 prevalence in sinonasal tissues and the postoperative (post-op) LMK-CT score (D), and the ILC2 prevalence in pre-op blood and the post-op LMK-CT score (E). eCRS, eosinophilic chronic rhinosinusitis.

Figure 2. Correlation analysis of the ILC2 prevalence in sinonasal tissues and in pre-op blood with the post-op outcomes in the eCRS and non-eCRS patients. Correlations between the ILC2 prevalence in sinonasal tissues and the post-op LMK-CT score improvement rate (A), and the ILC2 prevalence in pre-op blood and the post-op LMK-CT score improvement rate (B).

Figure 3. Changes in the blood ILC2 prevalence (A), the blood eosinophil count (B), and the LMK-CT score (C) before and after ESS.

Figure 4. Changes in the ILC2 prevalence in blood (A) and the blood eosinophil count (B) before and after ESS in the good outcome group and the poor outcome group.

Figure 1

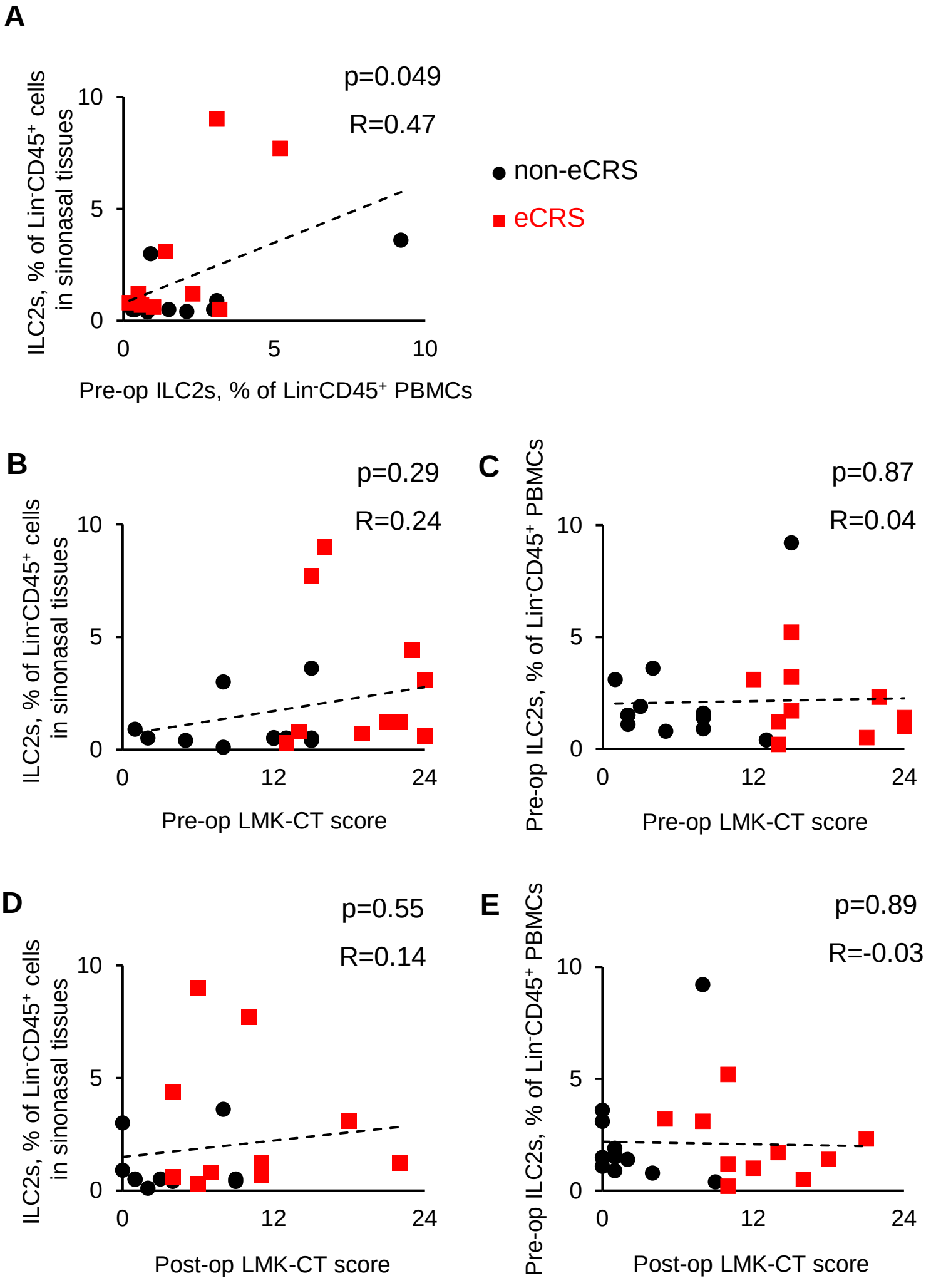

A
p=0.049
R=0.47
non-eCRS
eCRS
ILC2s, % of Lin-CD45+ cells in sinonasal tissues
Pre-op ILC2s, % of Lin-CD45+ PBMCs
B
p=0.29
R=0.24
Pre-op LMK-CT score
C
p=0.87
R=0.04
Pre-op ILC2s, % of Lin-CD45+ PBMCs
D
p=0.55
R=0.14
Post-op LMK-CT score
E
p=0.89
R=-0.03
Post-op LMK-CT score

Figure 2

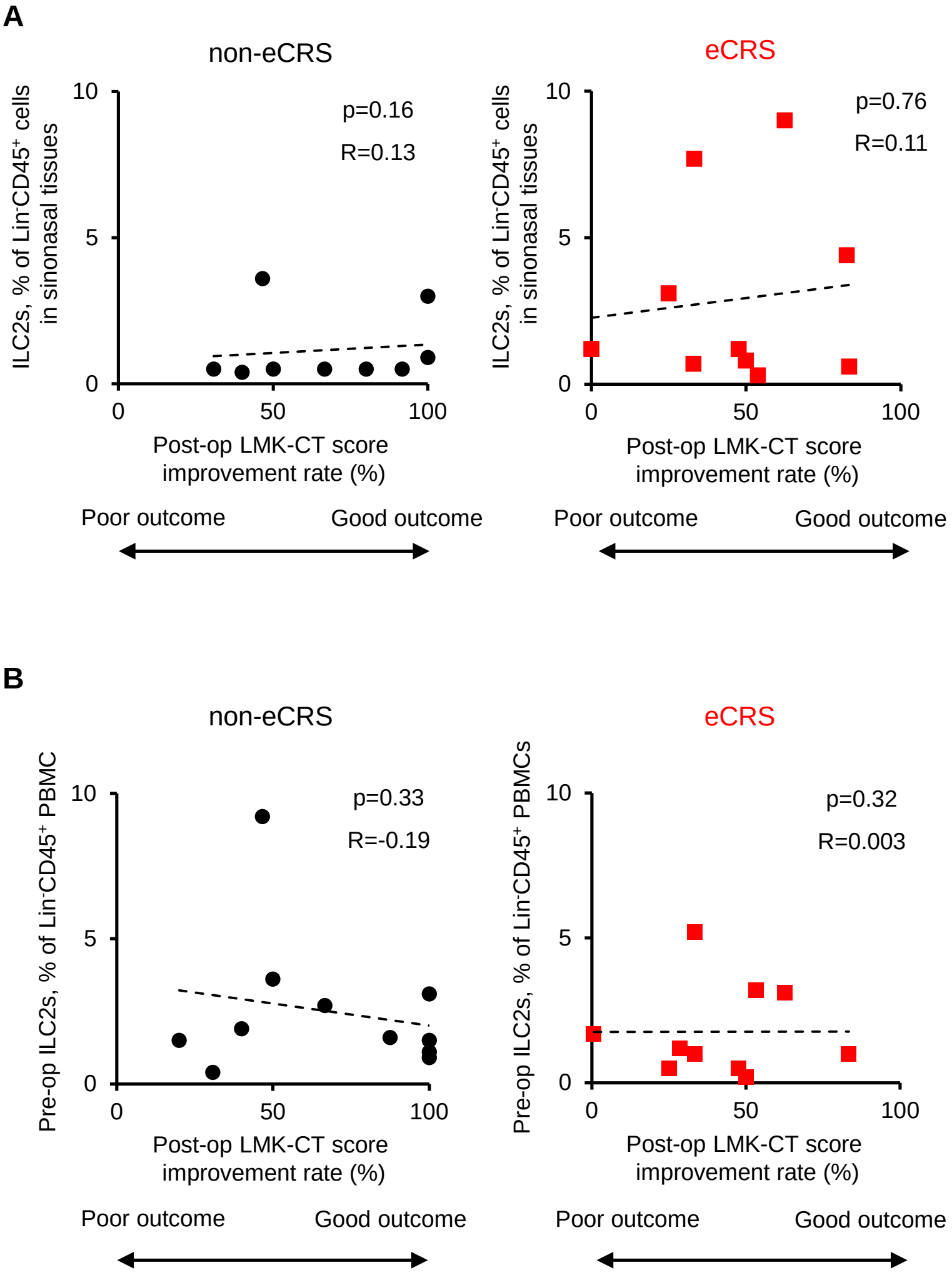

A
non-eCRS
eCRS
ILC2s, % of Lin-CD45+ cells in sinonasal tissues
p=0.16
R=0.13
p=0.76
R=0.11
Post-op LMK-CT score improvement rate (%)
Poor outcome
Good outcome
B
Pre-op ILC2s, % of Lin-CD45+ PBMC
p=0.33
R=-0.19
p=0.32
R=0.003

Figure 3

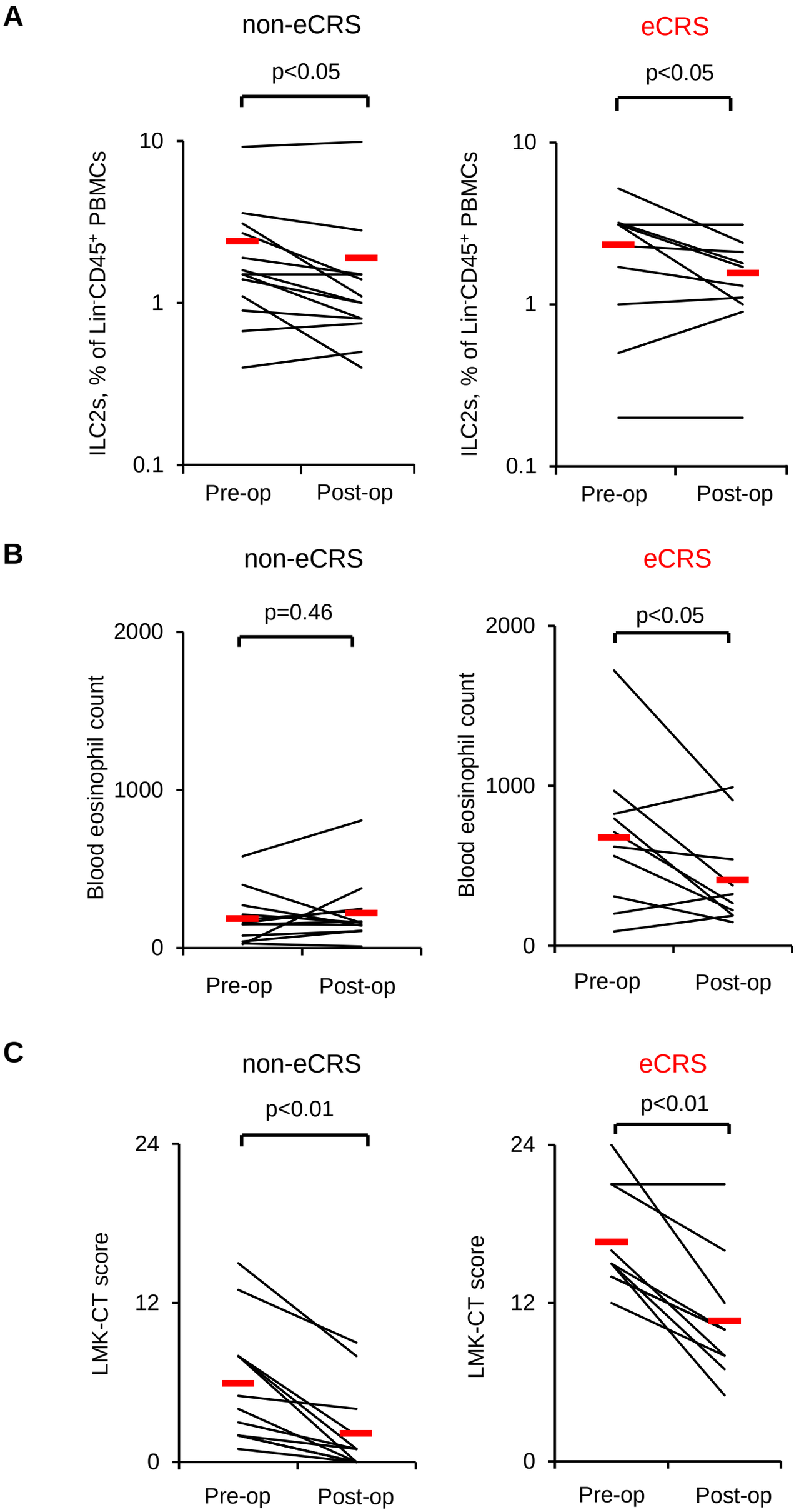
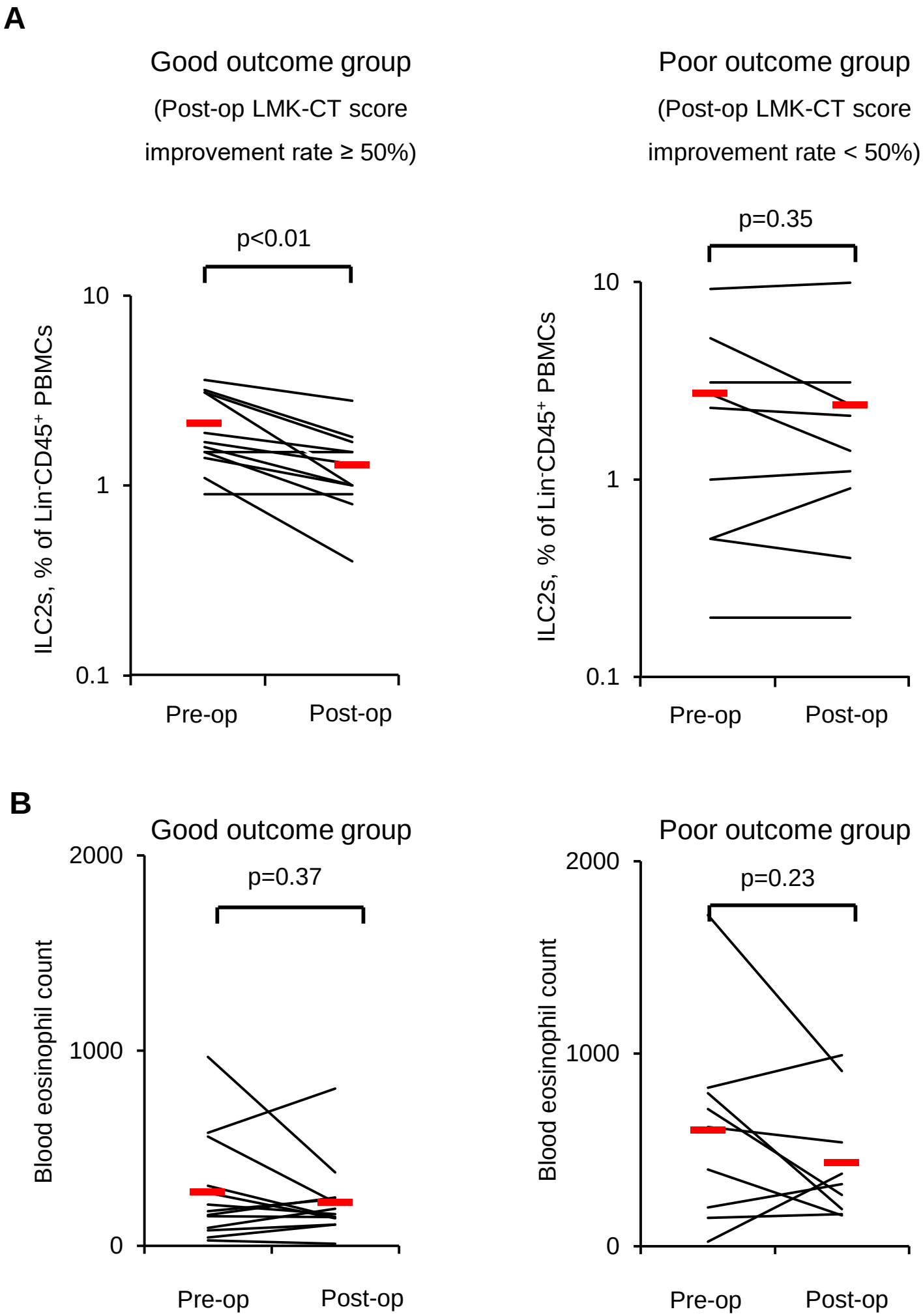

A
non-eCRS
p<0.05
10
1
0.1
ILC2s, % of Lin-CD45+ PBMCs
Pre-op
Post-op
eCRS
p<0.05
10
1
0.1
ILC2s, % of Lin-CD45+ PBMCs
Pre-op
Post-op
B
non-eCRS
p=0.46
2000
1000
0
Blood eosinophil count
Pre-op
Post-op
eCRS
p<0.05
2000
1000
0
Blood eosinophil count
Pre-op
Post-op
C
non-eCRS
p<0.01
24
12
0
LMK-CT score
Pre-op
Post-op
eCRS
p<0.01
24
12
0
LMK-CT score
Pre-op
Post-op

Figure 4


A
Good outcome group
(Post-op LMK-CT score
improvement rate ≥ 50%)
p<0.01
10
1
0.1
ILC2s, % of Lin-CD45+ PBMCs
Pre-op
Post-op
Poor outcome group
(Post-op LMK-CT score
improvement rate < 50%)
p=0.35
10
1
0.1
ILC2s, % of Lin-CD45+ PBMCs
Pre-op
Post-op
B
Good outcome group
p=0.37
2000
1000
0
Blood eosinophil count
Pre-op
Post-op
Poor outcome group
p=0.23
2000
1000
0
Blood eosinophil count
Pre-op
Post-op

Supplementary Figure 1

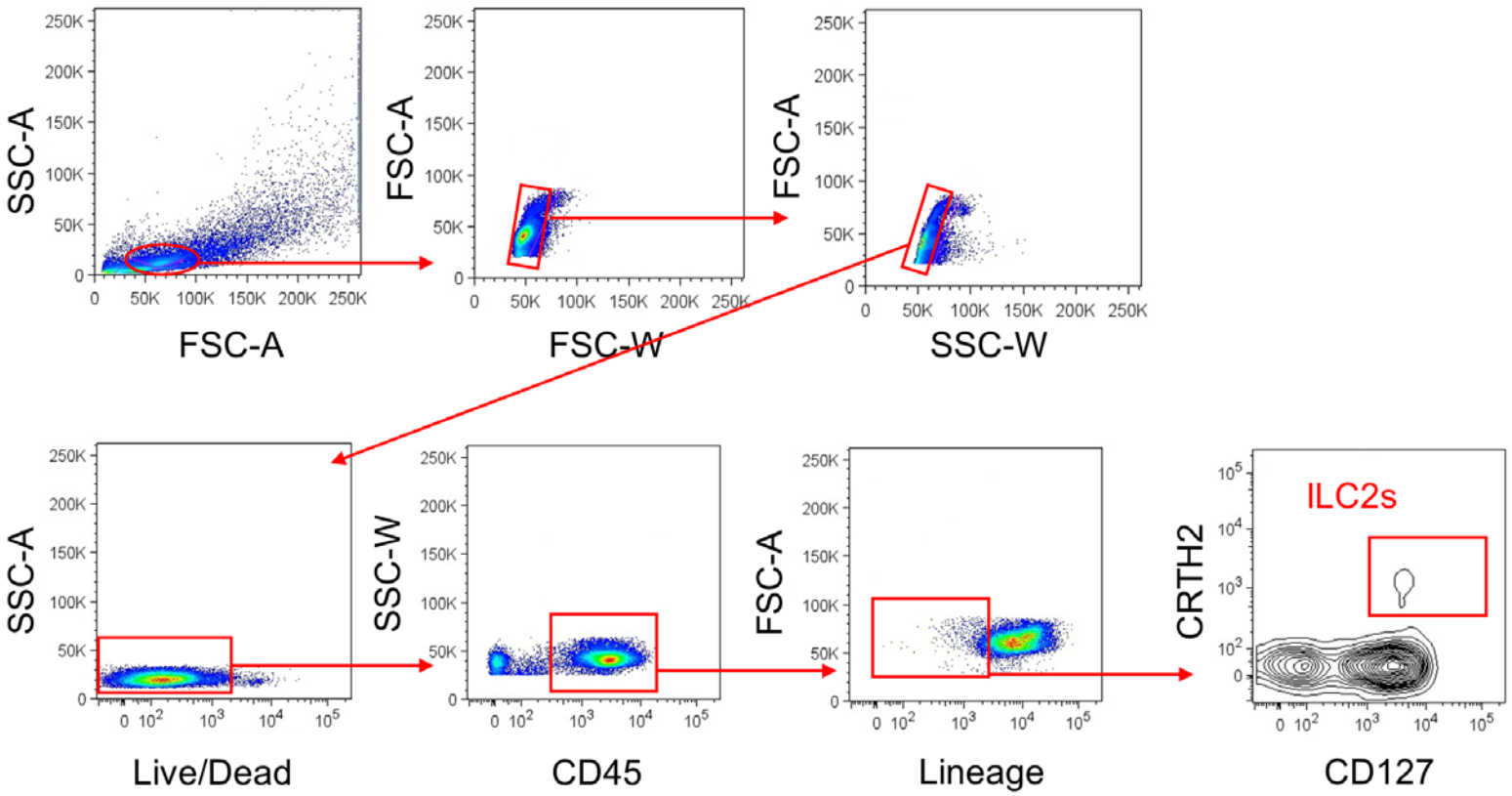


Table 1

	non-eCRS (n = 12)	**eCRS** (n = 10)	
Age (yrs)	56.7 ± 5.7	57.5 ± 4.8	p=0.92
Sex (M/F)	7/5	6/4	p=0.93
Nasal polyp (n)	4	10	p<0.01
Asthma (comorbid N-ERD, n)	1 (0)	7 (2)	p<0.01
AR (n)	8	7	p=0.87
Eosinophil count in pre-op blood	189 ± 47	680 ± 147	p<0.01
Eosinophil count in post-op blood	223 ± 59	416 ± 96	p=0.04
Total IgE (IU/mL)	815 ± 72	278 ± 54	p=0.25
JESREC score, range 0-17	4.6 ± 1.2	14 ± 1.2	p<0.001
Pre-op LMK-CT score, range 0-24	5.9 ± 1.4	16.7 ± 1.2	p<0.001
ILC2s in sinonasal tissues (% of Lin^-CD45^+ cells)	1.0 ± 0.4	2.9 ± 1.0	p=0.02
ILC2s in pre-op blood (% of Lin^-CD45^+ PBMCs)	2.4 ± 0.7	2.3 ± 0.5	p=0.64
ILC2s in post-op blood (% of Lin^-CD45^+ PBMCs)	1.9 ± 0.8	1.6 ± 0.3	p=0.39

Table 2

	non-eCRS (n = 12)	**eCRS** (n = 10)	
INCS	4/12	10/10	p<0.01
SCS	0/12	6/10	p<0.01
Anti-leukotriene	1/12	6/10	p<0.01
Oral anti-histamine for AR	5/8	3/7	p=0.45
Macrolide therapy	12/12	10/10	NS
Muco-active drug	12/12	10/10	NS
Nasal saline irrigation	12/12	10/10	NS