

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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June 1, 2022

Abstract

Objectives: The aim of this study was to investigate whether the prevalence of group 2 innate lymphoid cells (ILC2s) in sinonasal tissues or in peripheral blood is associated with the postoperative outcome in chronic rhinosinusitis (CRS) patients. **Design:** A cross-sectional study of CRS patients undergoing endoscopic sinus surgery (ESS). **Setting:** The Department of Otorhinolaryngology-Head and Neck Surgery at Shiga University of Medical Science Hospital. **Participants:** Eleven patients with eosinophilic CRS (eCRS) and ten patients with non-eCRS were recruited. **Main outcome measures:** We examined the ILC2 prevalence in sinonasal tissues and in peripheral blood before and after ESS. Lund-Mackay computed tomography (LMK-CT) scores were used to evaluate 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 the 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 the eCRS and non-eCRS patients, and the decrease was associated with the good outcome group, but not the poor outcome group. **Conclusion:** The decreased ILC2 prevalence in blood may be related to good postoperative outcomes after ESS in eCRS and non-eCRS patients.

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Results: The ILC2 prevalence in sinonasal tissues was correlated with that in preoperative blood in the 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 the eCRS and non-eCRS patients, and the decrease was associated with the good outcome group, but not the poor outcome group.

Conclusion: The decreased ILC2 prevalence in blood may be related to good postoperative outcomes after ESS 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

Key points

- The ILC2 prevalence in sinonasal tissues was correlated with that in preoperative blood in eosinophilic chronic rhinosinusitis (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.
- The ILC2 prevalence in blood was decreased postoperatively in eCRS and non-eCRS patients.
- The ILC2 prevalence in blood was decreased in the good outcome group (postoperative LMK-CT score improvement rate [?]50%), but not in the poor outcome group.
- A decreased ILC2 prevalence in blood after ESS may predict a good postoperative outcome in eCRS and non-eCRS patients.
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Main body

1. objectives

Chronic rhinosinusitis (CRS) is characterized by persistent symptomatic inflammation of the nasal and paranasal mucosa that lasts longer than 12 weeks¹. 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 inflammation², 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 risk of polyp recurrence when compared to non-eCRS⁵.

The Lund-Mackay CT scan (LMK-CT) score⁶ is the most commonly used metric for evaluating the radiological severity of CRS¹. Preoperative LMK-CT scores have been shown to be positively correlated with a nasal component of the 22-Item Sino-Nasal Outcomes Test (SNOT-22), a validated disease-specific survey for the quality of life⁷. For the treatment of CRS, intranasal corticosteroids (INCS) and nasal saline irrigation are the mainstays¹, and low-dose and long-term 14- and 15-membered macrolides (macrolide therapy) are widely used in Japan⁸. When such conservative treatments fail, endoscopic sinus surgery (ESS) is performed. Due to the high postoperative recurrence rate in 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, such as eCRS, allergic rhinitis (AR), and bronchial asthma⁹. Environmental allergens induce the rapid release of epithelial-derived cytokines, such as interleukin (IL)-33, IL-25, and thymic stromal

lymphopoietin; in response to these cytokines and to lipid mediators, such as prostaglandin D₂ and cysteinyl leukotrienes, ILC2s in mucosal tissues quickly produce large amounts of IL-5 and IL-13, leading to airway eosinophilia, mucus production, and tissue repair¹⁰. We previously reported that the ILC2 prevalence in sinonasal tissues is increased in eCRS patients, and is positively correlated with the number of infiltrating eosinophils, but that the ILC2 prevalence is not increased in the peripheral blood of eCRS patients¹¹. Although the importance of ILC2s in sinonasal tissues is well-known, the impact of ILC2s in peripheral blood has not yet been thoroughly investigated in CRS patients.

This study aimed to determine whether the ILC2 prevalence in sinonasal tissues and in peripheral blood is associated with the postoperative outcomes in CRS. The CRS cases were classified as eCRS or non-eCRS according to the JESREC study. The disease severity and postoperative outcome were evaluated using the LMK-CT scores at an average of 14 months after ESS. The ILC2 prevalence in postoperative blood was examined at an average of 6 months after ESS to determine whether the changes in the ILC2 prevalence in blood are associated with the postoperative outcome.

2. methods

2.1 Design, setting, and participants

A cross-sectional study of CRS patients undergoing ESS was conducted. CRS patients were recruited from "Blinded for review" according to a protocol approved by the Institutional Review Board of "Blinded for review" (ethics approval number 25-36) as described previously¹¹. 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) 2020¹. A detailed list of the patient characteristics is presented in Table 1. The CRS cases were classified as eCRS or non-eCRS according to a published clinical scoring system (JESREC score)⁵. 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.

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 81% (9/11) of them used INCS for more than 12 months after ESS. Two patients with non-eCRS used INCS, but they stopped by 6 months after ESS. In addition, 54.5% (6/11) 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. 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^{10,11}. 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- μ m 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 stained with the following antibody cocktail: fluorescein isothiocyanate-labeled antibodies to lineage markers (CD3, CD11b, CD11c, CD14, CD16, CD19, CD20, CD56, CD123, TCR $\alpha\beta$, TCR $\gamma\delta$, and Fc ϵ R1 α (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). ILC2s were identified as Lineage⁻CD45⁺CD127⁺CRTH2⁺ cells using FACSaria (BD Biosciences, San Jose, CA). The ILC2 prevalence was calculated as the number of ILC2s divided by the total number of Lineage⁻CD45⁺ cells.

2.4 Main outcome measures

Postoperative blood collection for the measurement of blood ILC2s was performed at 6.3 ± 1.3 months after ESS in the non-eCRS patients, and at 6.0 ± 1.1 months after ESS in the eCRS patients. The radiographic severity of CRS was assessed using the LMK-CT score⁶, and postoperative CT imaging was performed at 14.7 ± 5.0 months after ESS in the non-eCRS patients, and at 13.6 ± 3.9 months after ESS in the eCRS patients. We calculated the postoperative LMK-CT score improvement rate by dividing the preoperative score by the postoperative score. In addition, using the postoperative LMK-CT score improvement rate, cases with more than 50% ([?] 50%) improvement were classified into the good outcome group, and cases with less than 50% (< 50%) improvement were classified into the poor outcome group.

2.5. Statistical analyses

Data are presented as the means $\pm$ standard error of the mean (SEM) for the number of subjects or experiments indicated. Statistical analysis was performed using the Mann-Whitney *U* test. Correlations were assessed using Spearman's rank correlation. A Student's two-tailed *t* test was used to determine the level of significance for differences between two groups. $P < 0.05$ was considered to be statistically significant.

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 ($P = 0.012$, $R = 0.56$, 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 the 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 the eCRS and non-eCRS patients (Figure 2B).

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

The ILC2 prevalence 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 the eCRS and non-eCRS patients (Figure 3A and B). The ILC2 prevalence in postoperative blood was significantly decreased in the good outcome group, but not in the poor outcome group (Figure 4).

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 eosinophils¹⁶. It has also been reported that the ILC2 prevalence in sinonasal tissues is positively correlated with nasal symptom scores in CRS patients¹³, and that SCS reduce the ILC2 prevalence in eosinophilic nasal polyps¹⁴. In the present study, the ILC2 prevalence in sinonasal tissues was correlated with that in preoperative blood in the 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 drug-exacerbated respiratory disease (N-ERD), the ILC2 prevalence is increased in nasal scraping samples, but decreased in blood at the time of cyclooxygenase-1 inhibitor reactions¹⁵. ECRS is often comorbid with N-ERD. Similarly, segmental allergen challenge in patients with allergic asthma results in increased numbers of ILC2s in bronchoalveolar fluids and decreased numbers of ILC2s in blood¹⁶. These results suggest that ILC2s may be recruited from blood to the airway in response to allergen exposure. In the present study, the ILC2 prevalence in blood did not differ between the eCRS and non-eCRS patients before and after ESS, whereas the ILC2 prevalence in sinonasal tissues was increased in the eCRS patients when compared to the non-eCRS patients (Table 1), and was positively correlated with the ILC2 prevalence in preoperative blood.

We previously revealed that the ILC2 prevalence in blood is increased in patients with AR¹¹, and it also has been reported that the prevalence is positively correlated with the serum IL-13 levels¹⁷. The ILC2 prevalence in blood is increased during pollen season in patients with grass pollen-induced AR, and it is decreased after subcutaneous allergen-specific immunotherapy¹⁸. Increased levels of blood ILC2s have also been reported in asthmatic patients^{19,20,21}, and the increased levels of IL-13⁺ ILC2s in the blood of uncontrolled asthmatic patients decrease when the asthma is well-controlled²⁰. These results suggest that effective therapeutic interventions result in a decrease of blood ILC2s in patients with AR and bronchial asthma. Similar to previous studies that reported no increase in the ILC2 prevalence in the blood of eCRS patients^{11,22}, our present study also showed that the ILC2 prevalence in preoperative blood was not increased in the eCRS patients when compared to the non-eCRS patients. All patients received macrolide therapy, muco-active drug, and nasal saline irrigation, and there were apparent differences in the postoperative treatments between the eCRS and non-eCRS patients. All eCRS patients used INCS for a long time, and more than half of them used anti-leukotrienes and SCS. Despite these differences, the ILC2 prevalence in blood and the LMK-CT score were significantly decreased after ESS in both the 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 the 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-eCRS²³, and surgical intervention may attenuate 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. These results indicate that a decreased ILC2 prevalence in postoperative blood may predict a good postoperative outcome after ESS. Further studies using symptom scores, such as SNOT-22, to evaluate the clinical efficacy after ESS are of interest.

In conclusion, 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. These results suggest that a decreased ILC2

prevalence in blood after ESS may predict a good postoperative outcome in eCRS and non-eCRS patients.

Data availability statement

The data that support the findings of this study are available upon request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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Table 1. Patient characteristics

Note: Data are presented as the mean $\pm$ 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; 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 post-operative (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 ILC2 prevalence in blood (A) and in the LMK-CT score (B) before and after ESS.

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

Table 1 Subject characteristics

	non-eCRS (n = 10)	eCRS (n = 11)	
Age (yrs)	56.5 $\pm$ 6.2	58.6 $\pm$ 4.5	$P = 0.97$
Nasal polyp (n)	2	11	$P < 0.001$
Asthma (comorbid N-ERD, n)	0	8 (2)	$P < 0.001$
AR (n)	6	8	$P = 0.53$
Blood eosinophil (%)	4.2 $\pm$ 1.4	8.9 $\pm$ 1.5	$P < 0.05$
Total IgE (IU/mL)	189 $\pm$ 80	579 $\pm$ 99	$P = 0.08$
JESREC score, range 0-17	4.0 $\pm$ 1.1	13.1 $\pm$ 1.4	$P < 0.01$
Pre-op LMK-CT score, range 0-24	4.8 $\pm$ 1.2	15.9 $\pm$ 1.4	$P < 0.001$
ILC2s in sinonasal tissues (% of Lin ⁺ CD45 ⁺ cells)	0.5 $\pm$ 0.1	3.8 $\pm$ 1.9	$P < 0.05$
ILC2s in pre-op blood (% of Lin ⁺ CD45 ⁺ PBMCs)	1.7 $\pm$ 0.3	2.0 $\pm$ 0.5	$P = 0.86$
ILC2s in post-op blood (% of Lin ⁺ CD45 ⁺ PBMCs)	1.2 $\pm$ 0.2	1.4 $\pm$ 0.2	$P = 0.38$

Table 2 Postoperative treatments

	non-eCRS (n = 10)	eCRS (n = 11)	
INCS	2/10	11/11	$P < 0.001$
SCS	0/10	6/11	$P < 0.01$
Anti-leukotriene	0/10	7/11	$P < 0.01$
Oral anti-histamine for AR	3/10	4/11	$P = 0.76$
Macrolide therapy	10/10	11/11	NS
Muco-active drug	10/10	11/11	NS
Nasal saline irrigation	10/10	11/11	NS

Figure 1

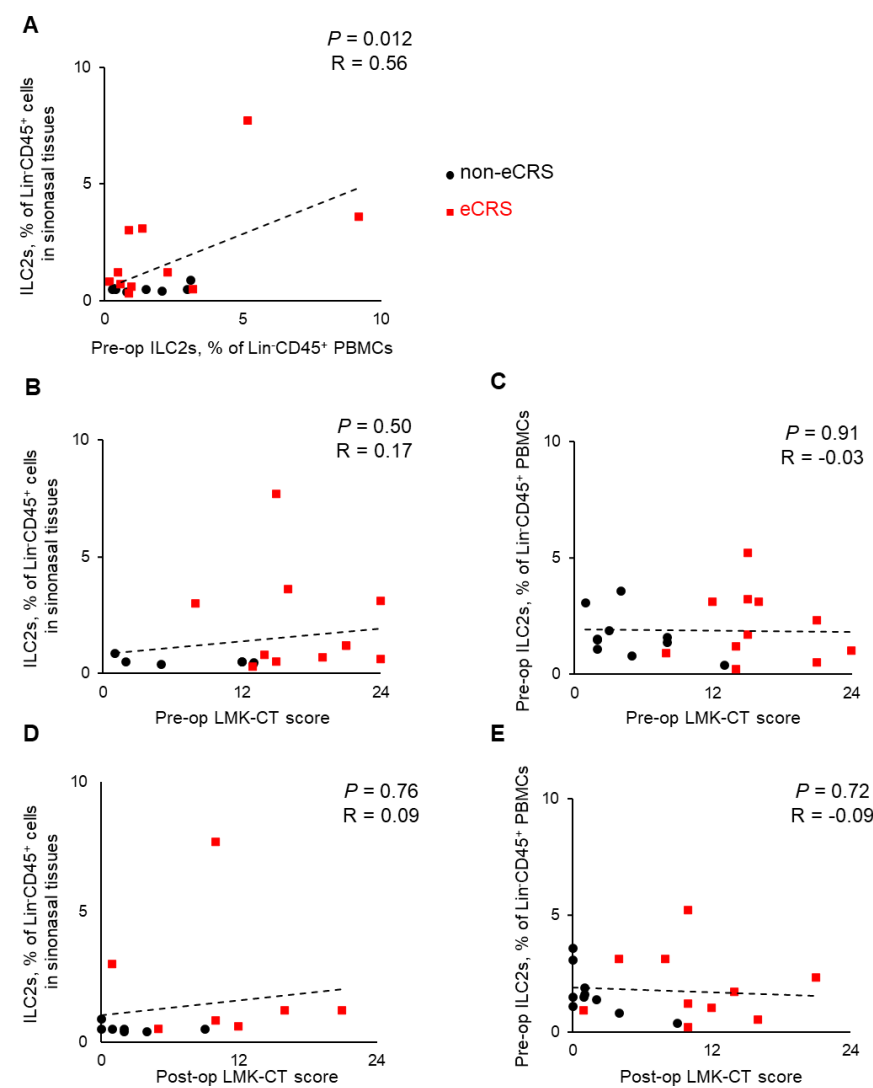


Figure 2

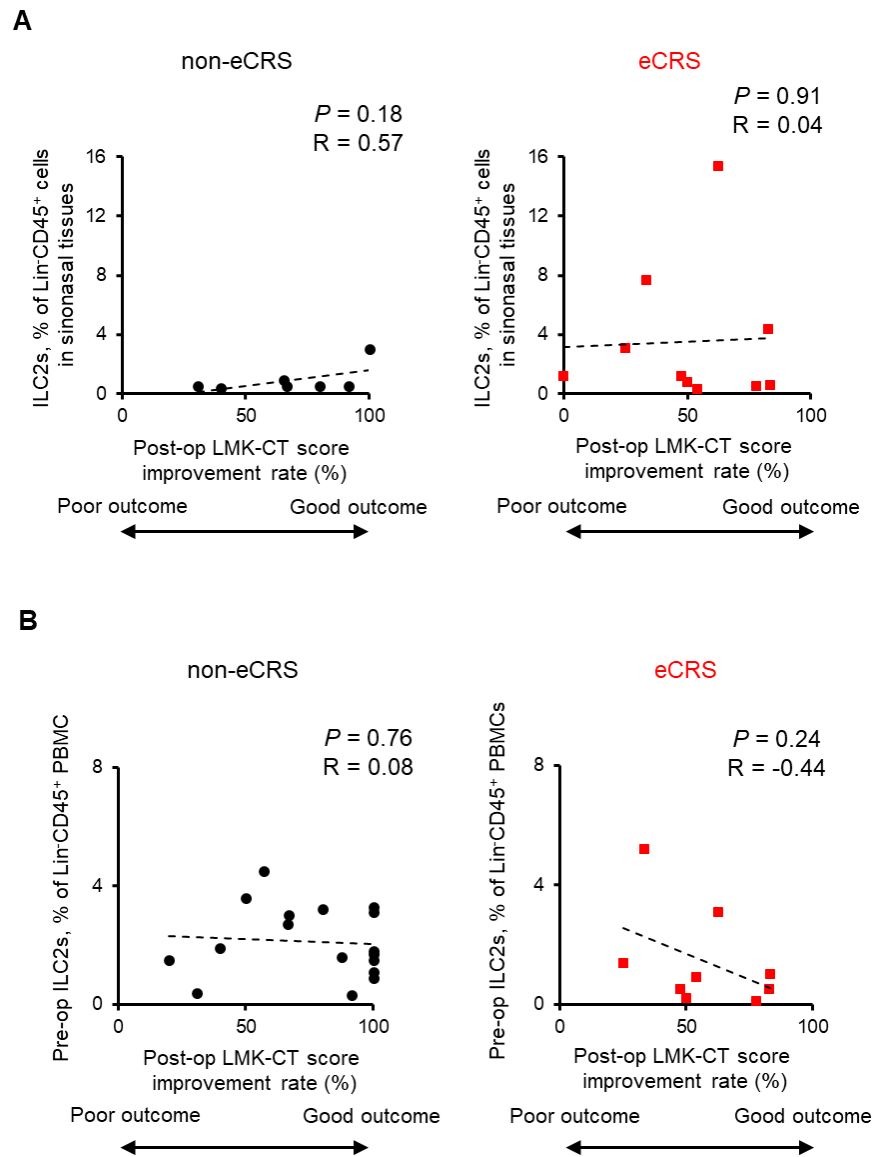


Figure 3

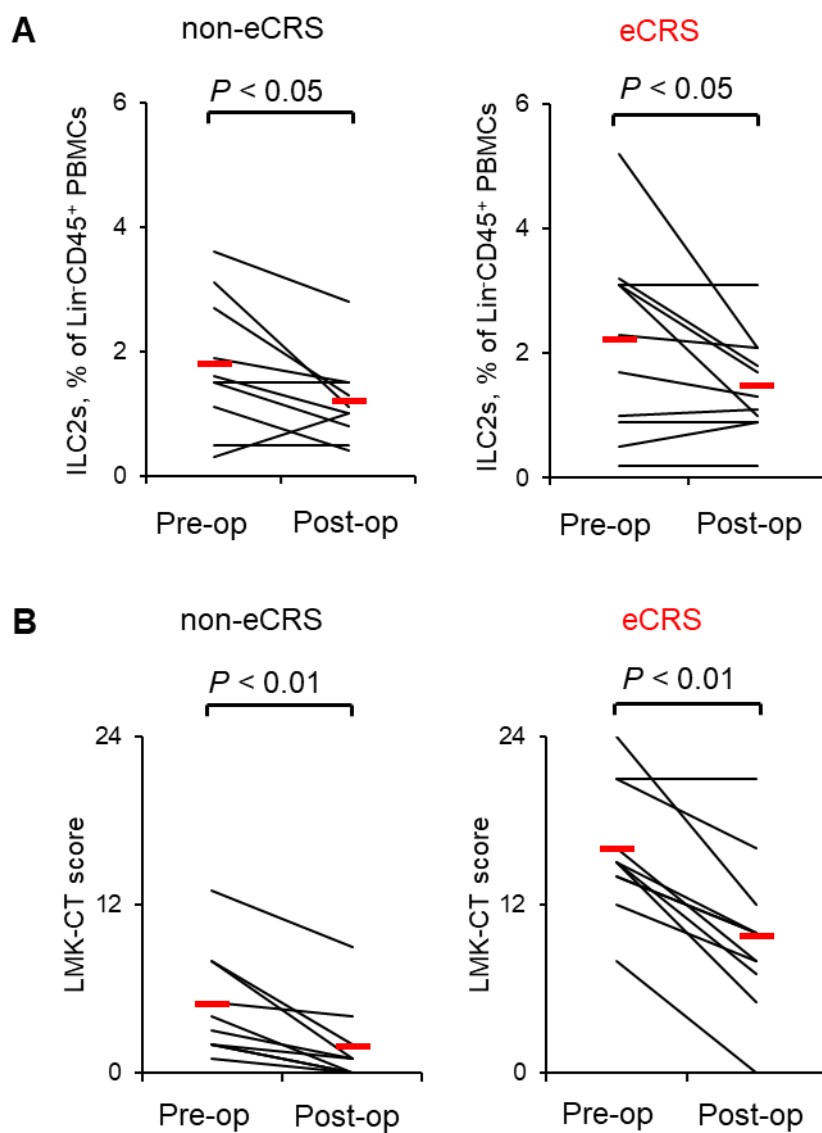


Figure 4

