

Tissue eosinophilia and computed tomography features in paediatric chronic rhinosinusitis with nasal polyps requiring revision surgery*

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Abstract

Background: Endoscopic sinus surgery (ESS) is an effective and safe treatment modality for medically recalcitrant chronic rhinosinusitis (CRS) in the paediatric population, especially in older children or those with nasal polyposis (CRSwNP). We aimed to elucidate the inflammatory pattern and clinical characteristics of CRSwNP related to revision surgery after ESS in a paediatric population.

Methods: We retrospectively enrolled 146 patients with bilateral CRSwNP. Twenty-two patients had recurrent nasal polyps that required revision surgery. The clinical characteristics, computed tomography (CT) features, tissue eosinophil count, and immunoreactivity of signature cytokines in the two groups were analysed.

Results: Tissue eosinophil infiltration and immunoreactivity of eosinophilic cationic protein and IL-5 in the sinus mucosa were higher in patients that required revision surgery. The revision surgery group was significantly younger and had positive aeroallergen test results, higher total Lund-Mackay scores, and ethmoid/maxillary sinus ratio on CT images than those without revision surgery. A nomogram was developed to predict the probability of the requirement of revision surgery according to the logistic regression analysis results.

Conclusions: We developed a nomogram model using clinical characteristics, tissue eosinophilia, and CT features for the preoperative identification of patients vulnerable to revision surgery in paediatric CRSwNP. This could help clinicians predict the probability of recurrence and perform intensive postoperative adjunct therapy and follow-up.

Key words: computed tomography, eosinophil, endoscopic sinus surgery, nasal polyp, paediatric chronic rhinosinusitis

Introduction

The prevalence of chronic rhinosinusitis (CRS) in the paediatric population is lower than that in adults, with an estimated prevalence

of 2–4%⁽¹⁾. However, paediatric CRS is a relevant disease in terms of quality of life, such as missed classes, poor concentration in school, impaired sleep quality, and impaired physical and

emotional health⁽²⁾.

Chronic rhinosinusitis can be classified as CRS without nasal polyps (CRSsNP) or CRS with nasal polyps (CRSwNP) based on the presence of nasal polyps⁽³⁾. CRS has also been classified into three major categories of cell-mediated effector immunity (type 1, 2, and 3) based on elevated inflammatory cytokines endotypically⁽⁴⁾. The type 1 endotype is characterised by elevated expression of cytokines, such as IFN- γ . The type 2 endotype is characterised by elevated expression of cytokines, including IL-4, IL-5, and IL-13, as well as eosinophil activity (eosinophilic cationic protein [ECP])⁽⁵⁾. The type 3 endotype is characterised by elevated levels of IL-17A⁽⁶⁾. CRS with type 2 inflammation is vulnerable to polyp formation, and severe type 2 nasal polyps are usually refractory to treatment^(7,8).

CRSwNP is uncommon in children, with a prevalence of 0.1%⁽⁹⁾. The histopathology of paediatric CRSwNP is rarely reported because of the low prevalence or unavailability of tissue samples. For medically recalcitrant CRS in the paediatric population, endoscopic sinus surgery (ESS) is considered an effective and safe treatment modality to remove nasal polyps and restore physiological sinus ventilation and drainage, especially in older children or those with nasal polyposis⁽¹⁰⁻¹²⁾. Although ESS has shown promising outcomes in improving sinonasal symptoms and quality of life, the recurrence rate and necessity for revision surgery have been reported to be 9–13%^(13,14). In the present study, we retrospectively analysed paediatric patients who underwent ESS for bilateral CRSwNP to elucidate the inflammatory pattern and clinical characteristics related to revision surgery after ESS in a paediatric population. Endotyping patients with CRSwNP is of increasing importance as a mechanism to identify patients likely to benefit from biologic treatments, especially those who experience frequent recurrence after surgery.

Materials and methods

Patients

Paediatric patients (age < 18 years) who underwent ESS for bilateral CRSwNP at the Chang Gung Memorial Hospital between 2004 and 2017 were identified via an automated search of the histopathology database and confirmed by manual chart reviews. The diagnosis of CRSwNP was based on the EPOS 2020 definition⁽¹²⁾. The presence of nasal polyps was established based on endoscopic findings and histopathologic analysis. The exclusion criteria were as follows: 1) patients with benign or malignant sinonasal neoplasms; 2) patients with a concomitant diagnosis of cystic fibrosis, primary ciliary dyskinesia, or immunologic complications; 3) patients whose preoperative computed tomography (CT) data were unavailable in the hospital computer network; and 4) patients with a follow-up period of <12 months. A total of 146 patients with bilateral CRSwNP were enrolled, among whom 22 had recurrent nasal polyps requiring revision surgery during the follow-up period. The endoscopic

sinus surgeries targeted the abnormalities observed on the CT images in primary CRSwNP, and more extensive surgery was performed in those requiring revision surgery at our institute. The surgeries were performed by seven experienced rhinologists. No difference in the recurrent rate was observed among the surgeons.

To characterise the inflammatory features of recurrent CRSwNP, 22 age- and sex-matched patients who did not undergo revision surgery during the follow-up period were selected to perform a case-control comparison in the immunostaining study. The clinical characteristics of the patients were collected. The requirement for informed consent was waived due to the retrospective nature of the study and anonymity of the data. This study was approved by the Institutional Review Board of the Chang Gung Medical Foundation (IRB number: 202201253B0). All study procedures were performed in accordance with relevant guidelines and regulations.

Tissue eosinophil quantification

Mucosal specimens obtained during ESS were fixed in formalin and embedded in paraffin. Standard 5- μ m sections were stained with haematoxylin and eosin. The number of eosinophils was counted in three microscopic fields with intact mucosal epithelium and the most severe inflammatory cell infiltration in each tissue section at $\times 400$ magnification (high-power field, HPF) by a person who was blinded to the clinical information of the patients.

Immunohistochemistry (IHC) for ECP, IL-5, IFN- γ , and IL-17A expression

Paraffin sections of sinus mucosa were de-waxed in xylene and rinsed in absolute alcohol, microwaved for 8 min in 1 mM Ethylenediaminetetraacetic acid (pH 8.0), and incubated in 3% H₂O₂ for 10 min to block endogenous peroxidase activity. The sections were incubated with specific antibodies against ECP, IL-5, IFN- γ , and IL-17A (dilutions of 1:300, 1:300, 1:1000, and 1:1000, respectively) (Bioss Antibodies, MA, USA) (Genetex, CA, USA) for 18 h at 4 °C. The slides were rinsed three times with phosphate-buffered saline before incubation with the appropriate secondary antibodies and further processed for IHC using the IHC Select HRP Detection Set (Merck KGaA, Darmstadt, Germany). The slides were stained with DAB (Dako, CA, USA) and counterstained with haematoxylin. Images of IHC were captured using the Olympus BX50 microscope equipped with an Olympus E5 camera (Olympus, Tokyo, Japan). Quantitation of the immunostaining results was performed using the Image J Fiji Software (version 1.2; WS Rasband, National Institute of Health, Bethesda, MD, USA) as described previously⁽¹⁵⁾. The areas with positive staining in the three regions of interest (ROIs, intact mucosa with the most severe inflammatory cell infiltration) of each sample were identified in the captured images by setting a threshold

Table 1. Clinical characteristics of control and CRSwNP.

| | Total (n = 146) | Revision surgery (n = 22) | No revision surgery (n = 124) | P value [†] |
|--|--------------------|------------------------------|----------------------------------|----------------------|
| Age (years) | 15.0 ± 2.1 | 13.8 ± 2.3 | 15.2 ± 2.0 | 0.009** |
| Female : male | 57:89 | 10:12 | 47 : 77 | 0.503 |
| Comorbid asthma | 3 (2.1%) | 1 (4.5%) | 2 (1.6%) | 0.390 |
| Adenoidectomy | 13 (8.9%) | 2 (9.1%) | 11 (8.9%) | 1.000 |
| Positive Phadiatop | 35 (45.5%) | 10 (45.5%) | 25 (20.2%) | 0.010* |
| Serum IgE (KU/L) ^a | 73.2 (118.3) | 76.8 (150.6) | 70.0 (114.8) | 0.887 |
| Lund-Mackay score ^a | 20.0 (6) | 22.0 (5.2) | 16.0 (11.5) | <0.001*** |
| Ethmoid/maxillary ratio | 1.8 ± 0.6 | 2.1 ± 0.4 | 1.8 ± 0.6 | 0.011* |
| Olfactory cleft opacification score ^a | 4.0 (3.0) | 4.0 (3.3) | 4.0 (3.0) | 0.130 |
| WBC (1000/ μ L) | 7.9 ± 2.4 | 7.9 ± 1.9 | 7.9 ± 2.5 | 0.558 |
| Neutrophil (%) | 59.5 ± 11.0 | 57.5 ± 8.6 | 60.0 ± 11.4 | 0.276 |
| Lymphocyte (%) | 33.0 ± 9.7 | 35.4 ± 7.9 | 32.5 ± 10.0 | 0.188 |
| Eosinophil (%) ^a | 1.4 (1.3) | 1.3 (2.1) | 1.4 (1.3) | 0.868 |
| Neutrophil/lymphocyte ratio ^a | 1.8 (1.4) | 1.5 (1.0) | 1.9 (1.1) | 0.244 |
| Absolute serum eosinophil count (/ μ L) ^a | 100.6 (112.8) | 101.6 (130.4) | 100.6 (113.3) | 0.798 |
| Tissue eosinophil count, (/HPF) ^a | 100.6 (112.9) | 33.0 (66.5) | 13.0 (27.5) | <0.001*** |

Data passing the normality test are represented as mean ± standard deviation. [†] Comparison between the participants who did and did not require revision surgery was performed using the Mann–Whitney U test for continuous variables and χ^2 test or Fisher exact test for categorical variables.

^a Data failing the normality test are represented as median (interquartile range). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

value and computed according to the software instructions. The percentage of the area with positive staining was calculated. The mean intensity of each ROI was calculated as the optical density of all areas/total area of each ROI. The evaluation was performed by a person who was blinded to the clinical information of the patients.

Features on sinonasal computed tomography

The sinonasal CT images were independently reviewed by two experienced rhinologists repeatedly; however, the patients' information was not blinded due to the nature of the reviewing process. The Lund-Mackay score was used for the radiologic quantification of CRS severity⁽¹⁶⁾. The sinuses were grouped into the frontal sinus, anterior ethmoidal cells, posterior ethmoidal cells, maxillary sinus, sphenoid sinus, and ostiomeatal complex. Each sinus was assigned a score of 0 (no abnormality), 1 (partial opacification), or 2 (complete opacification), whereas the ostiomeatal complex was assigned a score of 0 (not obstructed) or 2 (obstructed). Each side was graded separately, and the combined bilateral score was up to 24.

The ethmoid/maxillary (EM) ratio was calculated by dividing the sum of the CT scores of the bilateral ethmoid sinuses by those of the bilateral maxillary sinuses⁽¹⁷⁾. A higher ratio indicated a

higher disease proportion in the ethmoid sinus area.

The olfactory cleft (OC) opacification on CT images was rated on a scale of 0–3, indicating clear (score 0), less than half (score 1), more than half (score 2), and total (score 3) opacification at each side of the OC (Figure S1 in Supplementary Material).

Statistical analyses

The data are presented as mean ± standard deviation and statistically analysed using GraphPad Prism 5 (GraphPad Prism Software, Inc., San Diego, CA, USA) and RStudio v2022.02.1 (RStudio, Boston, MA, USA). Categorical variables were compared using the χ^2 test or Fisher's exact test as appropriate. Continuous variables were analysed using the Mann–Whitney U test when comparing the two groups. Univariate and multivariate logistic regression analyses were used to assess the associations between revision surgery and the variables by calculating the odds ratios (ORs) with 95% confidence intervals. To identify the cut-off values for the prediction of revision surgery, the receiver operating characteristic (ROC) curves were analysed, and the area under the ROC curve (AUC) was calculated. Based on the results of the logistic regression model, a nomogram model was plotted to predict the probability of revision surgery. The ROC curve, AUC, and calibration curve were constructed to measure

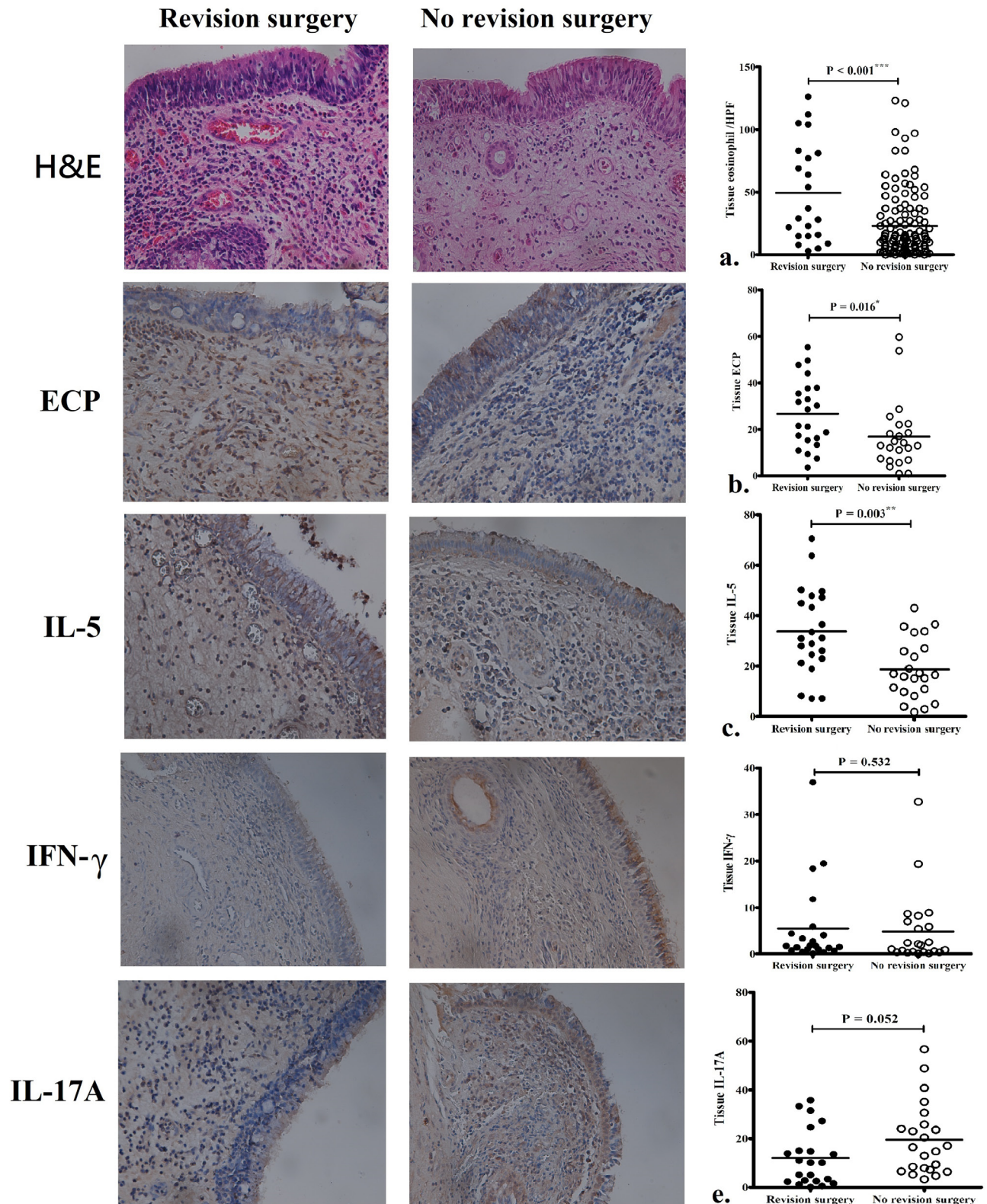


Figure 1. The tissue eosinophil infiltration (a), immunoreactivity of the eosinophilic cationic protein (ECP, b) and IL-5 (c) in the sinus mucosa were higher in patients requiring revision surgery than those who did not require revision surgery. There was no difference in the immunoreactivity of IFN- γ (d) and IL-17A (e) between the two groups. Magnification: 400X; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

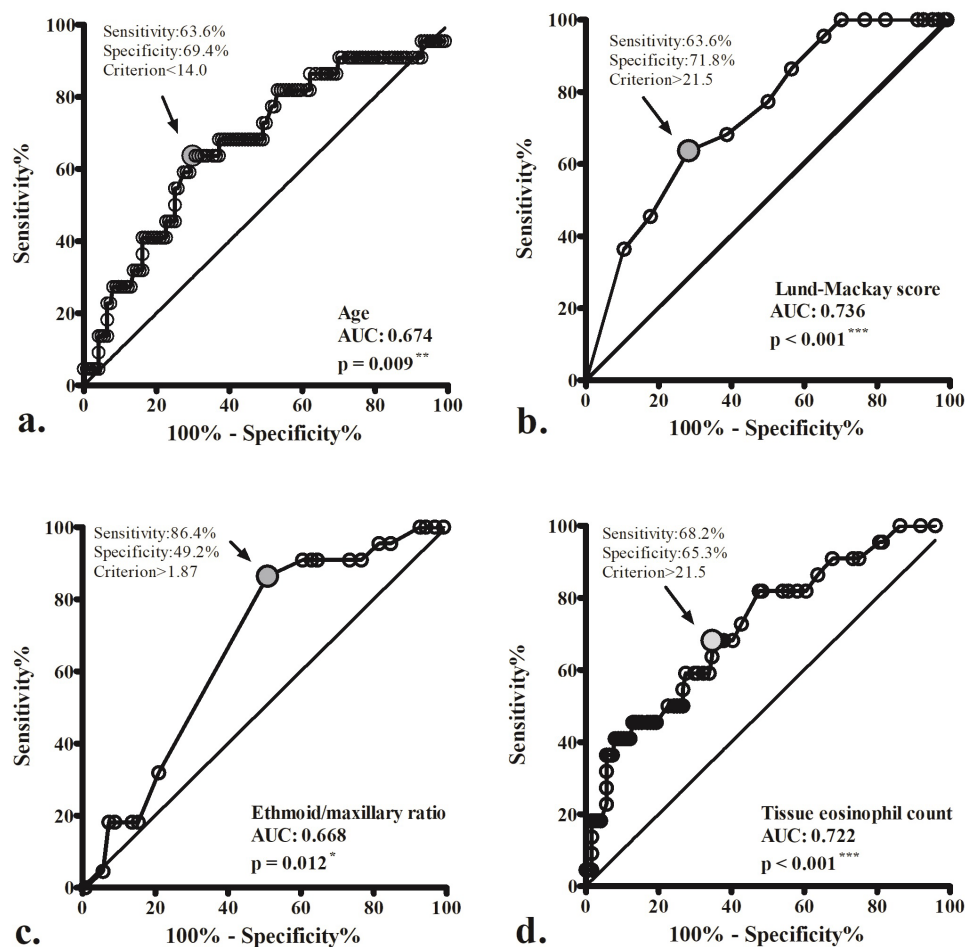


Figure 2. Receiver operating characteristic curves were generated to detect the probability of revision surgery using the variables of age (a), Lund-Mackay score (b), ethmoid/maxillary sinus ratio (c), and tissue eosinophil count (d). The optimal cutoffs for these metrics (maximising the sum of the sensitivity and specificity) are indicated. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

the predictive performance of the nomogram model. Statistical significance was set at $p < 0.05$. The power was calculated as 85.1% from the difference between the primary outcomes in the study groups.

Results

Clinical characteristics of the study population

A total of 146 paediatric patients with bilateral CRSwNP, including 89 males and 57 females, were enrolled. The average patient age was 15.0 ± 2.1 years. Twenty-two patients (15.1%) underwent revision surgeries. The mean time between the initial and first revision surgery was 46.2 ± 29.7 months. The demographic data are shown in Table 1. Patients who required revision surgery were younger and had more positive Phadiatop results than those who did not require revision surgery. There was no difference between the serum white blood cell and eosinophil counts of the two study groups.

Tissue eosinophil quantification

The average tissue eosinophil count in each HPF was 27.0 ± 29.7 for the total cases, 49.3 ± 39.5 in patients who required revision surgery group, and 23.1 ± 25.8 in patients who did not require revision surgery. The eosinophil infiltration in the sinus mucosa of patients requiring revision surgery was significantly more severe (Figure 1a).

Immunohistochemistry for ECP, IL-5, IFN- γ , and IL-17A expression

The expression of ECP and IL-5 in the sinus mucosa of patients who required revision surgery was higher than that in patients who did not require revision surgery (Figures 1b and c). However, there was no difference in the expression of IFN- γ and IL-17A between the two groups (Figures 1d and e).

CT features

In the comparison of CT features between the two study groups,

Table 2. Logistic regression analyses of the variables for revision surgery.

| Variables | Univariate analysis | | Multivariate analysis | |
|---------------------------------------|------------------------|-----------|------------------------|---------|
| | Odds Ratio (95% CI) | P value | Odds Ratio (95% CI) | P value |
| Age | 0.75 (0.60-0.93) | 0.008** | 0.86 (0.67-1.10) | 0.234 |
| Gender | 0.73 (0.29-1.83) | 0.505 | | |
| Positive Phadiatop results | 4.40 (1.25-15.50) | 0.021* | 3.66 (0.80-16.82) | 0.095 |
| Absolute serum eosinophil count (/uL) | 1.00 (0.99-1.00) | 0.798 | | |
| Lund-Mackay score | 1.35 (1.12-1.62) | 0.001** | 1.25 (1.00-1.57) | 0.050 |
| Ethmoid/maxillary ratio | 2.53 (1.10-5.78) | 0.028* | 2.38 (0.76-7.45) | 0.138 |
| Olfactory cleft opacification score | 1.23 (0.92-1.64) | 0.168 | 0.77 (0.52-1.13) | 0.178 |
| Tissue eosinophil count(/HPF) | 1.02 (1.01-1.04) | <0.001*** | 1.03 (1.01-1.04) | 0.004** |

CI, confidence interval. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

the patients who required revision surgery had significantly higher total Lund-Mackay scores and E/M ratio than those who did not require revision surgery (Table 1). There was no difference between the olfactory cleft opacification scores of the two study groups (Figure S2 in Supplementary Material).

Logistic regression analysis

Associations between the variables and revision surgery were examined using logistic regression analysis (Table 2). In the univariate analysis, age, positive Phadiatop results, the Lund-Mackay score, E/M ratio, and tissue eosinophil count were significant predictors of revision surgery. Tissue eosinophil count remained statistically significant in the multivariate analysis.

Using age, the Lund-Mackay score, E/M ratio, and tissue eosinophil count to predict the need for revision surgery

ROC curves were generated, and the AUC was calculated to evaluate the sensitivity and specificity of age, the Lund-Mackay score, E/M ratio, and tissue eosinophil count for predicting the probability of requiring revision surgery in our cohort (Figure 2). The ROC curves of age (AUC = 0.674, $p = 0.009$), Lund-Mackay score (AUC = 0.736, $p < 0.001$), E/M ratio (AUC = 0.668, $p = 0.012$), and tissue eosinophil count (AUC = 0.722, $p < 0.001$) had AUCs significantly greater than 0.5. The optimal cut-off values for these variables (maximising the sum of sensitivity and specificity) were age < 14.0 (sensitivity, 63.6%; specificity, 69.4%), Lund-Mackay score > 21.5 (sensitivity, 63.6%; specificity, 71.8%), E/M ratio > 1.87 (sensitivity, 86.4%; specificity, 49.2%), and tissue eosinophil count > 21.5 (sensitivity, 68.2%; specificity, 65.3%).

Nomogram for predicting revision surgery

A nomogram was developed to predict the probability of requiring revision surgery according to the results of the logistic regression analysis due to the lack of a single ideal predictor

in the ROC analysis (Figure 3). In the nomogram, each variable value represents a score. The total points can be obtained by adding the corresponding scores from the four predictors, and the predicted value of requiring revision surgery for an individual can be indicated. The ROC curve illustrated the good discrimination ability of the nomogram, with an AUC of 0.827 (95% CI, 0.741–0.949) (Figure S3 in Supplementary Material). Calibration curves for the unadjusted nomogram model and bias-corrected model were plotted and overlaid on a diagonal, representing a good model for prediction.

The nomogram total points of each patient were calculated and compared between the two study groups. Patients who required revision surgery had significantly higher points than those who did not require revision surgery (Figure 4a). The ROC curves of the nomogram total points for predicting the need for revision surgery had AUCs significantly greater than 0.5 (Figure 4b). The optimal cut-off was > 166.2 (sensitivity, 72.7%; specificity, 83.1%).

Discussion

ESS is considered a safe and effective surgical modality in children with medically recalcitrant CRS, especially in older children or those with nasal polyposis⁽¹⁰⁻¹²⁾. Although ESS has shown promising results in the improvement of sinonasal symptoms and quality of life, the need for revision surgery was reported as approximately 13% in previous investigations^(13,14) and the current study. In the present study, we evaluated the inflammatory patterns in paediatric CRSwNP by quantifying the tissue eosinophils and evaluating the expression levels of type 1, 2, and 3 signature cytokines, and compared the recurrence and non-recurrence groups. The results showed that the eosinophil infiltration was significantly more severe and the ECP and IL-5 expressions were in the sinus mucosa of the patients requiring revision surgery. These results indicate that more severe type 2

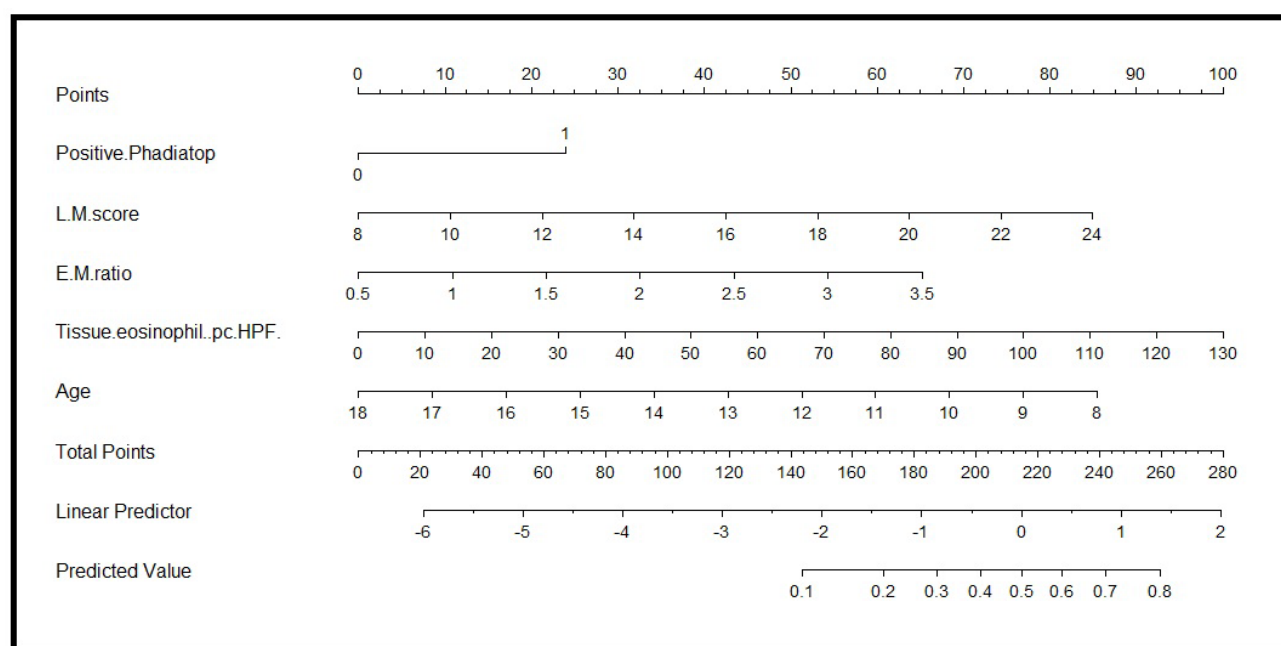


Figure 3. The nomogram developed to predict the probability of revision surgery according to the results of the logistic regression analysis. In the nomogram, each value of a variable represents its score. The total points can be obtained by adding the corresponding scores from the four predictors, and the predicted value of revision surgery for an individual can be indicated.

eosinophilic inflammation may contribute to a significant risk of recurrence. To the best of our knowledge, this is the first immunologic analysis of recurrent and non-recurrent CRSwNP in the paediatric population.

Studies on adult CRSwNP have shown that nasal polyps recurred immediately after ESS and presented with strong eosinophilic infiltration⁽¹⁸⁻²⁰⁾. Mucosal eosinophilia is associated with more severe symptoms and frequent comorbid asthma, and it often requires multiple surgeries⁽²¹⁾. As a result, severe type 2 eosinophilic CRSwNP is difficult to treat^(18,22). However, studies on the immunopathologic characteristics of paediatric CRSwNP are rare because of the low prevalence or unavailability of tissue samples⁽¹⁰⁾. The existing evidence in the literature is heterogeneous. Chan et al. found that eosinophilic tissue infiltration in CRS was more obvious in adults and older children than in younger children⁽²³⁾. Berger et al. reported diminished tissue eosinophilia in paediatric CRS compared with that in adult CRS⁽²⁴⁾. Coffinet et al. demonstrated that lymphocytes were more prevalent in young children with CRS than eosinophils⁽²⁵⁾. However, Jiang et al. revealed that tissue eosinophils were more abundant in the younger CRSwNP group than that in the older CRSwNP or CRSsNP groups and showed that Chinese paediatric CRSwNP presents as eosinophilic with mixed type 2/type 3 inflammation⁽¹⁾. Brown et al. recently reported that the majority (57.8%) of paediatric CRS cases had a lymphocyte-predominant inflammatory background, whereas the majority (66.5%) of adult CRS had a lymphoplasmacytic-predominant inflammatory background

⁽²⁶⁾. Differences in age, ethnic groups, environmental area, and the presence of polyps may be attributed to the different mechanisms and immunopathologic features of CRSwNP in the paediatric population⁽¹⁰⁾. Future large-scale studies are necessary to clarify the inflammatory pattern of CRSwNP in different age groups or areas. In the current study, our investigation focused on paediatric CRSwNP and demonstrated that more severe type 2 eosinophilic inflammation may contribute to a significant risk of recurrence. These results had a higher degree of similarity with those of the adult CRSwNP group, possibly because of the relatively older age and the presence of nasal polyposis in our study cohort. However, endotyping patients with CRSwNP, especially those with frequent recurrence after surgery, is increasingly important to identify patients who are likely to benefit from biologic treatments in the future⁽²⁷⁾.

The revision surgery group exhibited significantly higher total Lund-Mackay scores and E/M ratio on CT images than those who did not require revision surgery. The Lund-Mackay staging system was developed to quantify the extent of sinus inflammation⁽¹⁶⁾. A greater Lund-Mackay score is also associated with an increasing grade of polyposis and multiple sinus involvement, both of which may increase surgical difficulty and complexity^(14,28). Similarly, increased Lund-Mackay scores have been correlated with the need for revision surgery in adult CRS patients⁽²⁸⁾. In the present study, our results showed that the probability of revision was significantly higher in paediatric patients with increased Lund-Mackay scores.

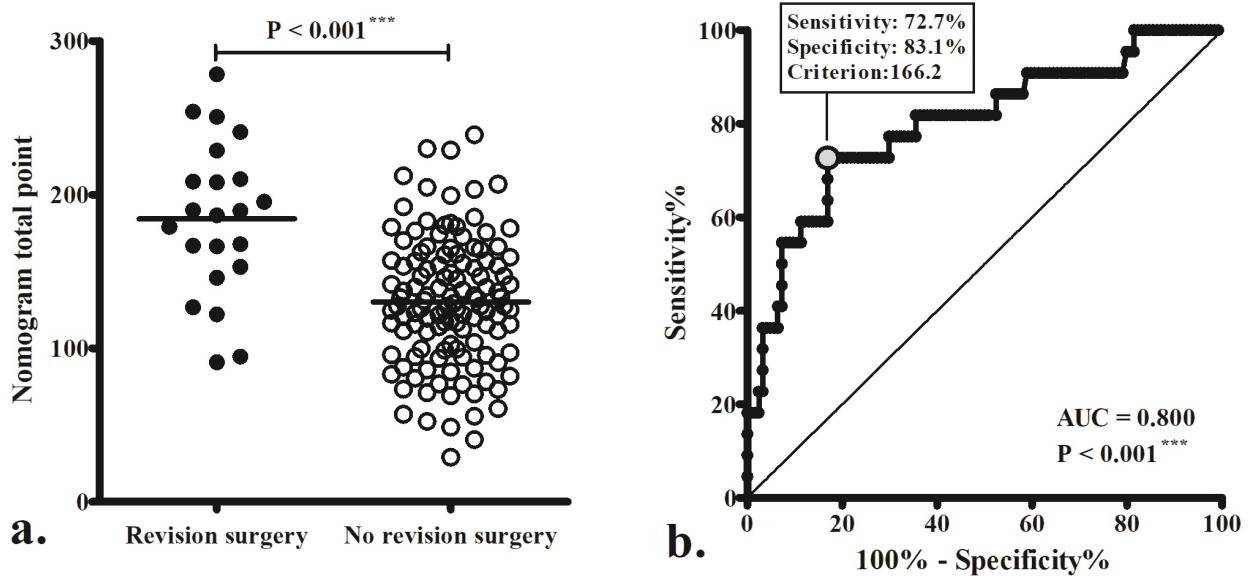


Figure 4. In the comparison of nomogram total points between the two study groups, the children who required revision surgery had significantly higher points than those who did not require revision surgery (a). The ROC curve was generated with nomogram total points to detect the need for revision surgery in our participants (b). The optimal cutoff is indicated. *** $p < 0.001$.

A previous study reported that CRS patients with dominant disease in the ethmoid sinus, compared with the maxillary sinus, on CT images were significantly refractory and susceptible to recurrence after surgery⁽²⁹⁾. Type 2 inflammation tends to involve the central part of the sinonasal area, including the nasal septum, OC, middle turbinates, and ethmoid cells^(6,17). However, the mechanism responsible for the predominance of ethmoid sinus inflammation in patients with type 2 CRS remains unclear. One possible explanation is that there are regional differences in the expression patterns of molecules in the nasal cavity⁽³⁰⁻³³⁾. Thus, the Japanese Epidemiological Survey of Refractory Eosinophilic Chronic Rhinosinusitis (JESREC) proposed ethmoid > maxillary sinus on CT shadow as one of the scoring criteria for refractory eosinophilic CRS⁽²⁹⁾. Meng et al. reported that the E/M ratio with a cut-off point of >2.59 is a more useful predictor in the diagnosis of adult eosinophilic CRSwNP from non-eosinophilic CRSwNP with a sensitivity of 94.2% and a specificity of 89.6%⁽¹⁷⁾. In the current study, our results showed that the E/M ratio with a cut-off value of >1.75 can distinguish recurrent CRSwNP from non-recurrent cases (sensitivity, 86.4%; specificity, 49.2%). Olfaction impairment is one of the most prevalent symptoms in adult type 2 eosinophilic CRSwNP due to the frequent involvement of OC in type 2 CRS inflammation⁽¹⁷⁾. However, paediatric patients or their caregivers may not frequently report olfaction-associated symptoms of CRSwNP^(2,34). Thus, we determined the OC opacification score on CT images to evaluate the severity of inflammation in the olfactory mucosa in paediatric patients with CRSwNP. Our results revealed a trend of increased OC opacification on CT images in patients requiring revision surgery; however, it was not statically significant.

Our previous study reported the association between revision surgery and age and positive aeroallergen test results⁽¹⁴⁾. Conservative surgical procedure; inadequate postoperative wound care, such as inadequate cleansing of the operative wounds in the outpatient department and nasal saline irrigation at home; and immature immune systems with frequent episodes of upper respiratory tract infections may contribute to the higher recurrence rate in younger children^(14,35). Moreover, the higher prevalence of positive aeroallergen test results in patients requiring revision surgery may be a result of the increased disease burden due to the presence of many comorbidities, such as atopic diseases, including allergic asthma, atopic dermatitis, allergic conjunctivitis, rhinosinusitis, and allergic ear diseases⁽³⁶⁻³⁸⁾. Adenoidectomy has been considered as the first-line surgical procedure for paediatric CRS refractory to conservative treatment^(11,12). However, in our study cohort, only 13 patients underwent adenoidectomy. The low adenoidectomy rate may have been caused by the participants' relatively older age (15.0 ± 2.1 years) and the presence of nasal polyposis. Adenoidectomy may be more beneficial in young children with CRSsNP, but such patients were not enrolled in our study.

A nomogram was developed to predict the probability of revision surgery because of the lack of a single ideal predictor in the ROC analysis. The predicted value of revision surgery was calculated by adding the corresponding scores from the five predictors in the nomogram (Figure S4 in Supplementary Material). The preoperative identification of patients vulnerable to recurrence after surgery is important for performing intensive postoperative adjunct therapy and follow-up.

This study has several limitations. First, missing data, loss to follow-up, the inclusion of different surgeons, and the lack of clinical history were unavoidable complicating factors owing to the retrospective study design. For example, the diagnosis of allergies and asthma was made by manually reviewing the patient's medical records based on clinical guidelines. Incomplete survey and lack of clinical information may have led to the underestimation of the results. Besides, due to the retrospective nature of our study, the data regarding the active/passive smoking exposure and the comprehensive evaluation of associated symptoms were unavailable. We enrolled 146 cases with detailed histopathological and clinical reviews to reduce these concerns. Second, the need for revision usually depends on the patient's symptoms, caregiver's concern, and surgeon's preference and may vary on a case-by-case basis. Further prospective studies are necessary to validate our results as predictors of recurrent CRSwNP. Third, only children with a histopathological diagnosis of CRSwNP who underwent sinus surgery were recruited for this study. Therefore, patients with CRSwNP who were ineligible for surgery were excluded from this study. This may have led to selection bias.

Conclusion

We found that high tissue eosinophilia and CT features of the Lund-Mackay score, E/M ratio, positive Phadiatop results, and young age were predictors for revision surgery in paediatric CRSwNP. We developed a nomogram model as a novel preoperative diagnostic tool for identifying patients vulnerable to re-

currence after surgery, according to the tissue eosinophil count and clinical and CT features. This could help clinicians predict the probability of revision surgery and perform intensive postoperative adjunct therapy and follow-up. In addition, endotyping patients with CRSwNP, especially those with frequent recurrence after surgery, is increasingly important to identify patients likely to benefit from biologic treatments in the future.

Authorship contribution

CCH and CHC designed this study. CCH, PWW, YLH and PHC performed data collection. YHF, PWW, and CCH performed data analysis and drafted the manuscript. YLH, TJL, CCH, and PHC helped with the enrolment of participants and collection of clinical data. PWW and CCH contributed to the data interpretation. All authors participated in scientific discussions and approved the final manuscript.

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Conflict of interest

The authors declare no conflicts of interest.

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SUPPLEMENTARY MATERIAL

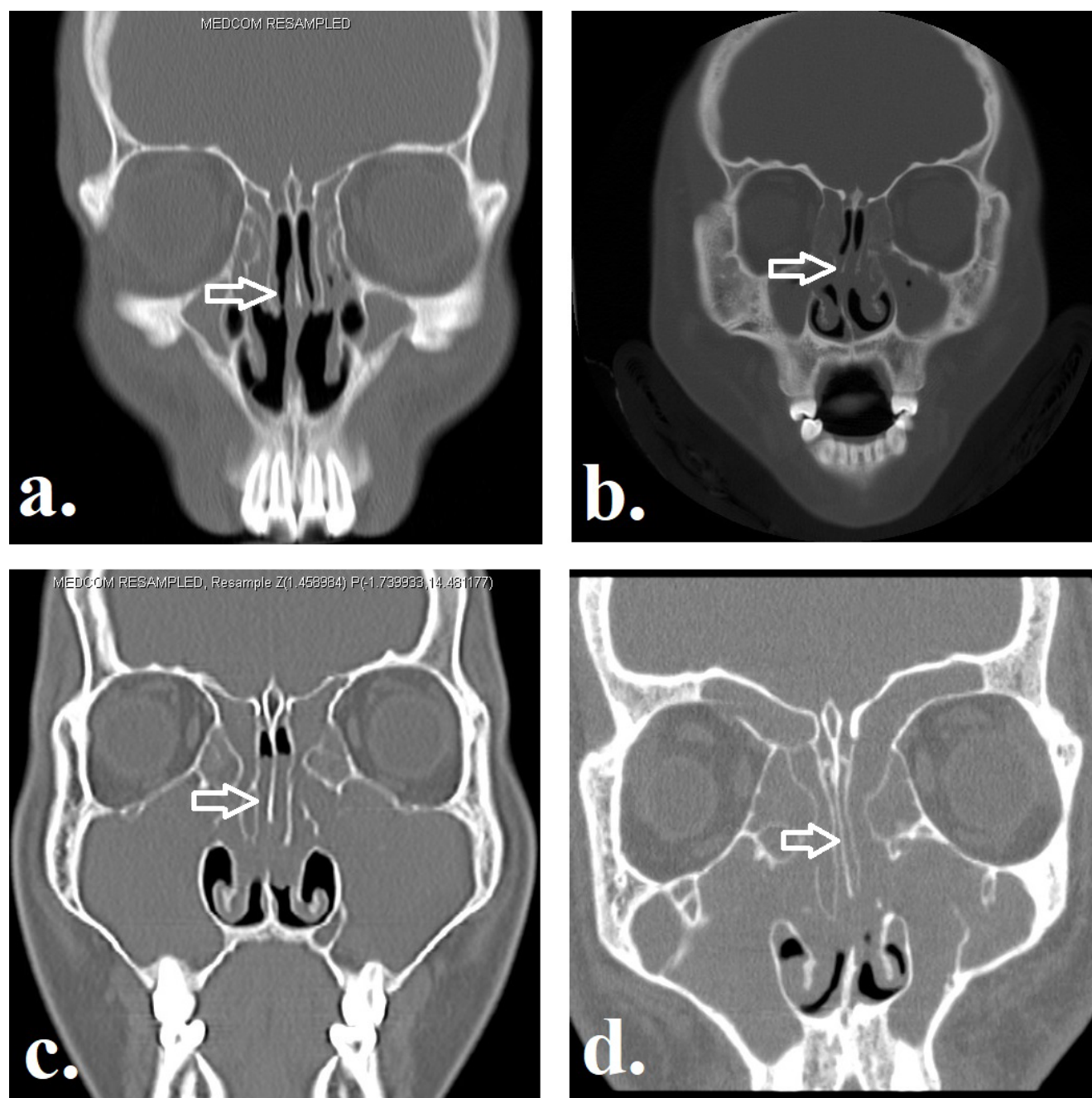


Figure S1. Grading of the olfactory cleft (OC) opacification. The OC opacification on CT image was rated on a scale of 0–3 indicating clear (a, score 0), less than half (b, score 1), more than half (c, score 2), and total (d, score 3) opacification at each side of the OC (the area between the middle turbinate and the nasal septum on the coronal plane section of the cribriform plate's CT imaging).

Table S1. Characteristics of the participants for immunohistochemistry study.

| | Revision surgery (n = 22) | No revision surgery (n = 22) | P value [†] |
|---------------------------------------|------------------------------|---------------------------------|----------------------|
| Age (years) | 13.6 ± 2.3 | 13.8 ± 2.1 | |
| Female : male | 10:12 | 10 : 12 | |
| Comorbid asthma | 1 (4.5%) | 2 (9.1%) | 1.000 |
| Adenoidectomy | 1 (4.5%) | 5 (22.7%) | 0.185 |
| Positive Phadiatop result | 10 (45.5%) | 5 (22.7%) | 0.116 |
| Serum IgE (KU/L) ^a | 76.8 (150.6) | 38.6 (83.4) | 0.090 |
| WBC (1000/ μ L) | 7.9 ± 1.9 | 8.0 ± 2.2 | 0.658 |
| Neutrophil (%) | 57.6 ± 8.6 | 57.2 ± 8.5 | 0.824 |
| Lymphocyte (%) | 35.4 ± 7.9 | 34.7 ± 8.2 | 0.833 |
| Eosinophil (%) | 1.6 ± 1.4 | 2.0 ± 1.4 | 0.291 |
| Absolute eosinophil count (/ μ L) | 117.7 ± 95.9 | 147.9 ± 96.7 | 0.302 |

Data are represented as mean ± stand deviation. [†] Mann-Whitney U test for continuous variables; χ^2 test or Fisher exact test for categorical variables;

^a Data are represented as median (interquartile range).

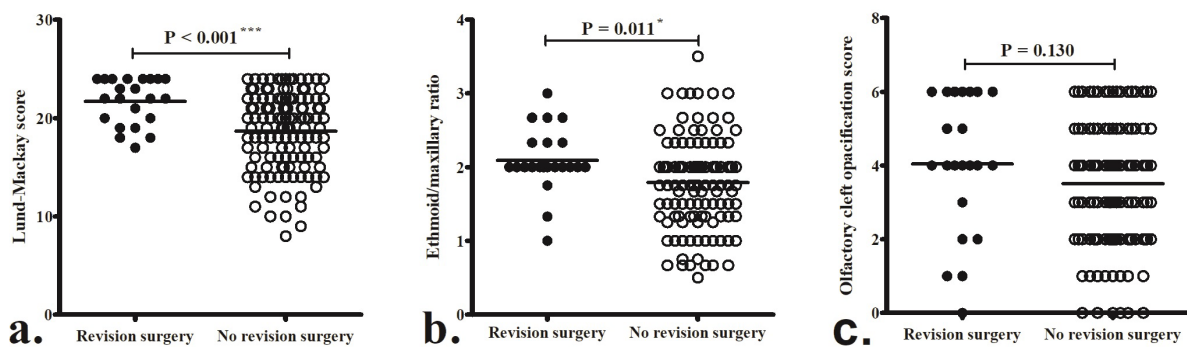


Figure S2. In the comparison of the computed tomographic features between the two study groups, the children with revision surgery exhibited significantly higher scores in total Lund-Mackay scores (a) and ethmoid/maxillary sinus (E/M) ratio (b) than those without revision surgery. There was no difference in the scores of olfactory cleft opacification between two study groups (c). * $p < 0.05$; *** $p < 0.001$.

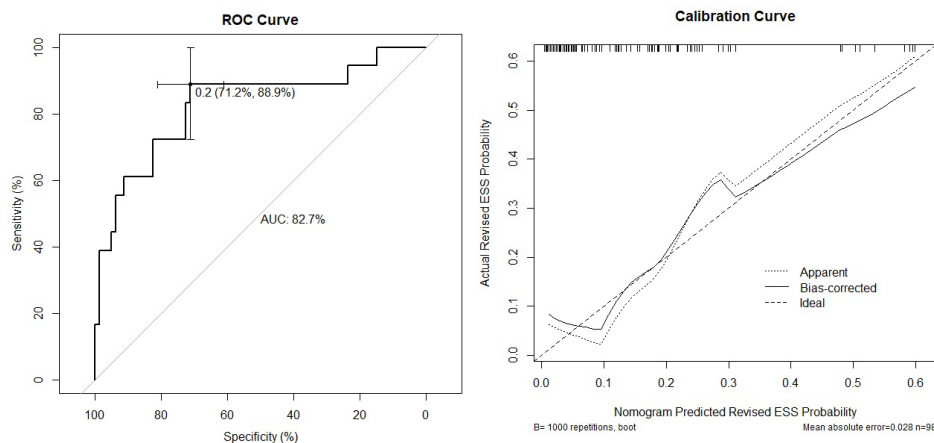
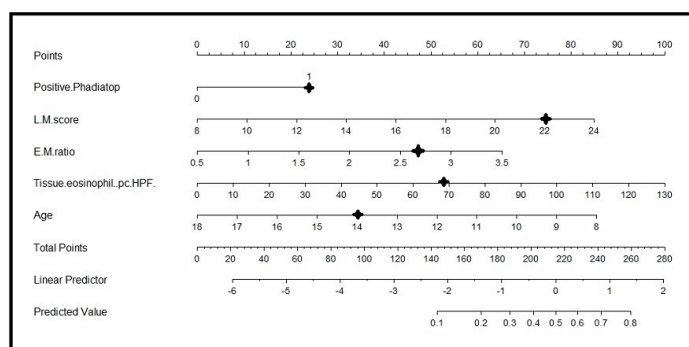
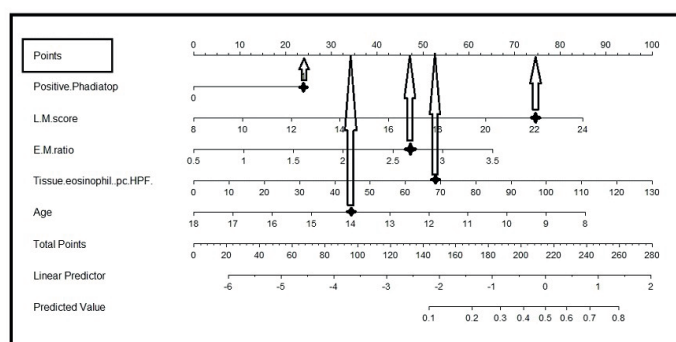


Figure S3. Receiver operating characteristic (ROC) curve of the nomogram model for predicting the probability of revision surgery. The area under the ROC curve (AUC) was 0.827 (95% confidence interval = 0.741-0.949) (a). Calibration curve of nomogram model for predicting the probability of revision surgery. The Ideal line represents the ideal model which predicts probabilities that perfectly match the actual probabilities. The Apparent line and bias-corrected line represent the nomogram model before and after bootstrap resampling method (b), respectively.

Step 1. Find the corresponding position of each variable according to the patient's clinical information, radiological and histological characteristics.



Step 2. Next, draw lines vertically upward to the Points axis above to obtain the respective points for each variable. After that, calculate the total points by summing up the respective points for each variable. For this patient, his total points are **232 points**.



Step 3. Finally, draw a line vertically downward from the corresponding position on the Total Points axis to the Predicted Value axis to obtain the probability of requiring revision surgery. For this patient, the probability is **about 63%**.

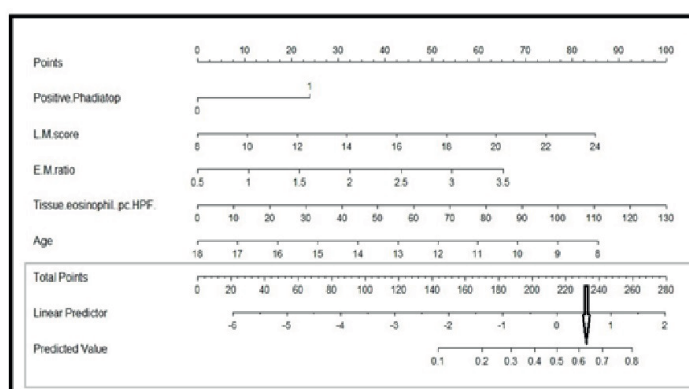


Figure S4. Steps for using a nomogram to predict the probability of a patient requiring revision surgery.

*Using a nomogram to predict the probability of a patient requiring revision surgery Before using the nomogram model, clinicians need to collect the patient's clinical information as shown below, including age, total Lund–Mackay (L.M.) score, ethmoid-to-maxillary sinus (E.M.) ratio on CT, tissue eosinophil count, and aeroallergen test results (Phadiatop). A study participant was chosen to exemplify the nomogram use. A 14-year-old boy presented with post-nasal dripping and nasal obstruction. His aeroallergen test was positive; the total L.M. score was 22, and the E.M. ratio was 2.67. Histology showed a tissue eosinophil count of 69/HPF. Therefore, based on these results, we used the nomogram to obtain the probability of requiring revision surgery for this patient, following the steps in Figure S4 in Supplementary Material; the probability obtained was approximately 63%.