Latent class analysis of structured histopathology in prognosticating surgical outcomes of chronic rhinosinusitis with nasal polyps in Singapore*

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Abstract

Background: Structured histopathology profiling is recommended when reporting chronic rhinosinusitis with nasal polyp (CRSwNP) tissue. The objective of this study is to identify features in structured histopathology that predict outcome after functional endoscopic sinus surgery (FESS) in a cohort of CRSwNP patients from Singapore.

Methods: Latent class analysis was performed on structured histopathology reports of 126 CRSwNP patients who had undergone FESS. Outcome measures were polyp recurrence, need for systemic corticosteroids, revision surgery or biologics, and disease control at 2 years post-FESS.

Results: Three classes were identified. Class 1 was characterised by mild, predominantly lymphoplasmacytic inflammation. Class 2 comprised of <100 eosinophils per high powered field (HPF) and moderate inflammation. Class 3 consisted of mainly severe inflammation, >100 eosinophils/HPF, hyperplastic seromucinous glands, mucosal ulceration and mucin containing eosinophil aggregates and Charcot-Leyden crystals. Classes 2 and 3 were significantly associated with uncontrolled disease at 2 years post-FESS. Class 3 was additionally associated with the need for systemic corticosteroids.

Conclusions: Eosinophil count, degree of inflammation, predominant inflammatory type, hyperplastic seromucinous glands, mucosal ulceration and mucin containing eosinophil aggregates and Charcot-Leyden crystals predicted need for systemic corticosteroids and uncontrolled disease at 2 years post-FESS. The presence of >100 eosinophils/HPF should be reported, as this subset of tissue eosinophilia was associated with less favourable outcomes after FESS.

Key words: sinusitis, nasal polyps, eosinophils, cluster analysis

Introduction

Chronic rhinosinusitis (CRS) is a heterogeneous inflammatory condition, which encompasses a range of clinical phenotypes and inflammatory mechanisms. In the updated European Position Paper on Rhinosinusitis and Nasal Polyps (EPOS) 2020, primary CRS is further considered by type 2 or non-type 2 endotype dominance [(1)]. Tissue eosinophilia, defined by EPOS 2020 as ≥10 eosinophils per high powered field (HPF)(x400), is one of the features of type 2 endotype and is predictive of greater disease burden [(2,3)].

A structured histopathologic report typically encompasses about 13 variables relating to the state of tissue inflammation and remodelling, including eosinophil count [(4)]. These histopathologic features may be useful in disease prognostication. There have been studies that analysed the impact of individual variables in relation to outcomes [(5-8)], but few that consider the effect of combinations of features [(9)]. This is relevant, given that

a structured histopathologic report consists of several variables that are closely related to one another. For example, basement membrane thickening, fibrosis and squamous metaplasia are markers of irreversible tissue remodelling, and eosinophils count, eosinophil aggregates and Charcot-Leyden crystals are related to eosinophilic infiltration. Knowing which variables in a structured report lend greater weight to prognostication is useful to clinicians. It could also potentially trim the structured report to include only the more relevant variables, which is practical in a busy pathology department.

The primary objective of this study was to investigate which of the features of structured histopathology reporting were useful in prognosticating 2-year outcomes of functional endoscopic sinus surgery (FESS) in our cohort of CRS patients with nasal polyps through latent class analysis. The secondary objective was to describe the inflammatory profile of our local population of CRS with nasal polyp (CRSwNP) patients, given that the inflammatory pattern of nasal polyps in Southeast Asia is not well established in the literature.

Materials and methods

A retrospective review of adult patients diagnosed with primary diffuse (bilateral) CRSwNP and underwent bilateral FESS (medial maxillary antrostomy, complete ethmoidectomy, sphenoidectomy and frontal sinusotomy) from January 2000 to January 2020 at our tertiary hospital in Singapore was performed. Exclusion criteria were primary localised (unilateral) CRS, secondary CRS, CRSsNP, history of radiation therapy to the head and neck, unable to retrieve histology slides from the FESS performed, post-operative follow-up less than 2 years and age less than 21 years old. The definitions of primary diffuse and secondary CRS are in accordance with EPOS 2020 [1]. Some of the patients received pre-operative low dose systemic corticosteroids (20-30mg/day) for 3-5 days leading up to their surgeries, at the discretion of the primary managing rhinologists. All patients received post-operative oral corticosteroids in tailing doses for 2 weeks and nasal saline irrigations twice a day, with budesonide (1mg/2ml) added to the wash at least once a day. This study was approved by our Institution Review Board.

Histology

All the histology slides from the patients' sinus surgeries were retrieved and reviewed by a senior head and neck pathologist, who was blinded to the patients' clinical data and outcomes. The findings were reported according to a recommended structured histopathology report format [4], consisting of 14 variables with slight modifications (Table 1): eosinophil count that was ≥10/HPF was further stratified into 10-50, 51-100 and >100/HPF to assess the severity of eosinophilic infiltration in our population (Figure 1). Similarly, neutrophil infiltration was categorised in terms of neutrophil count ≤10, 10-50 and >50/HPF. Basement membrane thickening was stratified into absent and present rather than measured qualitatively.

Demographic data including smoking, asthma (diagnosed by a respiratory physician), aspirin exacerbated respiratory disease (AERD), allergic rhinitis (confirmed by skin prick tests or allergen-specific serum IgE), previous FESS, serum total IgE, blood eosinophils and pre-operative Lund-Mackay computed tomography scores were recorded. Patients were classified as non-eosinophilic CRSwNP if eosinophil count was <10/HPF (x400) and eosinophilic CRSwNP if eosinophil count was ≥10/HPF [1].

Outcome measures

The outcome measures were polyp recurrence (defined as endoscopic nasal polyp score >2, maximum score 8), need for systemic corticosteroids (defined as >1 month of continuous use, or >2 courses of rescue steroids a year), need for revision surgery or biologics, and uncontrolled disease [1] (defined by EPOS 2020 as the presence of ≥3 of the following: nasal blockage; mucopurulent nasal discharge; facial pain or pressure on most days of the week; impaired sense of smell; presence of sleep disturbance or fatigue; nasal polyps on nasoendoscopy; persistent symptoms despite rescue treatment) at 2 years post-FESS. We defined polyp recurrence as polyp score >2, as we felt that this was more clinically significant than including grade 1 polyps. Polyp scores were determined independently by two rhinologists who were not the patient's primary physician, based on both written documentation as well as corroboration with outpatient endoscopic photographs, which were routinely taken during the patients' follow-ups. Discrepancies were resolved through review and discussion until consensus was reached.

Statistical analysis

Statistical analysis was performed using Stata version 17 (StataCorp LLC, College Station, TX, USA). Statistical significance was set at 2-sided $p<0.05$. Descriptive statistics for continuous variables were presented as mean (standard deviation [SD]) when normality and homogeneity assumptions were satisfied; otherwise, median (interquartile range [IQR]) and n (%) were presented for categorical variables. Differences in numerical variables were assessed using the 2-sample t test or one-way ANOVA when normality and homogeneity assumptions were satisfied; otherwise, Mann-Whitney U test or Kruskal Wallis test was used. Chi-square or Fisher exact was used for categorical variables. Latent class analysis (LCA) was performed to determine the appropriate number of clusters with distinct combinations of histopathologic characteristics. The best fitting model was selected using the Akaike information criterion (AIC) and the Bayesian information criterion (BIC). Features in structured histopathologic reporting were selected as input variables for the LCA. Association between classes and outcome measures were

Table 1. Structured histopathology variables and reported categories.

	Variable	Categories
Tissue	Degree of inflammation	Mild, moderate, severe
	Inflammatory predominance	Lymphoplasmacytic, lymphocytic, eosinophilic, neutrophilic, others
	Eosinophil count/HPF	≤10, 10-50, 51-100, >100
	Neutrophil count/HPF	≤10, 10-50, ≥51
	Squamous metaplasia	Absent, present
	Basement membrane thickening	Absent, present
	Hyperplastic/papillary change	Absent, present
	Hyperplastic seromucinous glands	Absent, present
	Mucosal ulceration	Absent, present
	Fibrosis	Absent, present
Mucin	Mucin	Absent, present
	Eosinophil aggregates	Absent, present
	Charcot-Leyden crystals	Absent, present
	Fungal elements	Absent, present

HPF = high power field

assessed using logistic regression, and adjusted for variables that were found to be significant in univariate analyses.

Results

Patient demographics

We identified 126 patients with primary diffuse CRSwNP who met the study criteria. Most of them (85/126; 67.5%) had eosinophilic CRSwNP, of whom 36.5% (31/85) displayed 10-50 eosinophils/HPF, 38.8% (33/85) had 51-100 eosinophils/HPF and 24.7% (21/85) had >100 eosinophils/HPF (Table 2). Pre-operative corticosteroids were prescribed to 41.3% (52/126) of the patients. Allergy testing was performed in 71 patients (56.3%), of whom 36 (50.7%) were positive for atopy. House dust mites (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae* and *Blomia tropicalis*) were the most common allergens (30/36, 83.3%), followed by German or American cockroach (14/36, 38.9%) and mold (6/36; 16.7%). Purulent discharge was present in 37 (29.4%) patients. The most common organism cultured was *Klebsiella pneumoniae* (6/37, 16.2%), followed by *Staphylococcus aureus* (5/37, 13.5%) and *Haemophilus influenzae* (4/37; 10.8%). The median follow-up period was 4.2 years (IQR 2.4-7.8 years).

Eosinophilic CRSwNP had significantly higher proportion of females (35.3% vs 12.2%, p=0.007), asthma (56.5% vs 12.2%, p<0.001), AERD (22.4% vs 0%, p=0.001), and higher median levels of serum total IgE (220.5 [72.5-412.0] kU/L vs 65.5 [45.0-207.5] kU/L, p=0.044) and blood eosinophils (0.57 [0.3-0.83] x10^9/L vs 0.22 [0.13-0.39] x10^9/L; p<0.001) compared to non-eosinophilic CRSwNP (Table 2). The incidence of allergic rhinitis was similar in eosinophilic and non-eosinophilic CRSwNP (52.8% vs 44.4% respectively, p=0.539). In terms of outcomes, eosinophilic CRSwNP had significantly higher proportions of patients who had polyp recurrence, required systemic corticosteroids, revision surgery/biologics and uncontrolled disease compared to non-eosinophilic CRSwNP.

Latent class analysis of histopathologic results

Latent class analysis identified three distinct clusters with significant internal homogeneity (Table 3). The membership for classes 1, 2 and 3 were 32.5%, 47.6% and 19.8% respectively (Table 4). Class 1 was characterised by eosinophil count mostly ≤10/HPF but not more than 50/HPF. The predominant inflammation was lymphoplasmacytic inflammation and the degree of inflammation was mild. Hyperplastic/papillary change, hyperplastic seromucinous glands and mucosal ulceration were uncommon and neutrophilic infiltration was absent. Class 2 was characterised by eosinophil count mostly >10/HPF but not more than 100/HPF. There was moderate degree of inflammation, fairly equal dominance of eosinophilic and lymphoplasmacytic inflammation and mucin was common, although eosinophil aggregates and Charcot-Leyden crystals were not frequently seen in the mucin. Class 3 was characterised by mainly eosinophil count >100/HPF. The predominant inflammation was eosinophilic, and the degree of inflammation was moderate to severe. The probability of hyperplastic seromucinous glands, mucosal ulceration and mucin containing eosinophilic aggregates and Charcot-Leyden crystals was the highest compared to the other two clusters (Figure 2). The Kruskal-Wallis test showed that the three clusters differed significantly with regards to seven variables: degree of inflammation, predominant inflammation, eosinophil count, hyperplastic seromucinous glands, mucosal ulceration, eosinophil aggregates and Charcot-Leyden crystals. Interestingly, none of our patients demonstrated neutrophilic inflammatory predominance or had fungal elements in their mucin.

When analysed in terms of clinical characteristics, the three classes differed in terms of gender, smoking status, tissue eosinophilia, asthma, median IgE and Lund-Mackay score (Table 4). Classes 2 and 3 had higher proportions of tissue eosinophilia, asthma, median IgE level and Lund-Mackay scores, whilst having fewer males and smokers compared to class 1.

Two-year post-operative outcomes

After adjusting for gender, smoking status, asthma and use of pre-operative steroids, class 3 had significant association with need for systemic corticosteroids at 2 years post-op (adjusted OR 4.4, 95% CI 1.2-16.4, p=0.029)(Table 5). Class 2 (adjusted OR

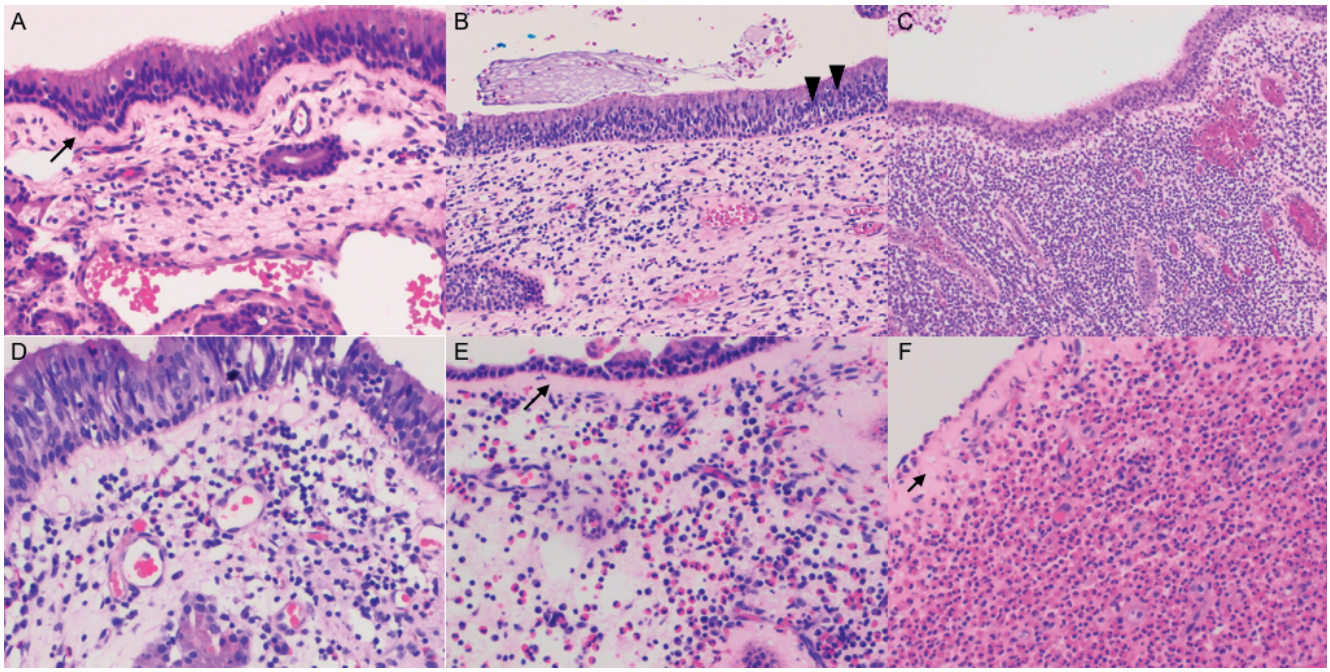

Figure 1. Non-eosinophilic (A-C) versus eosinophilic (D-E) CRSwNP. (A): Mild lymphoplasmacytic inflammation (B): Moderate lymphoplasmacytic inflammation (C): Severe lymphoplasmacytic inflammation (D): 10-50 eosinophils/HPF, moderate inflammation (E): 51-100 eosinophils/HPF, moderate inflammation (F): >100 eosinophils/HPF, severe inflammation. (Arrow: basement membrane thickening; Arrowhead: hyperplastic change).

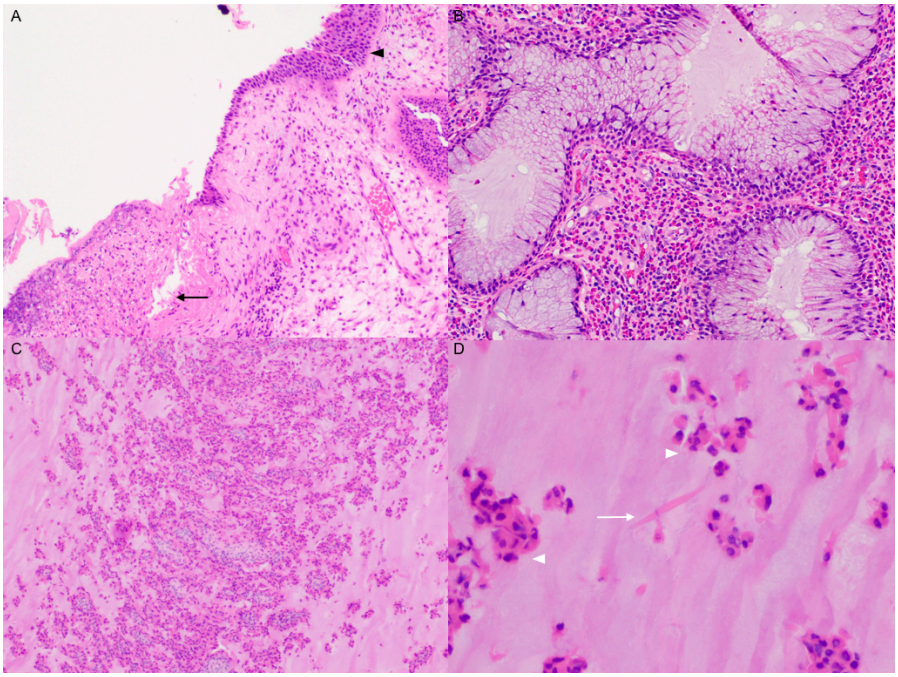

Figure 2. Histopathologic features highly associated with latent class 3, in addition to >100 eosinophils/HPF. (A): Mucosal ulceration (black arrow), squamous metaplasia (black arrowhead) (B) Hyperplastic seromucinous glands (C) Mucin containing eosinophil aggregates (D) Charcot-Leyden crystal (white arrow), eosinophil aggregates (white arrowhead).

4.4, 95% CI 1.1-18.1, p=0.041) and class 3 (adjusted OR 9.8, 95% CI 2.0-48.7, p=0.005) were significantly associated with uncontrolled disease. There was no significant difference in polyp recurrence and need for revision surgery or biologics among the three clusters.

Discussion

Our study identified three distinct latent classes based on similarities in combinations of histopathology features. These classes correlated with the predominant clinical characteristics in each class as well as post-FESS outcomes. The features in structured

Table 2. Patient demographics and 2-year post-op outcomes.

	Total (n=126)	Non-eosinophilic CRSwNP (n=41)	Eosinophilic CRSwNP (n=85)	p-value
Demographics				
Age (years)	49.2 (SD 11.7)	47.9 (SD 2.0)	49.8 (SD 1.2)	0.391
Males	91 (72.2%)	36 (87.8%)	55 (64.7%)	**0.007**
Race				0.458
Chinese	83 (65.9%)	31 (75.6%)	52 (61.2%)	
Malay	12 (9.5%)	4 (9.8%)	8 (9.4%)	
Indian	17 (13.5%)	3 (7.3%)	14 (16.5%)	
Caucausian	3 (2.4%)	1 (2.4%)	2 (2.4%)	
Others	11 (8.7%)	2 (4.9%)	9 (10.6%)	
Smoker	16 (12.7%)	7 (17.1%)	9 (10.6%)	0.306
Asthma	53 (42.1%)	5 (12.2%)	48 (56.5%)	**<0.001**
AERD	19 (15.1%)	0 (0%)	19 (22.4%)	**0.001**
Allergic rhinitis*	36 (50.7%)	8 (44.4%)	28 (52.8%)	0.539
Previous FESS	41 (32.5%)	16 (39.0%)	25 (29.4%)	0.281
Purulent discharge	37 (29.4%)	13 (31.7%)	24 (28.2%)	0.688
Total serum IgE* (kU/L)	160.5 (43.0-330.5)	65.5 (45.0-207.5)	220.5 (72.5-412.0)	**0.044**
Blood eosinophils* (x10^9/L)	0.41 (0.23-0.67)	0.22 (0.13-0.39)	0.57 (0.3-0.83)	**<0.001**
Lund Mackay score	16.1 (SD 5.0)	15.2 (SD 0.7)	16.5 (SD 0.6)	0.173
Eosinophil count/HPF				**<0.001**
<10	41 (32.5%)	41 (100%)	0 (0%)	
10-50	31 (24.6%)	0 (0%)	31 (36.5%)	
51-100	33 (26.2%)	0 (0%)	33 (38.8%)	
>100	21 (16.7%)	0 (0%)	21 (24.7%)	
2-year post-op outcomes				
Polyp recurrence	26 (20.6%)	4 (9.8%)	22 (25.9%)	**0.036**
Need for systemic corticosteroids	29 (23.0%)	2 (4.9%)	27 (31.8%)	**0.001**
Revision surgery	24 (19.1%)	3 (7.3%)	21 (24.7%)	**0.020**
Uncontrolled disease	30 (23.8%)	2 (4.9%)	28 (32.9%)	**0.001**

*Allergy testing performed in 71 patients; IgE performed in 48 patients; Blood eosinophils performed in 108 patients. AERD = Aspirin exacerbated respiratory disease; CRSwNP = Chronic rhinosinusitis with nasal polyps; FESS = Functional endoscopic sinus surgery; HPF = High powered field; SD = Standard deviation.

reporting that stood out in our CRSwNP population were degree of inflammation, predominant inflammation, eosinophil count, hyperplastic seromucinous glands, mucosal ulceration, and mucin containing eosinophil aggregates and Charcot-Leyden crystals. In particular, the main factors that distinguished the three classes were degree of inflammation, inflammatory predominance and eosinophil count/HPF. Class 1 was characterised by predominantly mild, lymphoplasmacytic (non-eosinophilic) inflammation. Classes 2 and 3 consisted mainly of eosinophilic inflammation, the key differences being that class 2 featured <100 eosinophils/HPF, while Class 3 consisted predominantly of >100 eosinophils/HPF. Hyperplastic seromucinous glands, mucosal ulceration and mucin containing eosinophil aggregates and Charcot-Leyden crystals had the highest probability of occurrence in class 3. This likely reflects the degree of tissue damage and remodelling associated with very severe eosinophilic inflammation, although individually they were not prevalent enough to define a certain latent class. This demonstrates the advantage of using latent class analysis to cluster subjects based on similarities in combinations of histopathology features and using these clusters to relate to outcomes. This form of analysis differs from other studies that analysed each histologic variable individually, as the results can be affected by the many collinear variables in the histopathology report which can result in a loss of power in the study.

This is the first study that reports the inflammatory pattern of Singaporean CRSwNP patients. Most of our CRSwNP patients exhibited a high degree of tissue eosinophilia, with 63.5% having eosinophil count >50/HPF and 24.7% having very high eosinophil counts of >100/HPF. These findings did not appear to be influenced by pre-operative oral corticosteroids, which

Table 3. Probabilities of histopathology features in each latent class.

	Total	Latent class 1	Latent class 2	Latent class 3	p-value
Membership	126 (100%)	41 (32.5%)	60 (47.6%)	25 (19.8%)	-
Histopathology features					
Degree of inflammation					**<0.001**
Mild	46 (36.5%)	41 (100.0%)	5 (8.3%)	0 (0.0%)	
Moderate	64 (50.8%)	0 (0.0%)	55 (91.7%)	9 (36.0%)	
Severe	16 (12.7%)	0 (0.0%)	0 (0.0%)	16 (64.0%)	
Inflammatory predominance					**<0.001**
Eosinophilic	61 (48.4%)	8 (19.5%)	32 (53.3%)	21 (84.0%)	
Lymphoplasmacytic	65 (51.6%)	33 (80.5%)	28 (46.7%)	4 (16.0%)	
Eosinophil count/HPF					**<0.001**
<10	41 (32.5%)	26 (63.4%)	14 (23.3%)	1 (4.0%)	
10-50	31 (24.6%)	15 (36.6%)	14 (23.3%)	2 (8.0%)	
51-100	33 (16.2%)	0 (0.0%)	32 (53.3%)	1 (4.0%)	
>100	21 (16.7%)	0 (0.0%)	0 (0.0%)	21 (84.0%)	
Neutrophil count/HPF					0.082
<10	117 (92.9%)	41 (100.0%)	53 (88.3%)	23 (92.0%)	
10-50	9 (7.1%)	0 (0.0%)	7 (11.7%)	2 (8.0%)	
>50	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Basement membrane thickening	119 (84.4%)	37 (90.2%)	57 (95.0%)	25 (100.0%)	0.239
Squamous metaplasia	31 (24.6%)	9 (22.0%)	14 (23.3%)	8 (32.0%)	0.626
Fibrosis	19 (15.1%)	7 (17.1%)	9 (15.0%)	3 (12.0%)	0.856
Hyperplastic/papillary change	27 (21.4%)	4 (9.8%)	16 (26.7%)	7 (28.0%)	0.086
Hyperplastic seromucinous glands	24 (19.1%)	2 (4.9%)	9 (15.0%)	13 (52.0%)	**<0.001**
Mucosal ulceration	10 (7.9%)	1 (2.4%)	3 (5.0%)	6 (24.0%)	**0.004**
Mucin	88 (69.8%)	24 (58.5%)	43 (71.7%)	21 (84.0%)	0.085
Eosinophil aggregates	28 (22.2%)	5 (12.2%)	11 (18.3%)	12 (48.0%)	**0.002**
Charcot-Leyden crystals	20 (15.9%)	4 (9.8%)	8 (13.3%)	8 (32.0%)	**0.044**
Fungal elements	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	NA

HPF = High power field

were received by nearly half the patients. The clinical utility of our findings is that class 3, with its high eosinophil count of >100/HPF, was associated with worse post-FESS outcomes. This has parallels with the Japanese CRSwNP population, who exhibit predominantly type 2 inflammation and >70 eosinophils/HPF significantly correlates with post-FESS recurrence [2]. A meta-analysis on the relationship between tissue eosinophilia and post-surgical recurrence in eosinophilic CRS found that a cut-off value of >55 eosinophils/HPF had the highest sensitivity and specificity in predicting recurrence [11]. This suggests that while 10 eosinophils/HPF is accepted as the cut-off value that distinguishes between eosinophilic and non-eosinophilic tissue inflammation, very high eosinophils counts have important clinical implications and should be reported when seen on routine histopathology.

Interestingly, features of airway remodelling such as squamous metaplasia, basement membrane thickening and fibrosis were not useful in prognosticating outcome in our population as their proportions did not differ significantly among the three classes. Basement membrane thickening was highly prevalent across all three classes, even in class 1, whereas squamous metaplasia and fibrosis were relatively uncommon findings, even in class 3. On the other hand, studies from Australia, China and the United States of America appear to have higher prevalences of fibrosis in their CRSwNP populations, ranging from 43.7 to 58.9% [5,6,8], compared to 15.1% in our population. An Italian study that conducted cluster analysis on structured histopathology profiling in their CRSwNP patients identified four distinct clusters which had different characteristics compared to the clusters in our study [9]. Cluster 1 was characterised by infrequent fibrosis. Cluster 2 consisted of hyperplastic/papillary changes in 70% of cases and fibrosis in 65% of cases. Cluster 3 showed fibrosis in 100% of cases. Cluster 4 was characterised by hyperplastic/papillary changes in 100% of cases and fibrosis in 92% of cases. While some of the differences among these studies are probably contributed by heterogeneity in methodologies, they also likely highlight the diversity in CRSwNP endotypes in different regions

Table 4. Demographics within each latent class.

	Latent class 1 (n=41)	Latent class 2 (n=60)	Latent class 3 (n=25)	p-value
Age (years)	47.6 (SD 11.6)	49.6 (SD 12.5)	50.8 (SD 9.9)	0.399
Males	36 (87.8%)	39 (65.0%)	16 (64.0%)	**0.025**
Race				0.676
Chinese	29 (70.7%)	41 (68.3%)	13 (52.0%)	
Malay	5 (12.2%)	5 (8.3%)	2 (8.0%)	
Indian	3 (7.3%)	9 (15.0%)	5 (20.0%)	
Caucasian	1 (2.4%)	1 (1.7%)	1 (4.0%)	
Others	3 (7.3%)	4 (6.7%)	4 (16.0%)	
Pre-operative oral corticosteroids	21 (51.2%)	22 (36.7%)	9 (36.0%)	0.408
Smoker	9 (22.0%)	7 (11.7%)	0 (0%)	**0.032**
Previous FESS	15 (36.6%)	17 (28.3%)	9 (36.0%)	0.629
Allergic rhinitis	11 (47.8%)	18 (60.0%)	7 (38.9%)	0.347
Asthma	10 (24.4%)	32 (53.3%)	11 (44.0%)	**0.015**
AERD	3 (7.3%)	10 (16.7%)	6 (24.0%)	0.165
Purulent discharge	7 (17.1%)	22 (36.7%)	8 (32.0%)	0.100
Blood eosinophils (x10^9/L)	0.34 (IQR 0.15-0.55)	0.51 (IQR 0.24-0.79)	0.52 (IQR 0.24-0.81)	0.112
Total serum IgE (kU/L)	65.5 (IQR 31.0-200)	235.0 (IQR 93.0-385.0)	220.5 (IQR 62.0-442.5)	**0.0388**
Lund-Mackay score	14.5 (IQR 10.25-18.00)	17.0 (IQR 13.00-19.75)	18.00 (IQR 13.50-23.00)	**0.021**
Tissue eosinophilia*	15 (36.6%)	46 (76.7%)	24 (96.0%)	**<0.001**

*≥10 eosinophils per high powered field. AERD = Aspirin exacerbated respiratory disease; FESS = Functional endoscopic sinus surgery; IQR = Interquartile range; SD = Standard deviation.

around the world and the need to tailor treatment according to the prevalent inflammatory endotype in each region. Neutrophilic infiltration was not a common finding in our CRSwNP patients. It was also not significantly different among the three classes, although we observed that >10neutrophils/HPF were seen only in classes 2 and 3. This may be in line with the observation in other studies that patients with severe or difficult-to-treat type 2 CRSwNP tended to exhibit neutrophilic inflammation. For example, machine-learning algorithms applied to a population of CRSwNP identified tissue neutrophilia (measured by subepithelial human neutrophil elastase-positive cell count) as a strong predictor of disease recurrence 12 months after FESS [12]. In another study, patients with tissue neutrophilia (defined as neutrophils comprising >50% of total cellularity on pre-operative nasal cytology) was found to be a significant predictor of poor disease control at 12 months after FESS [13]. Calprotectin (a protein secreted by neutrophils) levels in nasal secretions and tissue neutrophil count/HPF were found to be significantly higher in the nasal secretions of patients who had undergone 3 or more endoscopic sinus surgeries [14]. One of the reasons for the discrepancy between our cohort's prevalence of neutrophilic infiltration and that of other studies may be related to the definition of what constitutes "significant" neutrophilia, which there is currently no consensus. As seen in the above examples, the definition of what constitutes neutrophilia is heterogenous, with some studies using neutrophil count and others adopting surrogate markers of neutrophilic inflammation. The reporting of neutrophil count can also be qualitative (eg: absent/present or absent/focal/diffuse) [4,5,7,9] or semi-quantitative (eg: <20 and >20 neutrophils/HPF) [5,15,16]. In terms of absolute neutrophil counts, the median number of polyp neutrophils in three studies on Chinese CRSwNP patients was found to be as low as 0-6.3/HPF [17-19]. Another study found that a cut point of 4 neutrophils/HPF distinguished patients with significantly different baseline 22-item sinonasal outcome test (SNOT-22) scores [20]. Therefore, it is possible that utilising a different cut-off value for neutrophils may change the outcome of this analysis. The definition of clinically significant neutrophilia will need to be determined in further studies.

When faced with a CRSwNP patient, it can be difficult to determine which class he/she belongs to. Latent class analysis is a useful tool to detect latent subpopulations within a cohort, based on patterns of responses to a range of observed varia-

Table 5. Comparison of 2-year post-operative outcomes among clusters.

Outcome	Latent class	Outcome present	Outcome absent	Unadjusted		Adjusted*	
				OR (95% CI)	p-value	OR (95% CI)	p-value
Polyp recurrence	1	6 (14.6%)	35 (85.4%)	1.0		1.0	
	2	16 (26.7%)	44 (73.3%)	2.1 (0.8- 6.0)	0.156	1.9 (0.6- 5.8)	0.244
	3	4 (16.0%)	21 (84.0%)	1.1 (0.3- 4.4)	0.881	1.1 (0.2- 4.5)	0.941
Need for systemic corticosteroids	1	6 (14.6%)	35 (85.4%)	1.0		1.0	
	2	12 (20.0%)	48 (80.0%)	1.5 (0.5- 4.3)	0.490	1.0 (0.3- 3.2)	0.957
	3	11 (44.0%)	14 (56.0%)	4.6 (1.4- 14.8)	**0.011**	4.4 (1.2- 16.4)	**0.029**
Revision surgery	1	6 (14.6%)	35 (85.4%)	1.0		1.0	
	2	12 (20.0%)	48 (80.0%)	1.5 (0.5- 4.3)	0.490	1.0 (0.3- 3.7)	0.943
	3	6 (24.0%)	19 (76.0%)	1.8 (0.5- 6.5)	0.343	1.7 (0.4- 7.7)	0.463
Uncontrolled disease	1	3 (7.3%)	38 (92.7%)	1.0		1.0	
	2	17 (28.3%)	43 (71.7%)	5.0 (1.4- 18.4)	**0.015**	4.4 (1.1- 18.1)	**0.041**
	3	10 (40.0%)	15 (60.0%)	8.4 (2.0- 35.0)	**0.003**	9.8 (2.0- 48.7)	**0.005**

*Adjusted for gender, smoking status, asthma and pre-operative use of systemic corticosteroids. CI= Confidence interval; OR = Odds ratio

bles. As such, probabilities of class membership are obtained, but these are not clear cut assignments and some variation can exist. This study has identified that 7 of the 14 variables in a synoptic report differ significantly in prevalence among the three classes. Amongst these significant variables, eosinophil count/HPF is arguably the most clinically important as this is an objective measurement and it differentiates eosinophilic from non-eosinophilic CRS. Therefore, eosinophil count/HPF may be a useful starting point for assessment. If a patient has >100 eosinophils/HPF, this places him in class 3, as classes 1 and 2 have no membership of >100/HPF. If a patient has 51-100 eosinophils/HPF, the membership is likely to be class 2, since all patients with 51-100/HPF fell under this class, save for one (3.0%) in class 3. For patients with <10 eosinophils/HPF or 10-50 eosinophils/HPF, they are likely to belong either class 1 or 2. In this instance, it is useful to consider other supporting histologic and clinical features. For example, histologic findings of mild inflammation and lymphoplasmacytic inflammatory predominance, and clinical features of low to normal total serum IgE and lack of asthma would lean towards a class 1 rather than class 2 membership. Currently, there are some unanswered questions - for example, when would a patient with <10 eosinophils/HPF be "moved up" from class 1 to class 2, or even class 3? This would need verification with further studies. Nonetheless, the latent classes are not intended to be used as prescriptive formulas, but rather as part of precision medicine, and good clinical judgment is still required on a individual basis to provide personalised patient care.

Based on our findings, we propose that standard histopathology for CRSwNP should report the degree of inflammation, predominant inflammation and eosinophil count/HPF at minimum. When eosinophilic inflammation is present, reporting hyperplastic seromucinous glands, mucosal ulceration, eosinophil aggregates and Charcot-Leyden crystals is useful in characterising the severity of inflammation and prognosticating the likelihood of difficult-to-treat disease. This has implications on patient counselling or considerations in starting expensive therapy like biologics. While a detailed synoptic report is ideal, it may not always be feasible in a busy histopathology department and in a non-research setting. An abbreviated version of structured histopathology reporting may be more acceptable to our pathology colleagues, while at the same time providing the most important prognostic information to rhinologists.

There are several limitations in our study. This was a retrospective study and we did not have patient-reported quality of life measures. Some of our patients received a short course of low-dose oral corticosteroids prior to surgery. This could have influenced the histopathologic evaluation of inflammation, particularly the eosinophil count. Oral prednisolone 24mg/day for 2 weeks has been shown to reduce the percentage of tissue eosinophils by about 36% [21]. Even oral prednisone of 30mg/day for just 4 days followed by a 2-day taper and intranasal budesonide reduced the percentage of total tissues showing eosinophilic infiltration by 22% after 2 weeks [22]. On the other hand, there are also studies have reported that pre-operative low-dose corticosteroids did not significantly change tissue eosinophil count or degree of inflammation [6,23,24]. In our cohort, the distribution of patients who received pre-operative steroids was not significantly different between the three classes and yet significant differences in eosinophil count and other inflammatory features were demonstrated amongst the classes. Therefore, we feel that the results of our study are relevant especially in the context of a real-world setting, in which some clinicians may opt to give pre-operative corticosteroids to reduce intra-operative blood loss and improve visualisation of the surgical field [25]. We catego-

rised the eosinophil and neutrophil count instead of providing the actual number of cells per HPF. However, it may not be feasible for pathologists to count the number of inflammatory cells in daily clinical practice, thus the intention was to design a form that was practical and relatively easy for pathologists to complete whilst providing clinicians with histologic information that is clinically useful. None of the classes were associated with revision surgery, which may require a longer follow-up period to unfold. Finally, the findings of this study may only be applicable to patients from our region, and studies will need to be done in other populations to confirm if these findings may be extrapolated to them.

Conclusion

Eosinophil count, degree of inflammation, predominant type of inflammation, hyperplastic seromucinous glands, mucosal ulceration and mucin containing eosinophil aggregates and Charcot- Leyden crystals are helpful in predicting unfavourable outcomes at 2 years after FESS. In a non-research, clinical setting, where pathologists may be time-pressed to provide detailed reports, it is worthwhile focusing the reporting on these seven variables. While eosinophilic CRS is defined as ≥10 eosinophils/HPF, eosinophil counts of >50/HPF and >100/HPF are not uncommon in our cohort of CRWsNP patients in Singapore. In particular, the finding of >100 eosinophils/HPF should be reflected in histopathology reports as this group is significantly associated with systemic corticosteroids and uncontrolled disease.

Authorship contribution

XX wrote the first draft of the manuscript. XX and JES acquired the data. QVY analysed the data. YKO, DYW and XX interpreted the data. All authors critically revised the manuscript and approved the final manuscript.

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Conflict of interest

All authors declare no conflict of interest.

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