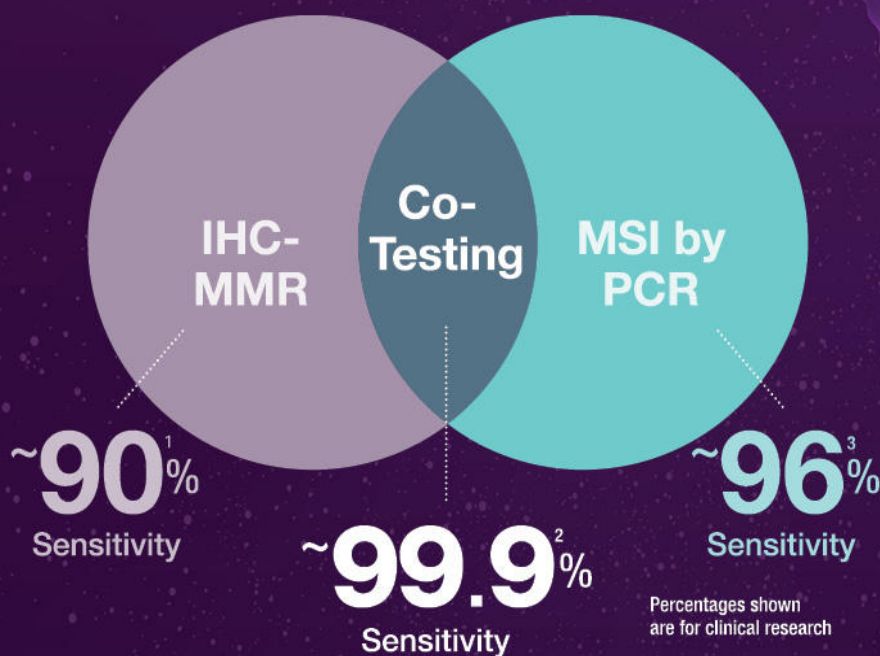


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¹Dudley (2016) *Clin. Cancer Res.* **22**, 813–820.

²Funkhouser *et al.* (2012) *J. Mol. Diag.* **14**, 91–103

³Based on an internal analysis of publications comparing MSI-PCR v. IHC-dMMR in colorectal cancer from 2004–2018. Literature bundle available from Promega Medical Affairs upon request.

A classification of chronic rhinosinusitis with nasal polyps based on structured histopathology

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A classification of chronic rhinosinusitis with nasal polyps based on structured histopathology

Aims: In chronic rhinosinusitis with nasal polyps (CRSwNP), tools based on objective evidence, such as histopathology, are needed to assist clinical decision-making. The main aim of this exploratory investigation was to determine whether structured histopathology could be used to classify CRSwNP in homogeneous histological clusters.

Methods and results: A cohort of 135 CRSwNP patients was assessed, on the basis of clinicopathological features: allergic fungal rhinosinusitis (17 patients); non-steroidal anti-inflammatory drug-exacerbated respiratory disease (19 patients); intrinsic asthma (18 patients); extrinsic asthma (21 patients); allergy (21 patients); histologically eosinophilic (22 patients); and histologically non-eosinophilic (17 patients). For structured histopathology, we considered: the degree of inflammation; eosinophil count; eosinophil aggregates; neutrophil infiltration; goblet cell hyperplasia; basement membrane thickening;

fibrosis; hyperplastic/papillary changes; squamous metaplasia; mucosal ulceration; and subepithelial oedema. Cluster analysis identified four distinct sets of cases. On discriminant analysis, the global error rate was 1.48%, and the stratified error rates were 4.34%, 0%, 0%, and 0% for clusters 1, 2, 3 and 4, respectively. Cluster 1 was characterised by infrequent fibrosis (<4.5% of cases). Cluster 2 mainly featured neutrophil infiltration in 100% of cases, hyperplastic/papillary changes in 70% of cases, and fibrosis in 65% of cases. Cluster 3 showed fibrosis in 100% of cases. Cluster 4 showed hyperplastic/papillary changes in 100% of cases, and fibrosis in 92% of cases.

Conclusions: This study shows that cluster analysis can identify different histotypes among CRSwNP patients. The next step will be to investigate, in a larger series, the clinical (e.g. prognostic) implications of identifying such homogeneous clusters of patients with CRSwNP on the basis of their structured histopathology.

Keywords: chronic rhinosinusitis, cluster analysis, endotyping, nasal polyposis, structured histopathology

Introduction

Endotyping of chronic rhinosinusitis with nasal polyps (CRSwNP) is a challenge for rhinologists. The

endotyping approach has given rise to the idea that some forms of this disease sharing the same mechanism of action could be treated with targeted therapies to prevent their progression.^{1–3} As our knowledge of pathophysiology evolves, treatment protocols must be adjusted to address specific underlying disease processes. Therefore, there is a need to develop tools to assist clinical decision-making based

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on objective evidence, such as histopathology.⁴ In routine practice today, histopathology on surgical samples offers limited information on the heterogeneous pathogenic mechanisms underlying different CRSwNP endotypes, but a potential role of structured histopathological profiling for CRSwNP has begun to attract attention.^{5–8} The few, but very intriguing, currently available studies^{5–8} support the concept that histopathology could provide very meaningful information to improve our endotyping capabilities.

In our structured histopathology approach, we currently consider: (i) overall degree of inflammation; (ii) eosinophil count; (iii) eosinophil aggregates; (iv) neutrophil infiltration; (v) goblet cell hyperplasia; (vi) basement membrane thickening; (vii) fibrosis; (viii) hyperplastic/papillary changes; (ix) squamous metaplasia; (x) mucosal ulceration; and (xi) subepithelial oedema. Cluster analysis is an unbiased, hypothesis-free method⁹ enabling a large number of variables to be considered without losing track of information that can reveal groups sharing similar characteristics. The main aim of the present exploratory investigation was to determine whether structured histopathology could serve as a tool for classifying patients with CRSwNP in homogeneous histological clusters.

Materials and methods

A consecutive series of adult patients treated with endoscopic sinus surgery (ESS) for CRSwNP at the Otolaryngology Section of the University of Padova was retrospectively assessed.

Patients with eosinophilic granulomatosis with polyangiitis or with inflammatory, autoimmune or haematological disorders, malignant neoplasms, or acute or chronic infectious diseases other than sinusitis were excluded from the study. Patients in whom surgery did not provide sufficient material for a structured histopathological analysis were also excluded. A final series of 135 patients met our inclusion criteria and were enrolled in the study.

The patients' histories of asthma and allergies, including sensitivity to acetylsalicylic acid or other non-steroidal anti-inflammatory drugs (NSAIDs), were obtained from their medical records. All patients underwent preoperative laboratory tests, all of which were performed at the same laboratory (Laboratory Medicine Service, Padova University Hospital), certified in compliance with ISO standard 15189. For allergic patients, data were also collected from their preoperative clinical reports concerning their total and specific

IgE for *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*, birch pollen, pellitory, grass mix, cat and dog dander, *Alternaria alternata*, *Aspergillus fumigatus*, and common ragweed. The diagnosis of asthma was based on reversible signs of obstruction during spirometry with methacholine challenge, according to the definition of the Global Initiative on Asthma.¹⁰ Asthma was defined as extrinsic when associated with allergy, or intrinsic when presenting with no known allergic origin. The diagnosis of NSAID-exacerbated respiratory disease (NERD) was clinically established from evidence of single NSAID-induced hypersensitivity reactions or asthma in patients with nasal polyps. Allergic fungal rhinosinusitis (AFRS) was diagnosed in patients with eosinophilic nasal polyps, allergic mucin detected at the time of surgery, and specific features on computed tomography (CT). Not all of these patients showed asthma, type I hypersensitivity, the presence of hyphae in the nasal sinuses, or Charcot–Leyden crystals.^{1,11}

Each patient underwent ESS involving ethmoidectomy, middle antrostomy, sphenoidotomy and/or frontal sinusotomy, depending on the site of the polyps and the inflammatory processes, with septoplasty if necessary, under general anaesthesia. The presence of any allergic mucin in the sinuses and nasal fossae was recorded in the surgeon's reports. All surgical specimens were submitted for histological and microbiological examination.

All patients were treated postoperatively with isotonic saline solution irrigations twice daily and nasal steroids; those with allergies were also treated with systemic antihistamines. Adequate therapy was prescribed for asthmatic patients. Endoscopic follow-up was scheduled 3, 6 and 12 months after ESS, and yearly thereafter, as previously reported.¹² Endoscopy was performed with rigid 0° or 30° endoscopes.

The cohort of 135 CRSwNP patients was distributed according to their clinicopathological features as follows: AFRS (17 patients); NERD (19 patients); intrinsic asthma (18 patients); extrinsic asthma (21 patients); allergy (21 patients); histologically eosinophilic (22 patients); and histologically non-eosinophilic (17 patients). Patients defined as histologically eosinophilic had a mean tissue eosinophil count of ≥ 10 , calculated on five high-power fields (original magnification 400 $\times$); those with a mean tissue eosinophil count of < 10 were considered to be histologically non-eosinophilic.

Before surgery, all patients signed a detailed informed consent form, and gave their written permission for clinical case publication. Data were

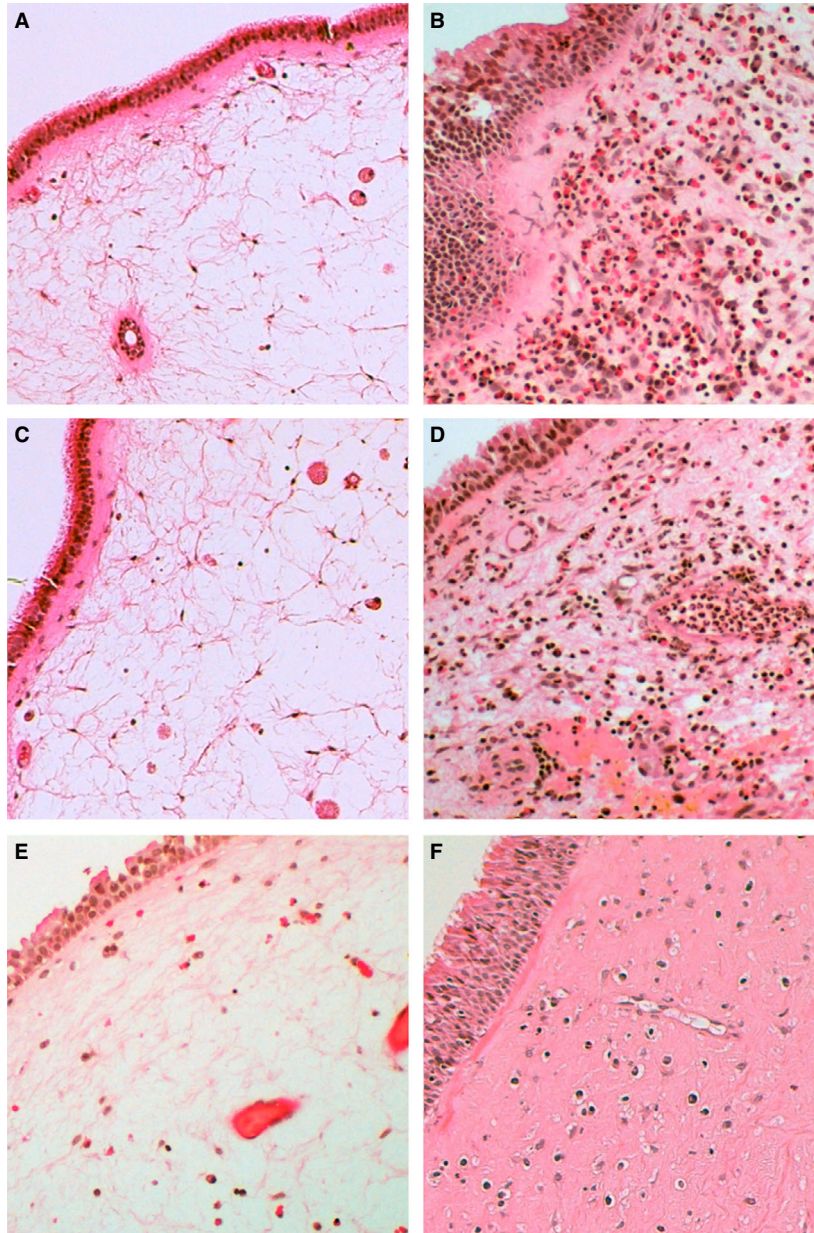


Figure 1. Representative histological images of: eosinophil aggregates (A, absent; B, present); neutrophil infiltration (C, absent; D, present); and fibrosis (E, absent; F, present).

examined in accordance with Italian privacy and sensitive data laws, and the in-house regulations of Padova University's Otolaryngology Section. The study complied with the principles of the Helsinki Declaration.

HISTOPATHOLOGICAL INVESTIGATIONS

Tissue samples were fixed in 10% buffered formalin, embedded in paraffin, cut into 4- μ m-thick sections, and routinely stained with haematoxylin and eosin (H&E). The same dedicated head and neck pathologist

(L.A.) and a general pathologist (V.C.) blindly analysed each specimen. Every H&E-stained slide was examined under a light microscope at low-power magnification ($\times 40$) to identify the most representative fields for each histological feature considered. Selected areas were then examined at high-power magnification ($\times 100$ or $\times 400$) and scored as described below.

The following microscopic parameters were recorded, according to the methodological approach used by Dhong *et al.*,⁵ Kuhar *et al.*,⁴ and Brescia *et al.*⁸ to describe CRSwNP: degree of inflammation;

neutrophil infiltration; basement membrane thickening; subepithelial oedema; hyperplastic/papillary changes; goblet cell hyperplasia; mucosal ulceration; squamous metaplasia; fibrosis; and eosinophil aggregates. With a magnification of $\times 100$, a semiquantitative two-tier score was assigned according to the presence or absence (presence = 1; absence = 0) of the following histopathological features: eosinophil aggregates, neutrophil infiltrate, fibrosis (Figure 1), hyperplastic/papillary changes, mucosal ulceration, and squamous metaplasia (Figure 2). A four-tier score was assigned according to the degree of visible

inflammation, basement membrane thickening, and subepithelial oedema, as follows: 0, none; 1, mild; 2, moderate; or 3, marked (Figures 3–5). Goblet cell hyperplasia was graded according to the number of goblet cells identifiable in a $\times 400$ field, as follows: 0, <3 cells; 1, 3–10 cells; 2, 11–20 cells; or 3, >20 cells (Figure 6). Five high-power fields (magnification $\times 400$) from each specimen were examined to count the eosinophils in the subepithelial connective tissue, and the mean of five counts was recorded. Intraepithelial and intravascular eosinophils were not counted.

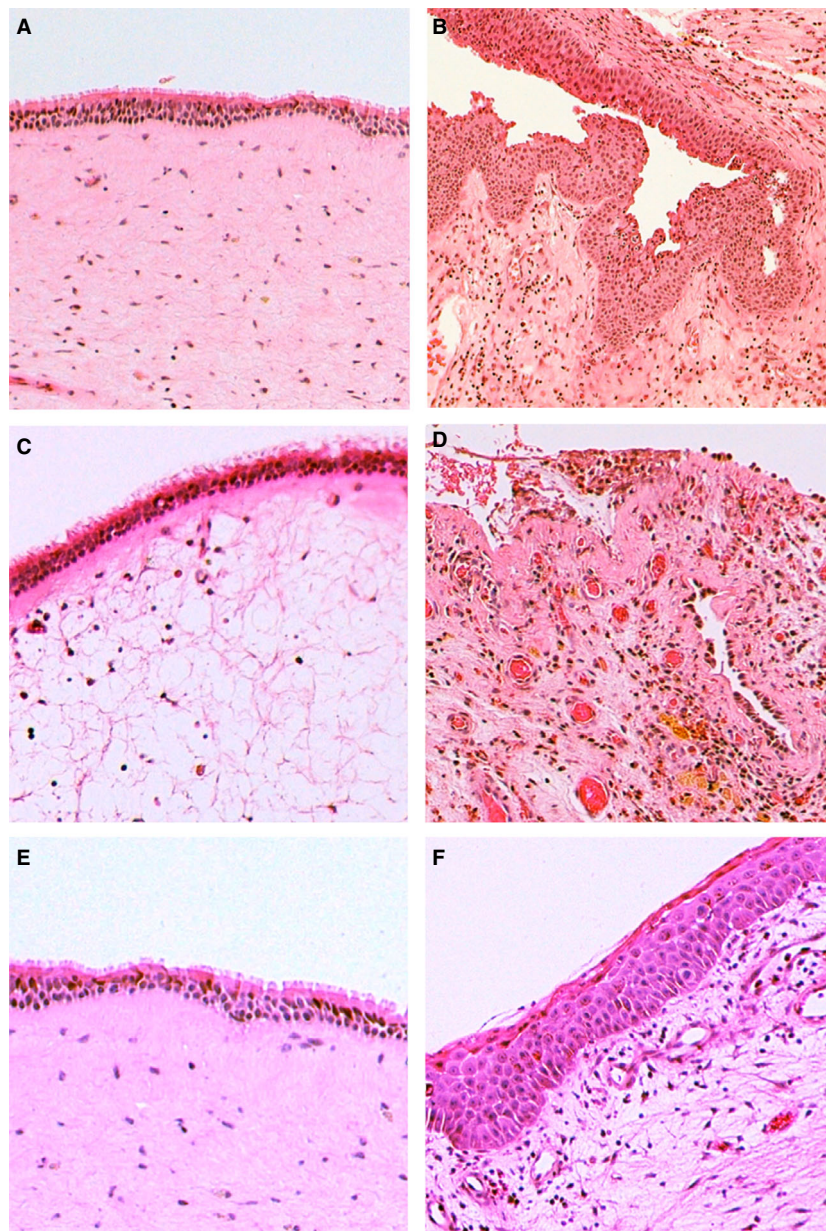


Figure 2. Representative histological images of: hyperplastic/papillary changes (A, absent; B, present); ulceration (C, absent; D, present); and squamous metaplasia (E, absent; F, present).

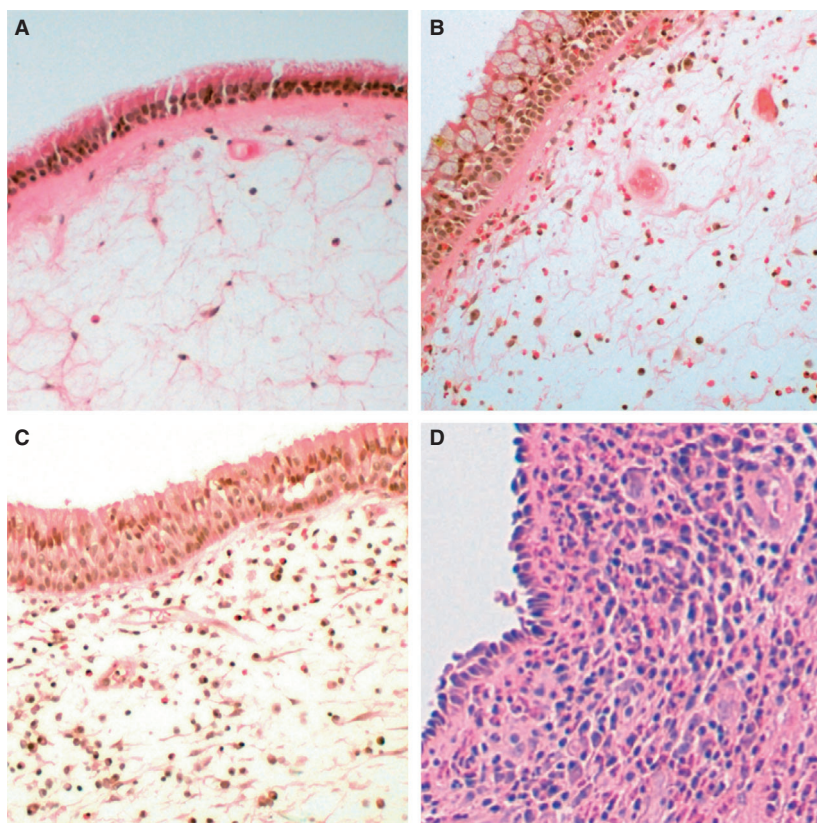


Figure 3. Representative histological images of degrees of connective tissue inflammation. A, Absent. B, Mild. C, Moderate. D, Marked. [Colour figure can be viewed at wileyonlinelibrary.com]

STATISTICAL ANALYSIS

The set of 135 CRSwNP cases, each described by a profile of 11 histopathological variables, was subjected to multivariate analysis. The cluster analysis approach was applied in the partitioning k median modality to classify patients on the basis of their structural histopathological features. The following modalities were applied: K number of classes (by use of the Calinsky–Harabasz pseudo- f stopping rule, $k = 4$), measure of dissimilarity (Gower), initial group centres (k unique, random), and maximum number of iterations (set at 10 000). The k nearest neighbour (KNN) discriminant analysis modality was applied to test the consistency of the classification calculated.¹³ The following procedure was then used: number of nearest neighbour (fixed $n = 1$), and group size (proportional prior probabilities). The ties in the groups' classification were randomly broken; the measure of similarity was Jacard, and the conversion from similarity to dissimilarity was obtained with the formula: $d(ij) = \sqrt{s(ij) + s(ii) - 2s(ij)}$. Global and stratified misclassification rates were calculated.

The Kruskal–Wallis test and Fisher's exact test were used to identify the most relevant variables (considering the P -value) that differentiated between

the clusters. The log rank test was also used, as appropriate.

A P -value of <0.05 was considered to be significant, and values in the range of $0.10 \geq P \geq 0.05$ were taken to indicate a statistical trend. All analyses were performed using the STATA 10.0 statistical package (Stata Corp LP, College Station, TX, USA).

Results

Table 1 summarises the structured histopathological features of the cohort of 135 CRSwNP patients grouped into the seven sub cohorts described above on the basis of their clinical or histopathological features.

CLUSTER ANALYSIS

Using the histopathological variables described above, cluster analysis identified four distinct sets of cases with significant internal homogeneity (Table 2). Table 3 summarises the histopathological features of these four clusters in terms of the 11 variables studied. Discriminant analysis gave a global error rate of 1.48%, and stratified error rates of 4.34%, 0%, 0%, and 0% for clusters 1, 2, 3, and 4, respectively (Table 4).

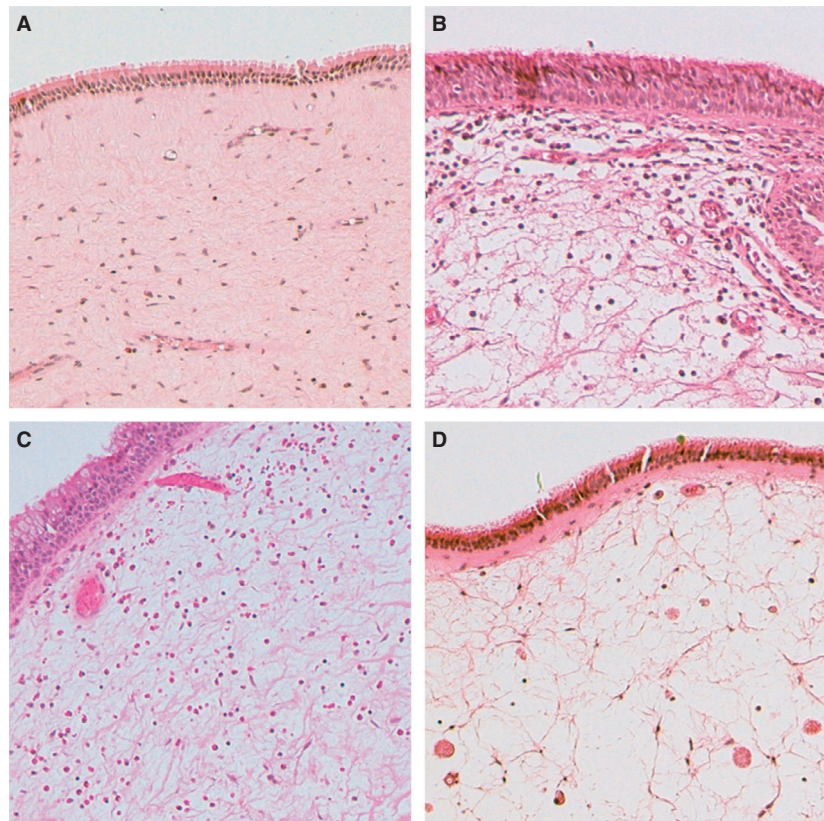


Figure 4. Representative histological images of connective tissue oedema. A, Absent. B, Mild. C, Moderate. D, Marked. [Colour figure can be viewed at wileyonlinelibrary.com]

ANALYSIS OF THE HISTOPATHOLOGICAL FEATURES MOST CHARACTERISTIC OF EACH CLUSTER

The Kruskal–Wallis test showed that the four clusters identified differed in: degree of inflammation ($P = 0.0001$); tissue eosinophil count ($P = 0.0235$); goblet cell hyperplasia (trend towards significance, $P = 0.0629$); basement membrane thickening ($P = 0.0001$); and subepithelial oedema (trend towards significance, $P = 0.0929$) (Table 3).

Fisher's exact test showed significant differences between the four clusters in terms of: neutrophil infiltration ($P < 0.0001$); fibrosis ($P < 0.0001$); hyperplastic/papillary changes ($P < 0.0001$); and mucosal ulceration (trend towards significance, $P = 0.089$) (Table 3). Statistical analysis excluded any significant difference between the clusters in terms of the presence of eosinophil aggregates or squamous metaplasia (Fisher's exact test, $P = 0.143$, and $P = 0.135$, respectively).

COMPARISON OF THE FOUR IDENTIFIED CLUSTERS IN CLINICAL AND PROGNOSTIC TERMS

Table 5 summarises the distribution of allergy, asthma and NERD in the four clusters. As shown in

Table 6, the distribution of these diagnoses did not differ statistically between the four clusters.

Regarding the prognostic features, statistical analysis showed no significant differences in the CRSwNP relapse rate (cluster 1, five of 46 cases; cluster 2, five of 20 cases; cluster 3, nine of 45 cases; cluster 4, six of 24 cases) (Table 6). Furthermore, the log rank test excluded a significant difference in terms of disease-free interval between the four clusters ($P = 0.490$).

Discussion

Chronic rhinosinusitis is a complex disorder characterised by persistent inflammation of the sinonasal mucosa.¹⁴ A better understanding of the pathological mechanisms underlying it has prompted an endotyping approach to the disease, with subtypes being defined functionally and pathologically according to specific cellular and molecular mechanisms.^{1,9,15} Cluster analysis can facilitate the subtyping of heterogeneous disorders, and has recently been used in various settings.¹⁶ Nakayama *et al.*¹⁷ used cluster analysis in a multicentre study on clinical data from chronic rhinosinusitis patients treated at five

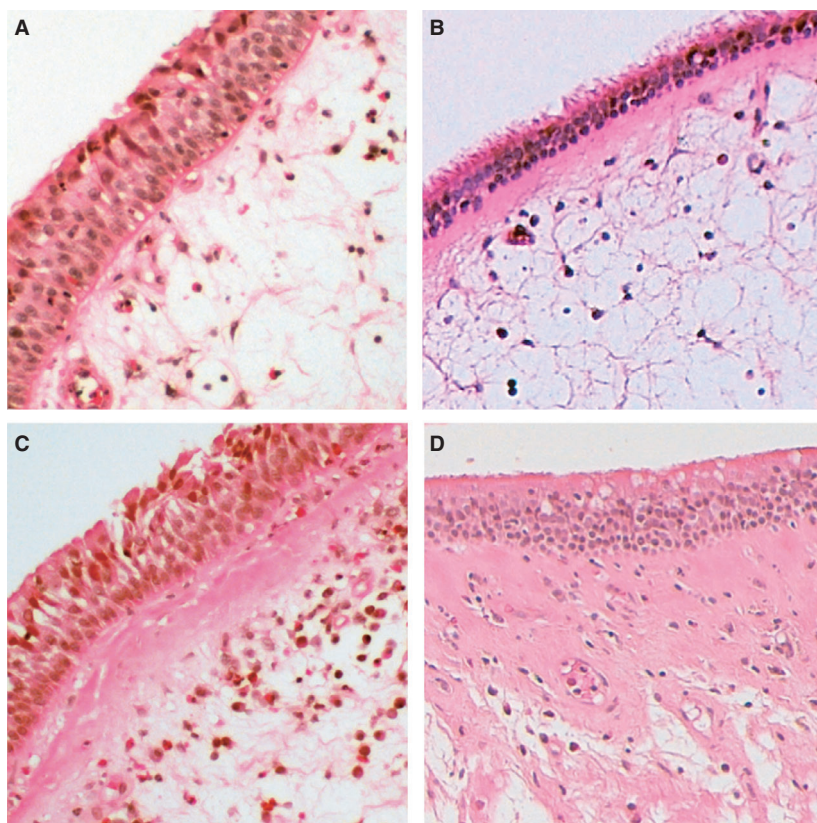


Figure 5. Representative histological images of basement membrane thickening. A, Absent. B, Mild. C, Moderate. D, Marked. [Colour figure can be viewed at wileyonlinelibrary.com]

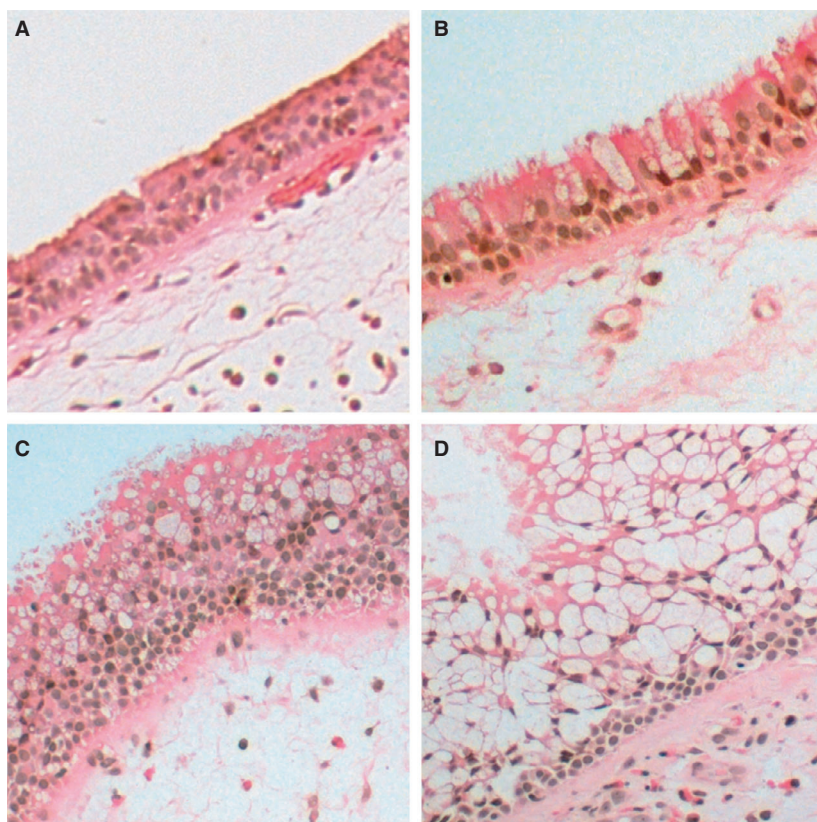


Figure 6. Representative histological images of goblet cell hyperplasia. A, Grade 0 (<3 cells). B, Grade 1 (3–10 cells). C, Grade 2 (11–20 cells). D, Grade 3 (>20 cells). [Colour figure can be viewed at wileyonlinelibrary.com]

Table 1. Structured histopathological features of the cohort of chronic rhinosinusitis with nasal polyps patients grouped into the subcohorts based on their clinical or histological features

Structured histopathological features	Score	AFRS (no. of cases)	NERD (no. of cases)	Intrinsic asthma (no. of cases)	Extrinsic asthma (no. of cases)	Allergy (no. of cases)	Histologically eosinophilic (no. of cases)	Histologically non-eosinophilic (no. of cases)
Degree of inflammation	0	0	1	2	3	4	0	4
	1	10	5	6	6	6	8	5
	2	4	6	9	10	8	12	3
	3	3	7	1	2	3	2	5
Eosinophil aggregates	0	16	11	17	19	15	13	17
	1	1	8	1	2	6	9	0
Neutrophil infiltration	0	12	14	14	18	17	17	9
	1	5	5	4	3	4	5	8
Goblet cell hyperplasia	0	6	7	7	7	6	2	10
	1	3	4	5	7	7	5	5
	2	4	4	3	2	4	5	2
	3	4	4	3	5	4	10	0
Basement membrane thickening	0	3	5	5	2	4	3	8
	1	4	7	5	8	5	10	6
	2	3	3	5	9	9	8	3
	3	7	4	3	2	3	1	0
Fibrosis	0	4	9	6	6	9	11	8
	1	13	10	12	15	12	11	9
Hyperplastic/papillary changes	0	12	14	10	13	13	15	12
	1	5	5	8	8	8	7	5
Squamous metaplasia	0	14	14	14	20	17	17	16
	1	3	5	4	1	4	5	1
Mucosal ulceration	0	13	14	13	19	20	19	15
	1	4	5	5	2	1	3	2
Subepithelial oedema	0	5	4	4	8	10	5	13
	1	7	6	7	7	3	5	3
	2	4	6	6	6	3	9	1
	3	1	3	1	0	5	3	0

AFRS, Allergic fungal rhinosinusitis; NERD, Non-steroidal anti-inflammatory drug-exacerbated respiratory disease.

hospitals. They identified four groups of patients: clusters 1 and 2 included those with low peripheral eosinophil and mucosal eosinophil counts (polyp scores were higher in cluster 2 than in cluster 1, however);

cluster 3 had very high mucosal eosinophil counts, but low polyp and symptom scores; and, cluster 4 showed severe polyposis. Soler *et al.*¹⁸ conducted a prospective multi-institutional study on patients with

Table 2. Numerical distribution of the study cohort among the clusters identified

Clusters	Frequency	Percentage
1	46	34.07
2	20	14.81
3	45	33.33
4	24	17.78
Total	135	100.00

chronic rhinosinusitis whose initial medical therapy had failed. They collected information with the Sino-Nasal Outcome Test (SNOT-22), the Rhinosinusitis Disability Index, Short Form-12, the Pittsburgh Sleep Quality Index, a patient health questionnaire, a Brief Smell Identification Test, CT, and endoscopy. Clustering was largely determined by the age of the patients, the severity of their reported outcome measures, depression, and fibromyalgia. In another multi-institutional study, Tomassen *et al.*¹⁹ applied hierarchical cluster analysis to 14 inflammatory mediators in tissues from 173 patients with chronic rhinosinusitis, and identified 10 distinct groups. Clustering was driven primarily by: eosinophilic and Th2-related markers; neutrophilic or proinflammatory mediators, or both; Th17/Th22 markers; and the Th1 cytokine interferon- γ . Approximately 60% of patients had a Th2-high signature, with a high prevalence of nasal

polyposis and asthma. In another cluster analysis setting, Liao *et al.*²⁰ considered 28 clinical variables and 39 mucosal cellular and molecular variables, and identified seven clusters of patients. In 2019, Kim *et al.*¹⁶ performed a cluster analysis on the clinical features of CRSwNP patients, including age, peripheral blood eosinophil count, tissue eosinophilia, CT scores, ratio of CT scores for the ethmoid and maxillary sinuses, and comorbid asthma. Two asthmatic clusters, and four non-asthmatic clusters emerged for CRSwNP. The number of clusters identified by such studies has varied, but the underlying characteristics of the clusters have been fairly consistent.²¹

The main strengths of the present study are methodologically homogeneous investigation and treatment of the patients described, because: (i) all surgical treatments were performed consecutively by the same team; (ii) all structured histopathological analyses were performed by a dedicated head and neck pathologist; (iii) postoperative medical treatments were mainly standardised; (iv) endoscopic follow-up after surgery was conducted by the same team; and (v) allergy tests were performed at the same laboratory. The main weaknesses of the study are its retrospective setting and the limited number of patients involved. Even though initially the structured histopathological analysis appeared to be time-consuming, once a dedicated pathologist had completed the training practice, the method proposed did not greatly increase the time needed to produce the pathology report. The parameters analysed can be quickly and easily detected by

Table 3. Distribution of structured histopathological features among the clusters identified

Structured histopathological features	Cluster 1 (46 cases)	Cluster 2 (20 cases)	Cluster 3 (45 cases)	Cluster 4 (24 cases)
Degree of inflammation (median value)	2	2	1	2
Tissue eosinophil count (median value)	16.5	20.5	9	32
Eosinophil aggregates* (%)	19.56	25.00	11.11	33.33
Neutrophil infiltration* (%)	21.74	100.00	8.89	0.00
Goblet cell hyperplasia (median value)	1	1	1	2
Basement membrane thickening (median value)	1	1	2	2
Fibrosis* (%)	4.34	65.00	100.00	91.67
Hyperplastic/papillary changes* (%)	15.22	70.00	2.22	100.00
Squamous metaplasia* (%)	21.74	30.00	8.89	12.50
Mucosal ulceration* (%)	21.74	30.00	8.89	8.33
Subepithelial oedema (median value)	1	1	0	1

*Positive cases.

Table 4. Error rate estimated from error count

	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Total
Error rate	0.0434783	0	0	0	0.0148148
Priors	0.3407407	0.1481481	0.3333333	0.1777778	

scanning the slides in a panoramic view while a 'traditional diagnosis' is made, and the semiquantitative scores applied to each variable are simple to evaluate at higher magnification, once the most significant areas have been identified.

To the best of our knowledge, this is the first cluster analysis based on structured histopathology on CRSwNP patients stratified according to well-codified clinical or pathological features. Our analysis identified four distinct histopathological clusters. With the use of KNN discriminant analysis to test the consistency of our classification, the global error rate was 1.48%, and the stratified error rates were 4.34%, 0%, 0% and 0% for clusters 1, 2, 3 and 4, respectively. Cluster 1 was characterised by infrequent fibrosis (<4.5% of cases). Cluster 2 was characterised by neutrophil infiltration in all cases, possibly related to the presence of ulceration in this group; hyperplastic/papillary changes in 70% of cases; and, fibrosis in 65% of cases. These histological parameters could reflect a continuous epithelial (papillary changes) and connective (fibrosis) tissue remodelling process as a consequence of injury due to ulceration. In cluster 3, 100% of cases showed fibrosis, possibly a long-term connective tissue response to damage, whereas all of the other variables were present at relatively low percentages, and there was no subepithelial oedema. None of the cases in cluster 3 showed any active tissue reactions (inflammation or epithelial response to

inflammatory damage). In cluster 4, 100% of cases showed hyperplastic/papillary changes and fibrosis was very common (92%), but none of the patients had neutrophil infiltration.

Table 5 compares the distribution of our CRSwNP patients according to the preliminary clinicopathological criteria and the new classification on histopathological grounds. The distribution of the cases stratified according to the former criteria among the four clusters identified by our analysis is rather complicated. This is because the initial classification criteria applied to the study population relied mainly on the concept of endotyping, which is based on pathophysiological definitions, whereas the information used in our cluster analysis came from histopathological morphological data. It is important to bear in mind that nasal mucosa remodelling due to inflammation is a dynamic process, so a given patient's histopathological patterns can change over time and after therapy and/or surgery.⁸ We analysed the distribution of allergy, asthma and NERD in the four clusters: no significant differences were found. Regarding the prognostic features, such as the CRSwNP relapse rate and the disease-free interval after ESS, statistical analysis ruled out significant differences between the clusters. This lack of association between clinical and prognostic parameters and the identified clusters may be reasonably explained by the limited number of cases considered.

Table 5. Relationship between subcohorts based on endotypes and the results of cluster analysis

Subcohorts based on clinicopathological criteria	Cluster 1 (46 cases)	Cluster 2 (20 cases)	Cluster 3 (45 cases)	Cluster 4 (24 cases)	Total (135 cases)
AFRS	3	3	9	2	17
NERD	8	3	4	4	19
Intrinsic asthma	6	1	5	6	18
Extrinsic asthma	6	2	9	4	21
Allergy	7	3	8	3	21
Histologically eosinophilic	10	4	4	4	22
Histologically non-eosinophilic	6	4	6	1	17

AFRS, Allergic fungal rhinosinusitis; NERD, Non-steroidal anti-inflammatory drug-exacerbated respiratory disease.

Table 6. Comparison of the four identified clusters in clinical and prognostic terms

Clinical and prognostic variables	<i>P</i> -value*
CRSwNP relapse rate	
Cluster 1 versus cluster 2	0.307
Cluster 1 versus cluster 3	0.574
Cluster 1 versus cluster 4	0.313
Cluster 2 versus cluster 3	0.751
Cluster 2 versus cluster 4	1.000
Cluster 3 versus cluster 4	0.550
Allergy	
Cluster 1 versus cluster 2	0.794
Cluster 1 versus cluster 3	0.297
Cluster 1 versus cluster 4	1.000
Cluster 2 versus cluster 3	0.294
Cluster 2 versus cluster 4	1.000
Cluster 3 versus cluster 4	0.309
Asthma	
Cluster 1 versus cluster 2	0.424
Cluster 1 versus cluster 3	0.532
Cluster 1 versus cluster 4	0.201
Cluster 2 versus cluster 3	0.124
Cluster 2 versus cluster 4	0.069
Cluster 3 versus cluster 4	0.446
NSAID-exacerbated respiratory disease	
Cluster 1 versus cluster 2	1.000
Cluster 1 versus cluster 3	1.000
Cluster 1 versus cluster 4	1.000
Cluster 2 versus cluster 3	1.000
Cluster 2 versus cluster 4	1.000
Cluster 3 versus cluster 4	1.000

CRSwNP, Chronic rhinosinusitis with nasal polyps; NSAID, Non-steroidal anti-inflammatory drug.

*Fisher's exact test.

In conclusion, this exploratory study has confirmed from a histopathological perspective that CRSwNP is not a single homogeneous condition. Differences in the structured histopathological data obtained in our

patient cohort subjected to cluster analysis identified different histotypes of CRSwNP. These histotypes could shed light on CRSwNP endotypes and mucosal remodelling due to the passage of time or medical and/or surgical treatments. The outcome of this study will be to investigate the prognostic implications of identifying groups of CRSwNP patients by structured histopathology in a larger series.

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Conflict of interest

The authors state that they have no conflicts of interest.

Author contributions

Study design: G. Brescia, G. Marioni, and L. Alessandrini. Data collection: D. Parrino, C. Zanotti, G. Tealdo, L. Franz, and V. Carraro. Data analysis and interpretation: G. Marioni, G. Brescia, and L. Alessandrini. Statistical analysis: L. Giacomelli. Drafting of the article: G. Marioni, G. Brescia, L. Alessandrini, and L. Franz. Critical revision of the article: G. Marioni, G. Brescia, L. Alessandrini, L. Giacomelli, and U. Barion. Final approval of the version to be published: all authors.

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