

Clinical Phenotypes of Nasal Polyps and Comorbid Asthma Based on Cluster Analysis of Disease History



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What is already known about this topic? Nasal polyps and comorbid asthma is a common unified airway disease.

What does this article add to our knowledge? Nasal polyps and comorbid asthma exhibits 3 clinical phenotypes with distinct natural courses.

How does this study impact current management guidelines? Identification of phenotypes of nasal polyps and comorbid asthma might be diagnostically and therapeutically useful.

BACKGROUND: Nasal polyps and comorbid asthma (NPcA) is a common united airway disease and is highly heterogeneous with respect to clinical, physiologic, and pathologic parameters. The clinical phenotypes of NPcA are poorly understood.

OBJECTIVE: We sought to explore clinical phenotypes in patients with NPcA.

METHODS: Patients first diagnosed with NPcA were recruited from Rhinological Clinics and Respiratory Clinics. We clustered patients with NPcA based on parameters regarding natural courses and demographic characteristics. Patients were also

evaluated with respect to clinical, functional, and inflammatory parameters in both upper and lower airways.

RESULTS: Clustering of 110 cases resulted in 3 clusters: cluster 1 (n = 16, 14.55%, atopic NPcA) was predominantly atopic patients with child-onset airway symptoms, intermediate disease duration, history of family asthma, better lung function, and less severe asthma; cluster 2 (n = 32, 29.09%, smoking NPcA) was characterized by more smokers, short disease duration, adult-onset airway symptoms, less atopy, nonsteroidal anti-inflammatory drug sensitivity, prior sinus surgery history, eosinophilic airway phenotypes, worse lung function, and severe computed tomography appearance; and cluster 3 (n = 62, 56.36%, older NPcA) consisted mostly of older patients with long disease duration, adult-onset airway symptoms, less atopy, more noneosinophilic airway phenotypes, and prior sinus surgery history.

CONCLUSIONS: Patients with NPcA with 3 distinct natural courses had different inflammatory status and disease severity. Determining the natural course of a patient might help clinicians predict the clinical aspects of NPcA and contribute to phenotype-guided management approaches in the future. © 2017 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2018;6:1297-305)

Key words: Chronic rhinosinusitis; Nasal polyps; Asthma; Phenotypes; Cluster analysis

Recent studies have provided consistent evidence to support the firm relationship between chronic rhinosinusitis (CRS) with nasal polyps (NP) and asthma.¹⁻⁵ Indeed, approximately 7% to 25.4% of patients with asthma have NP,⁶ and approximately 20% to 60% of patients with NP have asthma.^{7,8} Clinically, nasal polyps and comorbid asthma (NPcA) is a severe subtype of airway disease and it tends to be refractory to conventional medical and surgical treatment.⁹⁻¹² It is necessary to explore the natural course, clinical patterns, and pathophysiology of this entity.

Previous studies have shown that the adult-onset asthma phenotype is highly associated with NP^{2,4,13}—approximately

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Abbreviations used

AERD- Aspirin-exacerbated respiratory disease
 BHR- Bronchial hyperresponsiveness
 CRS- Chronic rhinosinusitis
 CT- Computed tomography
 FeNO- Fractional exhaled nitric oxide
 FEV1- Forced expiratory volume in 1 s
 FVC- Forced vital capacity
 GINA- Global Initiative for Asthma
 LM- Lund-Mackay
 PEF- Peak expiratory flow
 NP- Nasal polyps
 NPcA- Nasal polyps and comorbid asthma
 NSAID- Nonsteroidal anti-inflammatory drug
 SE- Staphylococcus aureus enterotoxin

19.30% to 38.64% patients with adult-onset asthma have NP.^{2,13,14} After evaluation of bronchial hyperresponsiveness (BHR) in adult patients with NP, approximately 28.17% to 40% patients with NP had newly diagnosed asthma.^{15–20} As a disease entity, the epidemiology of NPcA has partly been stated by previous studies.^{10,21} During the natural evolution of this disease combination, a clinical history of NP usually precedes asthma, and up to 45% of patients with NP will develop adult-onset asthma.^{4,22} It was shown that different risk factors, such as asymptomatic BHR, IL-9, IL-5, *Staphylococcus aureus* enterotoxin (SE)-specific IgE, atopy, sex, allergic rhinitis, and aspirin sensitivity, exert different influences on the formation and natural course of NPcA.^{17–19,21,23–25} A recent cluster analysis reported a certain endotype of patients with NPcA characterized by the presence of SE-specific IgE and high levels of IL-5 and IgE.¹ On the basis of these previous findings, we speculated that certain subtypes of NP would develop and coexist with defined phenotypes or endotypes of asthma. However, the natural history and clinical characteristics of this disease entity are poorly understood.

Notably, late-onset asthma rather than allergic asthma is associated with NP^{2,26}; however, the impact of NP treatment on asthma is controversial.^{12,27–30} After endoscopic sinus surgery, only 17% to 24% of patients with CRS and comorbid asthma achieved a well-controlled level of CRS^{31,32} and approximately half of patients with uncontrolled asthma showed an improvement.³³ However, another study reported that functional endoscopic sinus surgery (FESS) did not affect the asthma status of patients with NPcA.³⁰ Regarding medical therapy, our previous study identified 2 inflammatory phenotypes of NPcA with different responses to corticosteroid.³⁴ Potential causes of this discrepancy were the heterogeneity of this disease entity and the small number of patients enrolled in these studies.

The identification of heterogeneity and classification of NPcA may provide a foundation from which to understand the natural course, disease causality, and ultimately to develop phenotype-guided management approaches. Cluster analysis has been used to classify the phenotypes and endotypes of CRS or asthma,^{1,35,36} and it is a heuristic method allowing identification of a set of latent classes of individuals who are similar to each other according to the multiple variables used in the analysis. We hypothesized that patients with NPcA would cluster into groups and the groups would show different phenotypes. By clustering 110 patients with NPcA based on parameters including natural

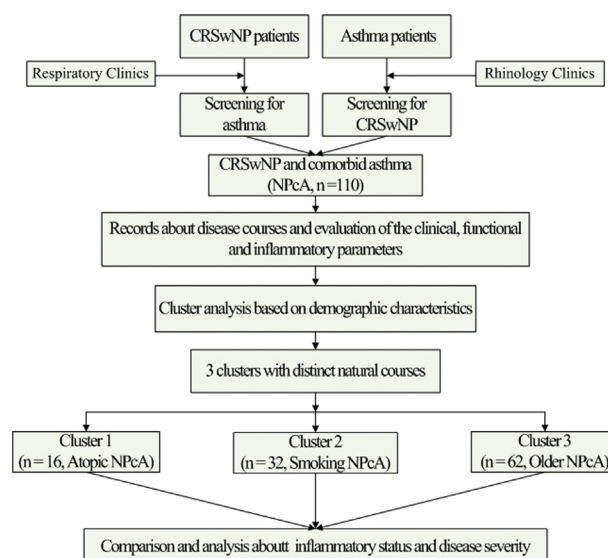


FIGURE 1. Flowchart. CRSwNP, Chronic rhinosinusitis with nasal polyps; NPcA, nasal polyps and comorbid asthma.

course and demographic characteristics, we aimed to identify phenotypes of NPcA and elucidate the natural course of united airway diseases.

METHODS

Study design

In this prospective cohort study, we enrolled 110 participants with NPcA in Rhinological Clinics and Respiratory Clinics from 2011 through 2016 and some of the characteristics of these subjects have been reported in our previous publications.^{34,37} Patients who were first diagnosed with NP underwent further examination of the lower airway. Similarly, patients who were first diagnosed with asthma would undergo a further examination of the upper airway. The study design is presented in Figure 1. This study was approved by the ethics committees of all individual institutions involved in data and tissue collection. Written informed consent was obtained from all the patients.

Patient population

Patients with NPcA (1) were diagnosed by pathology after biopsy and the results of computed tomography of the sinuses and (2) airway hyperreactivity (a 20% reduction in forced expiratory volume in 1 s [FEV1] after administration of methacholine up to 16 mg/mL) according to the criteria of the European Position Paper on Rhinosinusitis and Nasal Polyps 2012³⁸ and Global Initiative for Asthma (GINA) guidelines.³⁹ All subjects were aged 16 to 68 years. Excluded were subjects with noninvasive fungal balls and invasive fungal diseases, systemic vasculitis and granulomatous diseases, neoplasia, pregnancy, lactation, significant psychological diseases, acute upper or lower respiratory tract infections 2 weeks before inclusion, use of systemic corticosteroids within 4 weeks before the inclusion visit, systemic diseases preventing participation in the study, and medical and/or surgical treatments influencing the study.

If both of CRS with NP and asthma were diagnosed, related examinations including computed tomography (CT) scan, respiratory function test, and astograph methacholine provocation test should be performed within a week. Besides, biopsies of NP,

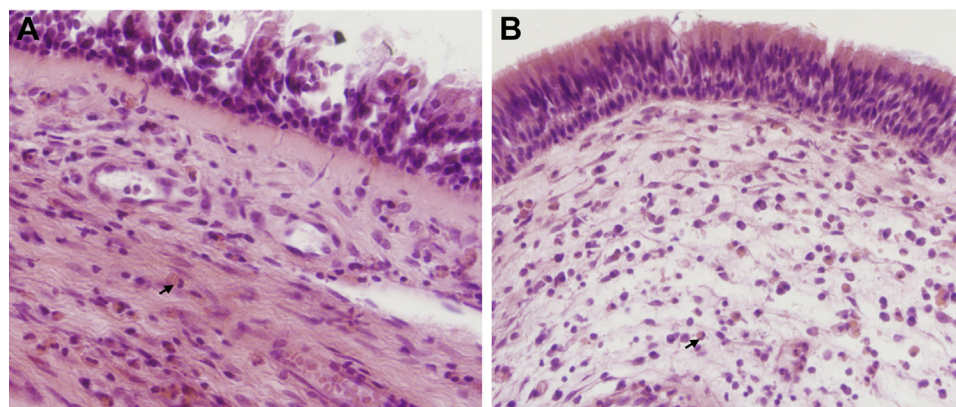


FIGURE 2. Representative histopathologic sections from patients with eosinophilic nasal polyps and noneosinophilic nasal polyps (hematoxylin-eosin, original magnification $\times 200$). **A**, Eosinophilic nasal polyps infiltrated by a large number of eosinophils (indicated by arrows); **B**, noneosinophilic nasal polyps infiltrated by large numbers of neutrophils, plasma cells, lymphocytes, and a few eosinophils (indicated by arrows).

peripheral venous blood, and fractional exhaled nitric oxide (FeNO) were collected on the same day within no more than a week of the diagnosis of NPcA.

Atopic status and inflammatory phenotype evaluation

The patients' atopic status was evaluated by the skin prick test to a standard panel of aeroallergens or by using the Immuno-CAP Phadiatop test (Phadia, Uppsala, Sweden) for detecting total IgE antibodies against various common inhalant allergens. Tissue specimens were obtained from the NP in the clinic, immediately formalin fixed, and then paraffin embedded. Paraffin sections (4 μ m thick) were stained with hematoxylin and eosin. A total of 5 randomly selected fields of view (FOVs) were evaluated for each stain at a magnification of $\times 400$ by 2 experienced pathologists. NP were classified as eosinophilic when the frequency of tissue eosinophils exceeded 10% of the total infiltrating cells per high-power field⁴⁰ (Figure 2). Exhaled nitric oxide was measured using an LR2000 analyzer (Logan Research, Rochester, UK) according to the European Respiratory Society task force.⁴¹ Eosinophilic asthma was defined when FeNO values were greater than 50 ppb.⁴²

Subjective assessment

At the time of enrollment in this study, patients with newly diagnosed NPcA were evaluated using standard questionnaires, medical histories, outside records, and examinations. The questionnaires included the following parameters: demographic data, age at first symptoms of rhinitis or asthma, duration of disease, age at asthma diagnosis, smoking status (never-, ex-, and current smokers), history of nonsteroidal anti-inflammatory drug (NSAID) sensitivity, history of nasal endoscopic surgery, family history of asthma, and medication use. The severity of asthma was defined by the GINA guidelines.³⁹ Patients were divided into 2 groups according to their age at asthma onset: childhood (<18 years old) or adult onset (≥ 18 years old) as previously described.¹³

Objective upper and lower airway evaluation

A CT scan was conducted to assess the inflammatory state and anatomical structure of the nasal cavity and sinuses. The CT scan was staged according to the Lund-Mackay (LM) scoring scale.⁴³ Respiratory function parameters, including the FEV₁

(% predicted), FEV₁/forced vital capacity ratio (FEV₁/FVC), and peak expiratory flow (PEF) (% predicted), were evaluated using a spirometer (Model 2160; Vitalograph, Maids Moreton, UK). The spirometric standards complied with the European Respiratory Society recommendations.⁴⁴ Astograph methacholine provocation test was conducted to assess the airway responsiveness as previously described.⁴⁵ Cumulative dose of inhaled methacholine measured at the inflection point at which respiratory conductance started to decrease (Dmin) was recorded.

Cluster analysis and discriminant analysis

Cluster analysis was conducted by applying a 2-step process as previously described.^{46,47} First, Ward's minimum-variance hierarchical clustering method was performed using an agglomerative (bottom-up) approach and Ward's linkage (Figure 3), and Calinski and Harabasz's pseudo-F index was calculated.⁴⁸ At each generation of clusters, samples were merged into larger clusters to minimize the within-cluster sum of squares or to maximize the between-cluster sum of squares.⁴⁹ The optimum number of clusters was determined by maximizing the Calinski and Harabasz pseudo-F index. Next, K-means analysis was carried out by using the prespecified number of clusters. Stepwise discriminant analysis was performed to identify variables discriminating between the prespecified clusters. Statistically significant results were expected for most of the comparisons because the objective of the cluster analysis was to differentiate the participants into distinct phenotypes of NPcA.

Statistical analysis

Continuous variables are presented as mean $\pm$ standard deviation, median (with interquartile range), or n (%) according to the data distribution. A *P* value of $<.05$ was considered statistically significant. The 2-sample *t*-test was applied for parametric data that followed a normal distribution. A nonparametric test (the Mann-Whitney *U* test) was applied for parametric data that did not follow a normal distribution. Differences in frequencies between groups were examined using the χ^2 test. Between-cluster differences of all parameters, as well as phenotype parameters, were tested by using the Kruskal-Wallis test with multiple group comparisons. Bonferroni corrections were conducted. Statistical analyses were

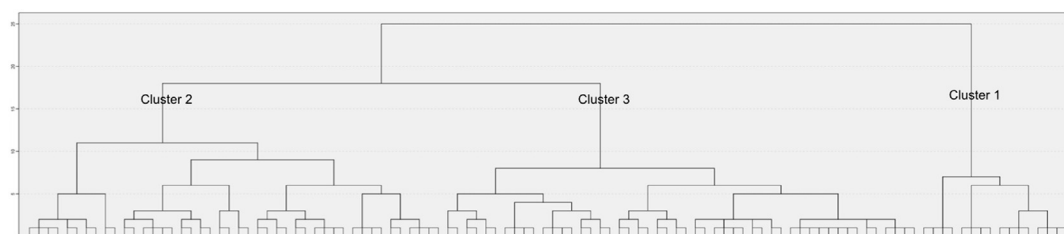


FIGURE 3. Dendrogram for the development of 3 clusters. Applying Ward's minimum-variance hierarchical clustering method and an agglomerative approach, 110 subjects were clustered to a single final group. At each formation of clusters, subjects were merged into larger clusters to minimize the within-cluster sum of squares or maximize the between-cluster sum of squares. Three clusters were generated.

TABLE I. Demographic characteristics of the total cohort and by clusters

Characteristic	Total cohort N = 110 NPcA	Cluster 1 N = 16 Atopy NPcA	Cluster 2 N = 32 Smoking NPcA	Cluster 3 N = 62 Older NPcA	P value among clusters
Age (y), mean $\pm$ SD	47.45 $\pm$ 10.08	45.25 $\pm$ 6.07	40.88 $\pm$ 11.52	51.13 $\pm$ 7.72	<.000
Male, n (%)	70 (63.64)	16 (100.00)	16 (50.00)	46 (74.20)	<.000
Atopy, n (%)	47 (42.73)	14 (87.50)	13 (40.63)	20 (32.26)	<.000
Age at first symptoms of rhinitis (y)	24.94 $\pm$ 4.17	9.25 $\pm$ 3.58	26.82 $\pm$ 5.17	23.81 $\pm$ 3.26	<.000
Age at first symptoms of asthma (y)	28.63 $\pm$ 4.76	15.80 $\pm$ 3.65	29.41 $\pm$ 5.27	34.20 $\pm$ 4.28	<.000
Disease duration (y), mean $\pm$ SD	19.43 $\pm$ 5.38	20.75 $\pm$ 4.21	8.44 $\pm$ 2.63	23.11 $\pm$ 5.22	.024
Age at asthma diagnosis (y), mean $\pm$ SD	41.94 $\pm$ 11.69	30.00 $\pm$ 12.62	35.26 $\pm$ 9.46	46.92 $\pm$ 9.75	<.000
Adult-onset, n (%)	78 (70.91)	4 (25.00)	20 (62.50)	54 (87.10)	<.000
Smoker (ex- and current smokers), n (%)	41 (37.27)	2 (12.50)	18 (56.25)	21 (33.87)	<.000
Prior sinus surgery, n (%)	71 (64.55)	5 (31.25)	23 (71.88)	43 (69.35)	<.000
History of NSAID sensitivity, n (%)	15 (13.64)	0 (0)	11 (34.38)	4 (6.45)	<.000
History of family asthma, n (%)	18 (16.36)	10 (62.50)	1 (3.13)	7 (11.29)	<.000

NPcA, Nasal polyps and comorbid asthma; NSAID, nonsteroidal anti-inflammatory drug; SD, standard deviation.

carried out using SPSS version 22.0 (IBM, Armonk, NY) and MATLAB, version 8.6 (MathWorks, Natick, Mass).

RESULTS

Study cohort and demographics

The study cohort included 110 patients with newly diagnosed NPcA ranging in age from 16 to 68 years. The characteristics of the total cohort are presented in Table I. Overall, 42.73% of patients had atopy, and the prevalence of the history of family asthma and NSAID sensitivity was 16.36% and 13.64%, respectively. The average age at asthma diagnosis, first symptoms of rhinitis, and first symptoms of asthma were 41.94, 24.94, and 28.63 years, respectively. There were 70.91% patients with adult-onset asthma and 37.27% patients with a smoking habit. Approximately 64.55% of patients with NPcA had prior sinus surgery.

Cluster analysis and natural course of NPcA by clusters

By performing cluster analyses in patients with NPcA based on parameters of natural course and demographic characteristics (listed in Table I), irrespective of clinical parameters, 3 clusters with distinct natural courses were identified (Table I, Figure 3). Significant differences existed between the clusters for demographics ($P < .001$). Cluster 1 ($n = 16$, 14.55%) predominantly contained atopic patients (87.50%) (atopic

NPcA) with the earliest onset of rhinitis symptoms (9.25 ± 3.58) and asthma symptoms (15.80 ± 3.65), intermediate disease duration (20.75 ± 4.21), lowest rate of history of NSAID sensitivity (0), and prior sinus surgery (31.25%) as well as the highest rate of history of family asthma (62.50%). Cluster 2 ($n = 32$, 29.09%) was characterized by the most smokers (56.25%) (smoking NPcA) with latest onset of rhinitis symptoms (26.82 ± 5.17), intermediate onset of asthma symptoms (29.41 ± 5.27), short disease duration (8.44 ± 2.63), highest rate of prior sinus surgery (71.88%), and history of NSAID sensitivity (34.38%) as well as the lowest rate of history of family asthma (3.13%). Cluster 3 ($n = 62$, 56.36%) consisted mostly of older patients (46.92 ± 9.75) (older NPcA) with intermediate onset of rhinitis symptoms (23.81 ± 3.26), latest onset of asthma symptoms (34.20 ± 4.28), mainly adult-onset asthma (87.10%), intermediate rate of prior sinus surgery (69.35%), and intermediate rate of history of family asthma (11.29%). Clinical parameters that were not used in the cluster analysis were analyzed within clusters and compared between clusters.

Inflammatory characteristics of patients with NPcA within the 3 clusters

The inflammatory characteristics of patients with NPcA in the 3 clusters are summarized in Table II. The level of total IgE

TABLE II. Inflammatory characteristics of subjects in total cohort and by clusters

Characteristic	Total cohort N = 110 NPcA	Cluster 1 N = 16 Atopic NPcA	Cluster 2 N = 32 Smoking NPcA	Cluster 3 N = 62 Older NPcA	P value among clusters
Dmin value, median (IQR)	0.47 (0.25-6.25)	0.30 (0.13-0.62)	0.29 (0.057-3.80)	2.60 (1.39-3.42)	.031
Total IgE, median (IQR)	60.00 (30.00-132.00)	54.00 (31.00-95.00)	136.00 (57.75-280.25)	30.00 (21.00-52.50)	<.000
FeNO, median (IQR)	85.00 (53.00-110.00)	79.00 (53.00-107.00)	106.50 (84.25-163.75)	36.30 (27.90-52.00)	.000
Blood eosinophil percentage (%), median (IQR)	5.40 (2.50-9.10)	5.50 (2.30-6.20)	7.30 (5.43-11.00)	3.15 (2.70-4.48)	.024
Tissue eosinophils (n), median (IQR)	17.54 (11.27-29.43)	16.71 (9.85-24.20)	22.45 (10.21-32.7)	9.46 (6.82-15.39)	<.000
Blood neutrophil percentage (%), median (IQR)	58.90 (52.50-62.90)	58.60 (51.90-61.80)	54.55 (52.43-59.90)	75.20 (63.10-80.90)	.039
Eosinophilia phenotypes of upper airway, n (%)	51 (46.36)	9 (56.25)	22 (68.75)	20 (32.26)	.002
Eosinophilia phenotypes of lower airway, n (%)	50 (45.45)	8 (50.00)	20 (62.50)	22 (35.48)	.014
Severe asthma, n (%)	45 (40.91)	3 (18.75)	18 (56.25)	24 (38.71)	<.000

Dmin, Cumulative dose of inhaled methacholine measured at the inflection point at which respiratory conductance started to decrease; *FeNO*, fractional exhaled nitric oxide; *IQR*, interquartile range; *NPcA*, nasal polyps and comorbid asthma.

TABLE III. Disease severity in total cohort and by clusters

Characteristic	Total cohort N = 110 NPcA	Cluster 1 N = 16 Atopic NPcA	Cluster 2 N = 32 Smoking NPcA	Cluster 3 N = 62 Older NPcA	P value among clusters
FEV1 (% predicted), median (IQR)	83.90 (73.20-97.40)	114.80 (103.28-118.33)	75.05 (61.55-82.20)	91.90 (83.90-97.30)	.017
FVC (% predicted), median (IQR)	100.20 (90.10-113.00)	112.00 (100.13-118.12)	97.95 (79.73-112.13)	103.90 (91.90-111.60)	.026
FEV1/FVC ratio (% predicted), median (IQR)	67.72 (62.23-79.56)	82.01 (80.67-87.40)	63.08 (57.06-66.90)	74.91 (62.92-79.05)	.023
PEF (% predicted), median (IQR)	95.40 (81.40-111.80)	120.9 (117.28-128.90)	82.85 (62.43-92.93)	103.50 (89.10-110.70)	.015
LM score, mean $\pm$ SD	21.44 $\pm$ 2.30	17.59 $\pm$ 1.72	21.44 $\pm$ 2.30	16.42 $\pm$ 2.21	.038

FEV1, Forced expiratory volume in 1 second; *FVC*, forced vital capacity; *FEV1/FVC*, the ratio of FEV1 to FVC; *IQR*, interquartile range; *LM score*, Lund-Mackay score; *NPcA*, nasal polyps and comorbid asthma; *PEF*, peak expiratory flow; *SD*, standard deviation.

and FeNO, the percentage of blood eosinophils, and the frequency of tissue eosinophils were significantly different among the 3 clusters ($P < .001$), with highest values in the smoking NPcA cluster and the lowest values in the older NPcA cluster. Notably, the percentage of blood neutrophils in the smoking NPcA cluster and atopic NPcA cluster were similar, whereas the older NPcA cluster had the highest percentage of blood neutrophils compared with the smoking NPcA cluster and atopic NPcA cluster ($P < .001$). Compared with the atopic NPcA cluster (18.75%), the percentage of severe asthma was significantly higher in the older NPcA cluster (38.71%) and smoking NPcA cluster (56.25%) (both $P < .01$). Furthermore, the smoking NPcA cluster had a significantly higher percentage of severe asthma compared with the older NPcA cluster ($P < .01$). The Dmin value was similar in the smoking NPcA cluster and atopic NPcA cluster, and there were no significant differences in these 2 clusters. Compared with these clusters, the older NPcA cluster had the highest Dmin value (both $P < .01$). Regarding the inflammatory phenotypes of NPcA, the proportions of eosinophilia phenotypes of the upper and lower airways in each cluster were similar, and there was a significant difference among the 3 clusters ($P = .002$ and $.014$, respectively). The smoking NPcA cluster had the highest number of eosinophilic airway phenotypes, whereas the older NPcA cluster had the least number of eosinophilic airway phenotypes.

Features of upper and lower airway disease severity in patients with NPcA within the 3 clusters

The features of upper and lower airway disease severity are shown in Figures 4 and 5. LM scores were calculated to evaluate the severity of upper airway disease. We found that the smoking NPcA cluster had significantly higher scores compared with the atopic NPcA cluster and older NPcA cluster ($P = .031$ and $.032$). However, LM scores were similar between the atopic NPcA cluster and older NPcA cluster (Figure 4). Regarding the pulmonary function of the lower airway in the 3 clusters (Table 3, Figure 5), FEV₁, FVC, FEV₁/FVC, and PEF were lowest in the smoking NPcA cluster and highest in the atopic NPcA cluster with a significant difference among the 3 clusters (all $P < .05$).

Discriminant analysis

By using a stepwise method of discriminant analysis, 21 of 26 variables were found to significantly discriminate between the clusters: apart from the 5 variables (age, atopy, FEV₁, FVC, and LM score), sex, age at first symptoms of rhinitis, age at first symptoms of asthma, disease duration, age at asthma diagnosis, adult-onset, smoker, prior sinus surgery, history of NSAID sensitivity, history of family asthma, Dmin value, total IgE, FeNO, blood eosinophil percentage, tissue eosinophil, blood neutrophil percentage, eosinophilia phenotypes of upper airway, eosinophilia phenotypes of lower airway, severe asthma,

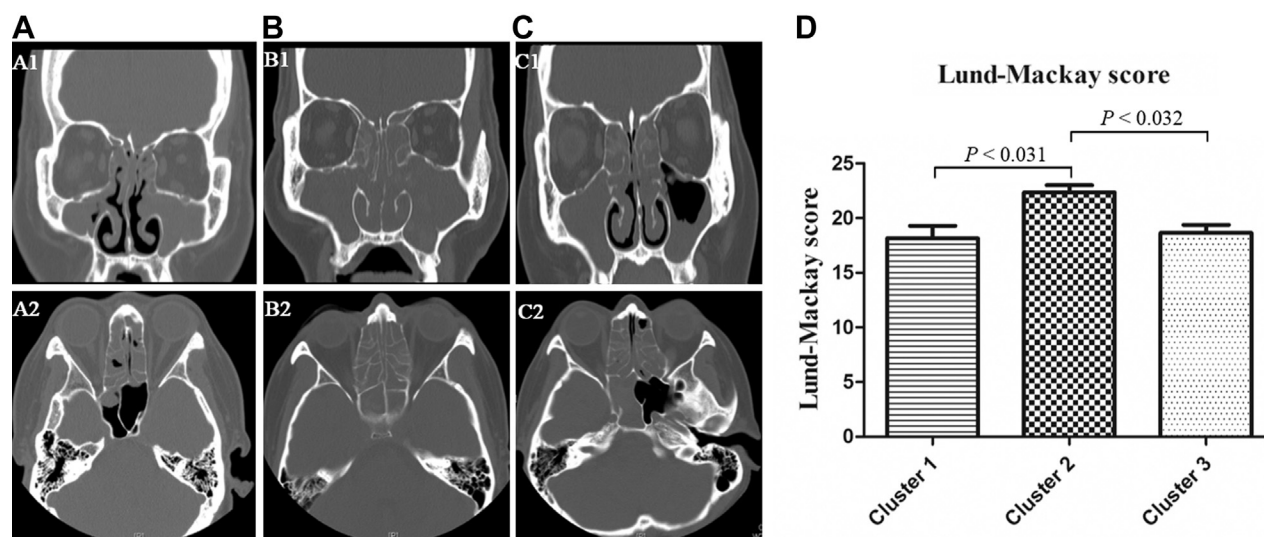


FIGURE 4. Computed tomography changes of the paranasal sinus among cluster 1 (A), cluster 2 (B), and cluster 3 (C). Coronal position scanning (A1, B1, and C1). Horizontal position scanning (A2, B2, and C2). (D) Comparison of Lund-Mackay scores among the 3 clusters. $P < .05$ was considered statistically significant.

FEV1/FVC ratio, and PEF were found to be statistically significant discriminants. Meanwhile, by applying the Fisher discriminant method, 2 canonical discriminant functions were generated to form a scatter plot. The clusters were well separated from each other, as shown in Figure E1 (available in this article's Online Repository at www.jaci-inpractice.org).

DISCUSSION

We identified different phenotypes of NPcA based on a cluster analysis of parameters for natural course and demographic characteristics, and we analyzed the clinical parameters within these clusters. The following 3 phenotypes with distinct natural courses and clinical characteristics were identified: cluster 1, atopic NPcA, child-onset airway symptoms, intermediate disease duration, history of family asthma, better lung function, and less severe asthma; cluster 2, smoking NPcA, adult-onset airway symptoms, short disease duration, less atopy, NSAID sensitivity, prior sinus surgery history, eosinophilia airway phenotypes, worse lung function, and severe CT appearance; and cluster 3, older NPcA, adult-onset airway symptoms, long disease duration, less atopy, more noneosinophilia airway phenotypes, and prior sinus surgery history. This observation indicated that NPcA is not a homogeneous inflammatory disease entity and that 3 subtypes of patients developed NPcA with different patterns of onset time. To our knowledge, this is the first study to reveal different patterns of the natural course of NPcA with different clinical characteristics.

The natural course of NPcA had not been comprehensively explored before. As a subtype of NPcA, aspirin-exacerbated respiratory disease (AERD) (also called aspirin triad) has a distinct natural course⁵⁰: onset of persistent rhinitis/rhinosinusitis (mean age 30 years), onset of asthma after 2 years (mean age 32 years), and aspirin-induced respiratory reactions and nasal polyposis after 4 years (mean age 34 years). AERD is characterized by intense eosinophilic infiltration,⁵¹ high NP recurrence rates,⁵²

severe asthma,⁵³ and less atopy.²³ In our study, this subtype had a similar pattern to the smoking NPcA cluster, which was the most severe phenotype among the identified 3 clusters. Compared with the atopic NPcA cluster and older NPcA cluster, the eosinophilic airway phenotype was more prevalent in the smoking NPcA cluster. Adult-onset eosinophilic asthma was recognized as a distinct profile and had a higher comorbidity of NP compared with adult-onset noneosinophilic asthma.² Furthermore, the smoking NPcA cluster had the most smokers, which might have contributed to the disease severity of this cluster. Cigarette smoking is treated as a risk factor for the development of chronic airway disease.⁵⁴ Cigarette smoke-induced airway inflammation was associated with NP formation and insensitivity to corticosteroids, resulting in increased severity and persistence of NP and asthma.^{55,56} These studies indicated that the smoking NPcA cluster had a distinct pattern of natural course and severe phenotype.

Previous studies showed that child-onset asthma, as a sub-phenotype of asthma, significantly differed from adult-onset asthma in many aspects.^{13,57} In this study, the atopic NPcA cluster was characterized by early onset of airway symptoms, child-onset asthma, history of family asthma, and good lung function. Atopic NPcA was processed differently and was significantly different to the other 2 adult-onset clusters (older NPcA cluster and smoking NPcA cluster) in terms of disease course. It was reported that atopic status in patients with asthma was associated with early onset of airway symptoms and a family history of asthma^{58,59} and patients tended to show early clinical symptoms of airway disease. In terms of disease severity, allergic asthma, especially child-onset allergic asthma, was highly associated with fully reversible airflow obstruction, responsiveness to inhaled corticosteroids and less severe asthma phenotypes.^{60,61} Regarding the disease entity of NPcA, we identified that the atopic NPcA cluster had similar traits with the atopic condition.

The older NPcA cluster was the largest ($n = 62$, 56.36%) and was characterized by less atopy, long disease duration, and

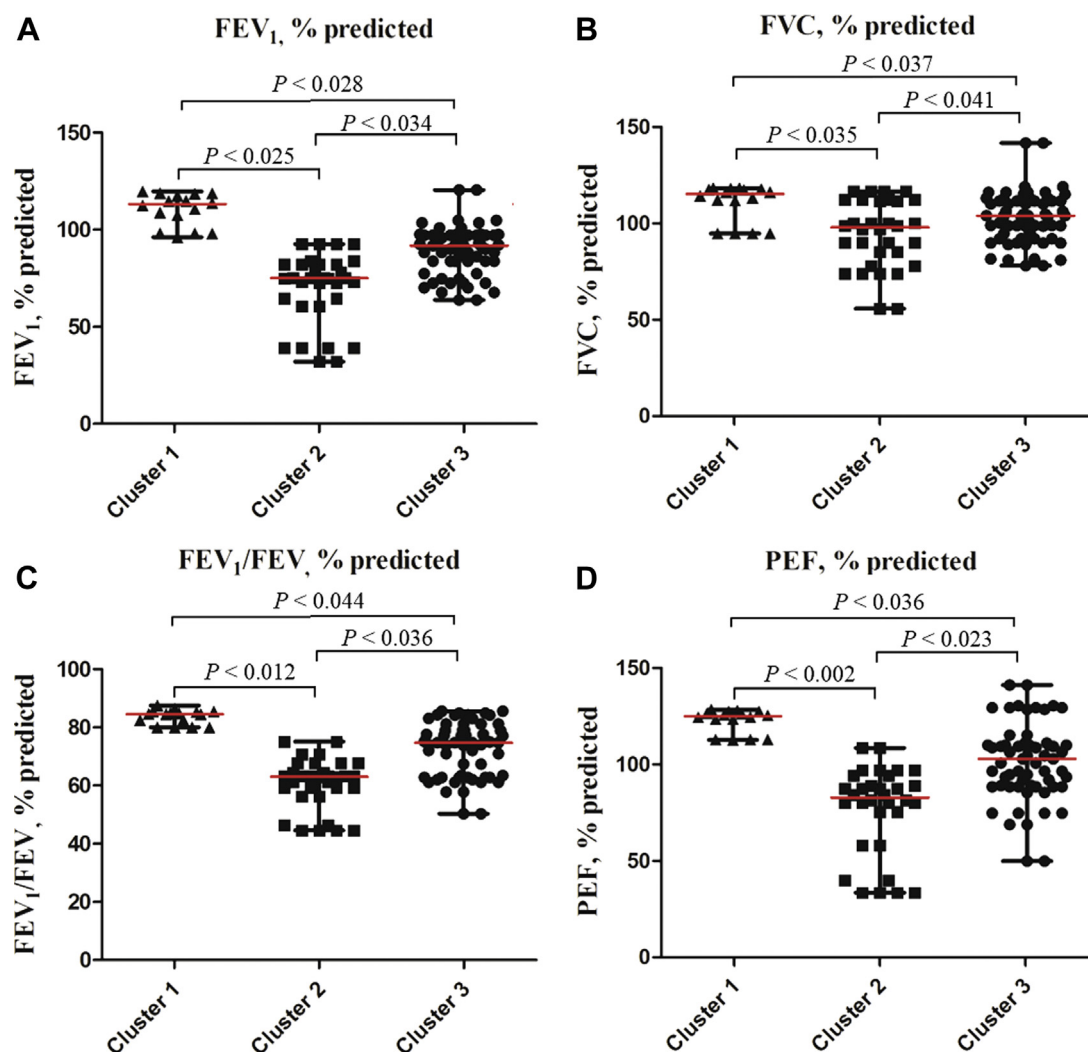


FIGURE 5. Lung function changes among cluster 1, cluster 2, and cluster 3. Comparison of forced expiratory volume in 1 second (FEV1), forced vital capacity (FVC), ratio of FEV1 to FVC, and peak expiratory flow (PEF) among the 3 clusters. $P < .05$ was considered statistically significant.

adult-onset asthma, which was similar with the disease course of adult-onset nonallergic asthma.⁶² Indeed, adult-onset asthma can be divided into 2 distinct phenotypes: adult-onset eosinophilic asthma and adult-onset noneosinophilic asthma²; furthermore, adult-onset eosinophilic asthma was identified as a severe phenotype. Similarly, our present study identified 2 adult-onset phenotypes in patients with NPcA with a distinct disease course and inflammatory phenotype: smoking NPcA cluster (eosinophilic inflammation, 29.09%) and older NPcA cluster (noneosinophilic inflammation, 56.36%). The smoking NPcA cluster included AERD and was also a severe phenotype. This suggested that older NPcA was a distinct subtype of NPcA and should be treated differently.

After identifying 3 clusters based on demographic characteristics, we evaluated and compared the clinical parameters among clusters to validate their clinical significance. We observed that total IgE and eosinophilic inflammatory parameters such as FeNO, blood, and tissue eosinophils were highest in the smoking NPcA cluster and lowest in the older NPcA cluster, which

revealed the different functionality of total IgE and eosinophilic inflammation in the pathogenesis of the 3 clusters. Anti-IgE treatment was effective in patients with NPcA irrespective of the presence of allergy, and this beneficial effect was not associated with a reduction of eosinophilic inflammation.⁹ High total IgE levels are a biomarker for severe asthma phenotype.⁶³ These findings are partly consistent with the characteristics of the smoking NPcA, which was the most severe phenotype among the 3 clusters. Our previous study of NPcA showed that an eosinophilic phenotype was more severe than a noneosinophilic phenotype.³⁴ Here, we expanded this finding by analyzing the natural course of NPcA and found that 3 NPcA clusters with different disease courses presented with distinct distributions in terms of eosinophilic phenotypes and level of total IgE. This is the first study to report the spectrum of NPcA, which may provide evidence for phenotype-guided management approaches.

The radiological features of the nasal cavity and sinuses as well as pulmonary function were assessed to characterize the severity of airway disease among atopic, smoking, and older NPcA

clusters. In this study, we found that paranasal sinus CT abnormalities in the smoking NPcA cluster were the worst compared with other 2 clusters. The atopic NPcA cluster and older NPcA cluster presented with similar upper airway severity. Previous studies showed that a comorbidity of asthma in patients with NP tended to increase LM scores.⁶⁴⁻⁶⁷ When stratifying NPcA based on the presence of atopy or eosinophilic inflammation, the nonatopic or eosinophilic phenotype showed significantly higher LM scores,^{34,65} indicating that the presence of nonatopy and eosinophilic inflammation was highly associated with worse abnormalities in patients with NPcA. This was further supported by the result that the smoking NPcA cluster with a higher prevalence of nonatopic (59.38%) and eosinophilic (approximately 65.63%) phenotypes had the highest LM scores. Regarding the severity of the lower airway in patients with NPcA, our previous study showed that the eosinophilic phenotype of NPcA had a worse lung function compared with those with a noneosinophilic phenotype.³⁴ After stratifying patients with NPcA based on the natural course, we found that lung function differed significantly among the 3 clusters with the worst in the smoking NPcA cluster and the best in the atopic NPcA cluster. Interestingly, we also found that a shorter disease duration was closely associated with worse lung function. For the first time, we have demonstrated that the severity of lower airways in patients with NPcA is associated with natural course.

Our study had several limitations. First, we clustered patients based on demographic information of natural course by using questionnaires, which might weaken the accuracy of the data. Further validation in independent cohorts including a wider variety of demographics is needed. Second, the sample size was relatively small; multicenter and large sample studies are needed. It was also important to point out that the sample sizes varied between clusters with cluster 1 being the smallest. It remained possible that a type II error existed within this group because of the small sample size and a lack of power. Third, this study did not explore the mechanism involved, and further studies are required to elucidate the molecular mechanisms responsible for our study findings. Finally, the use of cluster analysis was potentially a limiting factor in this study because of the inherent defects of this statistical method.¹ Because cluster analysis is not an inferential statistical technique, there is no probability calculated. Therefore, our aim is to illustrate the heterogeneity in the natural course of NPcA instead of claiming a final classification.

In summary, we identified distinct natural courses in patients with NPcA. Furthermore, the inflammatory status and disease severity were markedly different among atopic, smoking, and older NPcA clusters, which further supports the clinical significance of the results. By investigating the natural course, clinicians might predict the clinical aspects of NPcA and contribute to phenotype-guided management approaches.

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DW designed the study, analyzed the data, and wrote the manuscript. LL, XZ, LZ, and QL collected the clinical data and followed up with the patients. BSB and JW read and revised the

manuscript. YW designed the study and revised the manuscript. All authors have read and approved the final manuscript.

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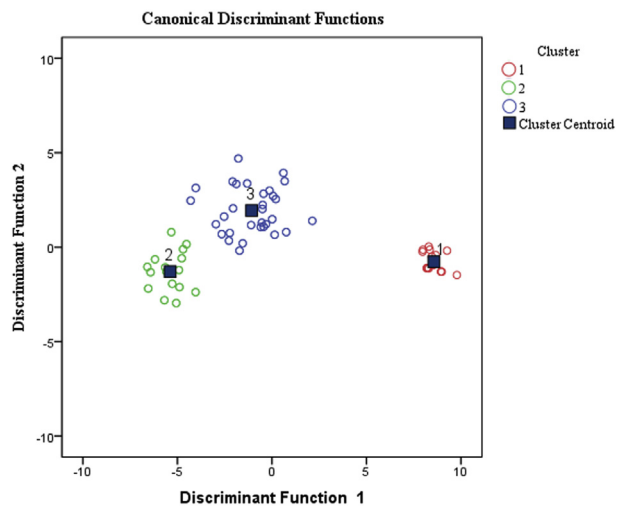


FIGURE E1. Canonical discriminant function analysis of the 110 patients with nasal polyps and comorbid asthma. By using a stepwise method of discriminant analysis, cluster 1, cluster 2, and cluster 3 were well separated.

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