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Original Article

Cluster analysis of patients with cough, mainly cough variant asthma, showing a good response to asthma therapy

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Abstract

Introduction: Cough variant asthma (CVA) and asthma account for a large proportion of patients with chronic cough. We used hierarchical cluster analysis (HCA) to characterize the phenotypes of patients with cough, mainly those with CVA, who showed a rapid response to asthma therapy, and sought to identify simple diagnostic markers.
Methods: Patients who initially presented with cough as the main symptom were retrospectively enrolled. Those who were clinically diagnosed with asthma/CVA were selected and analyzed. HCA was performed and the patients were classified by phenotype.
Results: HCA identified the five following phenotypes among the 221 participants: highly eosinophilic bronchitis [high fractional exhaled nitric oxide (FeNO) , n = 28]; atopic [high total serum IgE , n = 23]; non-atopic and non-eosinophilic [n = 104]; distal airway narrowing [low FEF75 ($\dot{V}_{25}$), n = 21] and severely atopic [extremely high total serum IgE and/or extreme high FeNO, n = 45]. No significant differences in long-term prognosis were found among the phenotypes.
Discussion: Five phenotypes were identified among patients with cough who showed a rapid response to asthma treatment. Wheezing was observed in about 24% of patients, and abnormal FeNO or $\dot{V}_{25}$ levels in about 30%.

Key Words: Cough variant asthma, Asthma, Phenotype, FeNO, Cluster analysis.

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Introduction

Cough is one of the most common chief complaints in general medicine. In the United States, "cough" was the sixth main reason for office visits after "progress visit," "general medical examination," "postoperative visit," "medication" and "counseling"; there were 18 million visits for cough in 2014[1]. In general practice in the UK, "cough" was the most common symptom in more than half of all new referrals[2]. In Japan, "cough" is also a common symptom. In 2012, 4,543 of the 35,768 patients who visited the general medical department in Japan presented with "cough," and this was the second most common symptom following "fever"[3]. Previous studies reported that asthma and cough variant asthma (CVA) were the most common causes of chronic cough[4-7]. In some patients, cough is so severe that it causes insomnia; these individuals require immediate symptom relief[8], and it is therefore important to establish simple diagnostic criteria to identify who will benefit from asthma treatments.

Patients with CVA have chronic cough as their principal, if not only, symptom, and it is associated with airway hyperresponsiveness [9]. Cough is often more problematic at night. Lung function may be normal[9]. Unless

adequately treated, 30 to 40% of adult patients with CVA may progress to classic asthma[10].

It has been widely considered that asthma is a collection of different phenotypes, rather than a single disease. By identifying these asthma phenotypes, it might enable more appropriate, effective, and safer managements or treatments of each phenotype in addition to better understanding of the underlying pathobiology that contributes to a particular phenotype. Therefore, it is valuable to identify patients to classify them according to recognizable clusters of demographic, clinical or pathophysiological characteristics. Hierarchical cluster analysis (HCA) has been used to identify these disease phenotypes[11]. In patients with more severe asthma, some phenotype-guided treatments are available[11], however, no research have been published in patients with CVA. In this study we used HCA to identify the phenotypes of patients with cough who showed rapid response to ICS, and also determine the diagnostic criteria for each cluster.

The earliest change associated with airflow obstruction in small airways is thought to be slowed airflow in the terminal portion of the spirogram, even when the initial portion is barely affected[12]. This slowing of expiratory flow is depicted as a concave shape on the flow-volume curve. Quantitatively, it is reflected by the fact that the instantaneous flow measured after 75% of the FVC has been exhaled (FEF75), known as $\dot{V}_{25}$ in Japan, shows a proportionally greater reduction than forced expiratory volume in 1 second (FEV_1)[12]. The value is effort-independent because an intrathoracic check valve is created. The predicted values of FEF75 ($\dot{V}_{25}$) in Japan, as reported by the Japanese Respiratory Society's lung physiology expert committee report[13], are as follows: predicted male FEF75 ($\dot{V}_{25}$) = 0.021 × standing height (cm) − 0.031 × age (yrs) − 0.073; predicted female FEF75 ($\dot{V}_{25}$) = 0.003 × standing height (cm) − 0.025 × age (yrs) + 2.155. Most normal subjects have $\dot{V}_{25}$ values greater than 50% of the predicted $\dot{V}_{25}$ value (Figure 1a, b).

Measuring the fractional nitric oxide concentration in an exhaled breath (FeNO) is a quantitative, noninvasive, simple, and safe method of assessing airway inflammation and airway disease, including asthma[14]. Measurement of FeNO has been covered by insurance since 2013 and is becoming more common in Japan. A high FeNO reflects airway eosinophilic inflammation[14]. Receiver operating characteristic (ROC) curve analyses indicated that a FeNO of 22 parts per billion (ppb) was the cut-off value with the highest combination of sensitivity (90.8%) and specificity (83.9%) for differentiating healthy subjects from asthma patients in a Japanese population[15]. In healthy Japanese adults, the geometric mean of FeNO was 15.4 ppb with a mean ± 2SD of 6.5 to 36.8 ppb. Therefore, the upper limit of FeNO in healthy adults was set at 36.8 ppb[16]. It is rare that healthy subjects have a FeNO value greater than 37 ppb in Japan (Figure 2).

We aimed to identify the phenotypes and investigate their prognosis of patients with cough, mainly CVA, showing a good response to asthma therapy, and sought to identify simple diagnostic markers.

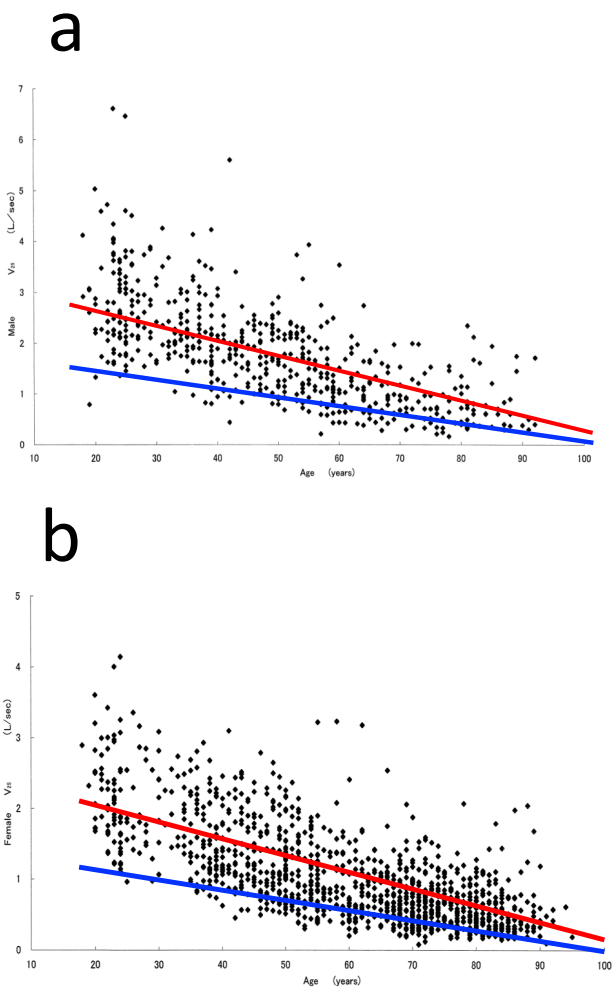

Figure 1.

The distribution of $\dot{V}_{25}$ levels in normal males (a) and females (b), adopted from "Spirogram and arterial blood gas partial reference values of Japanese," available at http://www.jrs.or.jp/quicklink/journal/nopass_pdf/039050000j.pdf (accessed on 2nd/July/2019). Red lines indicate the predicted $\dot{V}_{25}$ values based on the average height of a Japanese adult (167 cm for males and 158 cm for females). The half values of predicted $\dot{V}_{25}$ are shown with blue lines. Most normal subjects showed $\dot{V}_{25}$ values greater than 50% of the predicted $\dot{V}_{25}$ value (above the blue line).

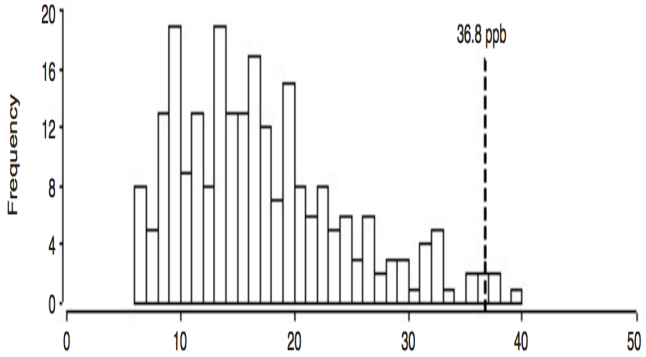

Figure 2.

The distribution of FeNO values in healthy subjects, adopted from "Reference ranges for exhaled nitric oxide fraction in healthy Japanese adult population. Allergol Int. 2010; 59: 363-7." The geometric mean was 15.4 ppb with a mean ± 2SD of 6.5 to 36.8 ppb, suggesting that it is unlikely that healthy subjects have FeNO values greater than 37 ppb.

Materials and Methods

Study Subjects

We retrospectively enrolled the first visit patients complaining of cough who were given final diagnosis of asthma/CVA. Patients with gastroesophageal reflux disease, rhinitis and sinus are excluded. They were not previously correctly diagnosed the cause of their symptoms and untreated for asthma/CVA. They initially presented to the Ikebukuro Otani Clinic, which is the specialized clinic in the treatment of cough, between March 2014 to July 2014. Pulmonary function tests (PFTs), blood tests and FeNO measurements were performed routinely. Patients were excluded if their CRP showed over 0.3 mg/dl (suspected of having comorbid infections) or if they did not have acceptable flow-volume curves in spirometry or did not undergo blood tests or FeNO measurements. Patients given a clinical diagnosis of asthma or CVA were included in this study.

Test and Laboratory Parameters

Information regarding patients' profiles, histories of presenting illness, previous medical histories, auscultatory findings, results of blood tests, PFTs results and clinical courses were obtained from medical records and evaluated. Blood tests were conducted at the laboratories of Showa Medical Science Corporation (Tokyo, Japan). FeNO concentrations were measured using a nitric oxide analyzer device (NIOX MINO Airway Inflammation Monitor, Aerocrine AB [Solna, Sweden]). PFTs measurements were performed using a spirometer (SP-370COPD, Fukuda Denshi [Tokyo, Japan]).

Phenotypic classification by cluster Analysis

HCA was performed using R (The R Foundation for Statistical Computing, version 3.2.2)[17]. Ward's minimum-variance hierarchical clustering method was performed using an agglomerative approach and Ward's linkage. Since cluster analysis cannot differentiate between relevant and irrelevant variables, the choice of variables must be based on conceptual considerations. In this study, HCA was performed repeatedly with different variables and weights. We decided to use the four chosen variables and divided them based on the upper limits of reference values so that they would have equal significance. The value of FeNO, total serum IgE level, FEF75 ($\dot{V}_{25}$), and peripheral blood eosinophil count were selected for HCA in order to generate a dendrogram, which produced characteristic clusters reflecting the clinical examinations. Variable values were divided based on the upper limit of the reference value, and a normal range of 1 or lower was used to calculate cluster distances (*i.e.*, FeNO (ppb) / 22, %$\dot{V}_{25}$ defined as predicted $\dot{V}_{25}$ / $\dot{V}_{25}$, total serum IgE (IU/ml) / 185, and eosinophil count (/μl) / 440 were used in the analysis in order to have equal significance). R with EZR (Saitama Medical Center, Jichi Medical University) [18], which is a graphical user interface for R, was used for comparing the differences between clusters. The Kruskal-Wallis test was used to compare differences between nonparametric continuous variables. P values less than 0.05 were regarded as statistically significant.

Decision tree analysis

The simple rule of decision tree to classify the patients into each cluster was established. The accuracy of the rule was also estimated.

Treatment period

The patients were followed-up for more than 2 years. The treatment periods of patients in each cluster were compared using the Kaplan–Meier method, and comparison of the clusters was carried out using the log-rank test.

Establishing simple diagnostic criteria

To identify patients with cough who would benefit immediately from asthma treatments, simple diagnostic criteria were established based on examinations.

Validation cohort study of the diagnostic criteria

To test the specificity of the diagnostic criteria, healthy adults who were enrolled in another study at Yokohama City Minato Red Cross Hospital and Ikebukuro Otani

Clinic were also recruited; as part of that study, they underwent FeNO and PFTs assessment as well as evaluation using MostGraph. For a validation cohort study of the diagnostic criteria, another group of patients who initially presented to the Ikebukuro Otani Clinic between April 2015 to May 2015 and who met our study criteria were also recruited.

This study was approved by the ethics committee of the Tokyo Medical and Dental University (approval numbers 1931, 2047 and M2016-032). The requirement for obtaining the participants' informed consent was waivered by the institutional review board, for this is the retrospective chart review study. By the poster for privacy notices and more information, the patients were able to choose to opt out of sharing their information for research.

Results

Subject Demographics

The initial dataset included 401 participants (male patients: n = 164, mean age = 41.8 ± 13.8; female patients: n = 237, mean age = 39.8 ± 14.4) who initially presented to the Ikebukuro Otani Clinic with a chief complaint of cough. Of the initially recruited patients, 221 were ultimately included in our study (male patients: n = 92, mean age = 39.2 ± 12.4; female patients: n = 129, mean age = 38.7 ± 13.0) (Figure 3), after excluding patients with other diseases like infection, gastroesophageal reflux disease, rhinitis, sinus, etc. Patients' demographic characteristics and clinical findings are displayed in Table 1.

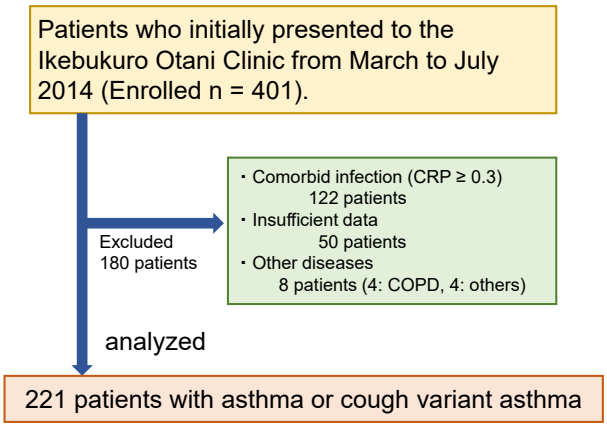

Figure 3.

Patient recruitment. Of 401 patients who initially presented to the Ikebukuro Otani Clinic, 221 were diagnosed with CVA without infection or comorbid diseases.

Table 1. Subjects' characteristics and clinical findings

N	221
Gender, M/F (%M)†	92/129 (41.6)
Age (years; mean ± SD)	38.94 ± 12.60
Smoking Status, n (%)†	
non-smokers	157 (71.0)
ex-smokers	35 (15.8)
current smokers	29 (13.1)
Past Medical History, n (%)†	
bronchial asthma (BA)	37 (16.7)
cough variant asthma (CVA)	7 (3.2)
allergic rhinitis (AR)	54 (24.4)
atopic dermatitis (AD)	24 (10.9)
Duration, n (%)†	
< 3 weeks	86 (38.9)
3-8 weeks	75 (33.9)
> 8 weeks	60 (27.2)
Wheezes/Rhonchi, n (%)†	53 (24.0)
Blood Eosinophil (/μl)	195 ± 148
Total serum IgE (IU/ml)	207 ± 435
Positive specific IgE for house dust, n (%)†	86 (40.4)
Positive specific IgE for house dust mite, n (%)†	84 (39.4)
FeNO (ppb)	28.0 ± 27.1
%VC‡ (%)	96.3 ± 13.5
$FEV_{1.0\%}$ (%)	83.3 ± 7.41
$\%\dot{V}_{50}$§ (%)	85.9 ± 22.9
$\%\dot{V}_{25}$¶ (%)	78.0 ± 31.3

Data are expressed as mean ± SD, except for †
VC, vital volume; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; $\dot{V}_{50}$, forced expiratory flow at 50% of FVC, FEF50; $\dot{V}_{25}$, forced expiratory flow at 75% of FVC, FEF75; $FEV_{1.0\%}$, FEV_1/FVC.
‡ %VC = VC / predictedVC
PredictedVC was calculated using the formulas below:
$predictedVC_{(male)} = 0.045 \times height\ (cm) - 0.023 \times age\ (yrs) - 2.258$
$predictedVC_{(female)} = 0.032 \times height\ (cm)\ 0.018 \times age\ (yrs) - 1.178$
§ $\%\dot{V}_{50} = \dot{V}_{50}$ / $predicted\dot{V}_{50}$
$Predicted\dot{V}_{50}$ was calculated using the formulas below:
$predicted\dot{V}_{50}(male) = 0.043 \times height\ (cm) - 0.046 \times age\ (yrs) - 0.385$
$predicted\dot{V}_{50}(female) = 0.014 \times height\ (cm) - 0.038 \times age\ (yrs) + 3.150$
¶ $\%\dot{V}_{25} = \dot{V}_{25}$ / $predicted\dot{V}_{25}$
$Predicted\dot{V}_{25}$ was calculated using the formulas below:
$predicted\dot{V}_{25}(male) = 0.021 \times height\ (cm) - 0.031 \times age\ (yrs) - 0.074$
$predicted\dot{V}_{25}(female) = 0.003 \times height\ (cm) - 0.025 \times age\ (yrs) + 2.155$

Cluster Analysis

A dendrogram was generated using an agglomerative approach and Ward's linkage. The cluster analysis was repeated and the most representative model was selected. FeNO, $\%\dot{V}_{25}$, total serum IgE levels and number of blood eosinophils were selected as variables, and five clusters were identified (Figure 4). All patients could be classified into 5 groups with distinctive phenotypes. The demographic and clinical characteristics of each cluster are displayed in Table 2. The clusters showed no significant differences in patients' ages, genders, smoking histories, past medical histories or symptom durations.

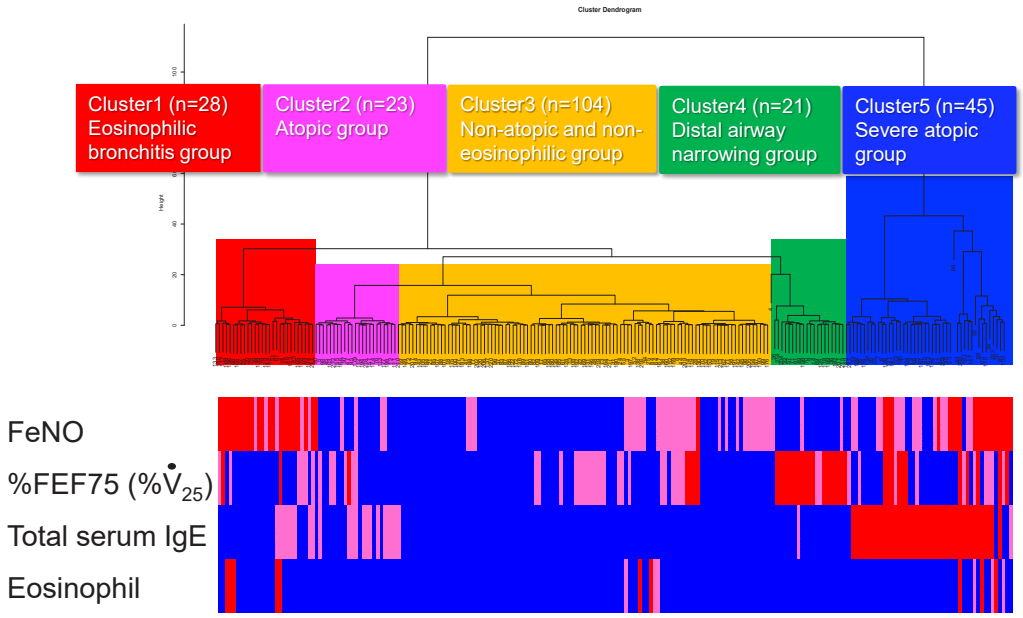

Figure 4.

Cluster dendrogram. The following five clusters were identified: Cluster 1: highly eosinophilic bronchitis group (high FeNO level only, n = 28); Cluster 2: atopic group (high total serum IgE only, n = 23); Cluster 3: non-atopic and non-eosinophilic group (n = 104); Cluster 4: small airway narrowing group (low $\dot{V}_{25}$, n = 21); and Cluster 5: severely atopic group (extremely high total serum IgE and/or FeNO levels, n = 45). Variable values are displayed as a heatmap. Red denotes an abnormal value, i.e., FeNO > 37 ppb, $\%\dot{V}_{25}$ < 50%, total serum IgE > 310 IU/ml and eosinophil > 500 /μl. Pink denotes a borderline value, i.e., FeNO = 22-37 ppb, $\%\dot{V}_{25}$ = 50-66%, total serum IgE = 185-310 IU/ml and eosinophil = 440-500 /μl. Blue denotes a normal value.

Table 2. Characteristics and clinical findings of each clusterData are expressed as mean ± SD, except for †

	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	p-value
N	28	23	104	21	45	
M/F (%M)†	15/13 (54)	10/13(43)	37/67(36)	7/14(33)	22/23(49)	0.217
Age (year; mean ± SD)	38.8 ± 10.9	34.6 ± 9.4	38.0 ± 12.1	47.0 ± 14.3	40.2 ± 14.6	0.063
Smoking Status, n (%)†						0.198
non-smokers	23 (82.1)	17 (73.9)	73 (70.2)	17 (81.0)	27 (60.0)	
ex-smokers	4 (14.3)	3 (13.0)	17 (16.3)	3 (14.3)	8 (17.8)	
cur-smokers	1 (3.6)	3 (13.0)	14 (13.5)	1 (4.8)	10 (22.2)	
Past Medical History, n (%)†						0.118
bronchial asthma	8 (28.6)	6 (26.1)	10 (9.6)	2 (9.5)	11 (24.4)	
CVA	0	2 (8.7)	4 (3.8)	0	1 (2.2)	
allergic rhinitis	5 (17.9)	8 (35.0)	21 (20.2)	5 (23.8)	15 (33.3)	
atopic dermatitis	1 (3.6)	2 (8.7)	11 (10.6)	1 (4.8)	9 (20.0)	
Duration, n (%)†						0.128
< 3weeks	11 (39.3)	9 (39.1)	37 (35.6)	5 (23.8)	25 (55.6)	
3-8 weeks	6 (21.4)	9 (39.1)	44 (42.3)	8 (38.1)	9 (20.0)	
> 8 weeks	11 (39.3)	5 (21.7)	23 (22.1)	8 (38.1)	11 (24.4)	
Wheezes/Rhonchi, n (%)†	10 (35.7)	7 (30.4)	18 (17.3)	4 (19.0)	14 (31.1)	0.153
Eosinophil (/μl)	258 ± 194	196 ± 106	154 ± 119	184 ± 110	256 ± 181	< 0.001
Total serum IgE (IU/ml)	111 ± 96.2	200 ± 46.8	38.9 ± 30.2	63.5 ± 59.6	724 ± 761	< 0.001
Positive specific IgE for house dust, n (%)†	13 (46.4)	12 (57.1)	19 (19.0)	8 (38.1)	34 (79.1)	< 0.001
Positive specific IgE for house dust mite, n (%)†	13 (46.4)	12(57.1)	19 (19.0)	8 (38.1)	32 (74.4)	< 0.001
FeNO (ppb)	47.9 ± 13.1	17.1 ± 5.7	17.9 ± 7.5	16.2 ± 4.8	50.0 ± 47.9	< 0.001
%VC‡ (%)	100 ± 12.0	95.2 ± 11.4	97.8 ± 14.1	91.9 ± 14.2	92.5 ± 12.9	0.085
$FEV_{1.0\%}$ (%)	83.4 ± 6.0	85.0 ± 6.0	84.9 ± 7.2	75.9 ± 4.7	81.9 ± 8.4	< 0.001
$\%V_{50}$§ (%)	86.2 ± 18.7	88.2 ± 15.0	92.7 ± 22.1	61.8 ± 19.3	80.0 ± 24.0	< 0.001
$\%V_{25}$¶ (%)	80.9 ± 29.1	80.1 ± 22.5	87.1 ± 29.6	39.1 ± 10.0	73.1 ± 31.6	< 0.001

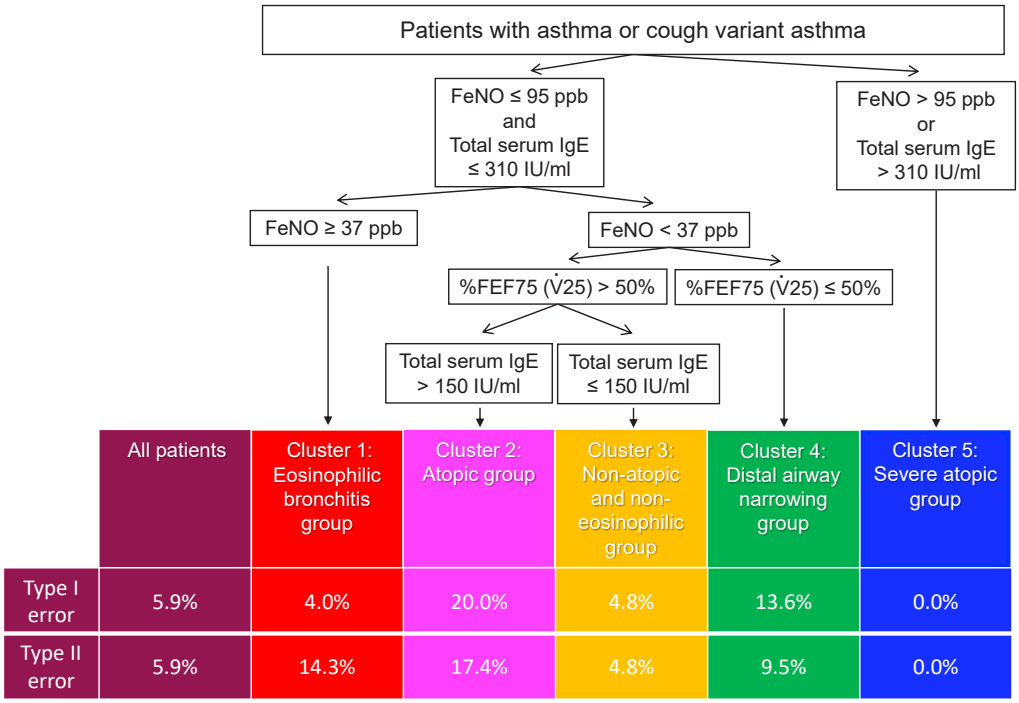

Figure 5.

Decision tree for cough variant asthma phenotypes using values of FeNO, total serum IgE and % $\dot{V}_{25}$. When the rules were applied to all participants, 94% of them were assigned to the original clusters defined using hierarchical cluster analysis, shown in the lower portion of the diagram.

Cluster 1: Eosinophilic bronchitis group

Cluster 1 comprised 13% of the patients (n = 28) and consisted of patients with high FeNO values and few abnormalities in the other parameters. Patients in this group tended to have higher values of blood eosinophils (Table 2).

Cluster 2: Atopic group

Cluster 2 consisted of a small group of patients (10%, n =23) with slightly elevated values of total serum IgE. These patients had a younger mean age than those in any other cluster, and a high proportion had a medical history of bronchial asthma (BA), allergic rhinitis (AR) or atopic dermatitis (AD) (Table 2).

Cluster 3: Non-atopic and non-eosinophilic group

Cluster 3 was the largest group (47%, n = 104). This cluster was predominantly female (male patients = 37; female patients = 67), and patients had fewer wheezes and a lower rate of blood test and PFTs abnormalities (Table 2).

Cluster 4: Distal airway narrowing group

Cluster 4 was the smallest group (10%; n = 21). Patients in this group had poorer pulmonary function and lower levels of VC, FEV1.0/FVC ($FEV_{1.0\%}$), $\%V_{50}$ (V_{50} / predictedV$_{50}$) and $\%V_{25}$. The mean age of the patients in this group was more than 5 years higher than the other groups (Table 2).

Cluster 5: Severely atopic group

Cluster 5 comprised 20% of the patients (n = 45). Patients in this group had extremely high levels of total serum IgE and/or extremely high values of FeNO.

Decision tree analysis

To make it easier and more straightforward to classify participants into these five clusters, a decision tree was made based on FeNO values, total serum IgE and $\%\dot{V}_{25}$ (Figure 5). Application of this method resulted in 94% of subjects being assigned to the original HCA cluster, although 11 subjects were categorized into a different cluster.

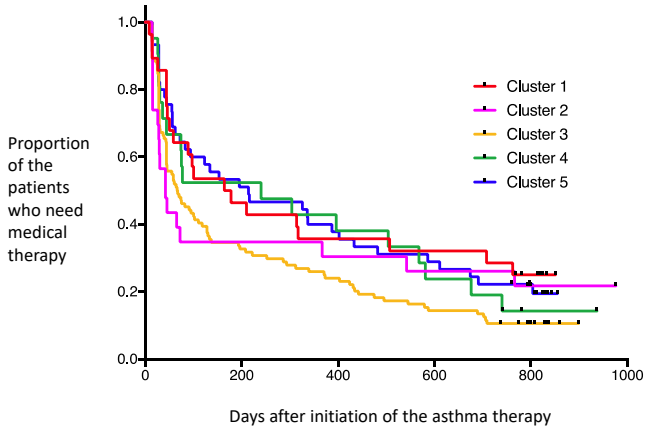

Figure 6.

Overall treatment period of each cluster. There are no significant differences among clusters (p = 0.080).

Treatment period

Patients were followed-up for more than 2 years, and no significant differences (p = 0.080) were found among clusters in terms of patients' asthma treatment periods (Figure 6).

Validation cohort study

To evaluate the validity of FeNO and $\%\dot{V}_{25}$ values as diagnostic markers, we evaluated 92 patients with cough (male patients: n = 23, mean age = 40.3 ± 12.5; female patients: n = 69, mean age = 40.0 ± 15.9) and 90 healthy adults (male adults: n = 49, mean age = 47.8 ± 13.6; female adults: n = 41, mean age = 31.6 ± 12.8). Twelve patients (13.0%) had wheezes. Seventeen patients (18.5%) had FeNO values over 37 ppb and 13 (14.1%) had $\%\dot{V}_{25}$ values lower than 50%. If at least one criterion was present, the sensitivity was 35.9%. If the second or third were present, the sensitivity was 30.4% in the validation cohort.

Ninety healthy adults (male adults: n = 49, mean age = 47.8 ± 13.6; female adults: n = 41, mean age = 31.6 ± 12.8) were also recruited to evaluate specificity. The specificities of a FeNO value over 37 ppb and a $\%\dot{V}_{25}$ lower than 50% were 88.9% and 88.9%, respectively. The overall specificity when at least one criterion was present was 80.0%; it was also 80% when the second or third criteria were present because no healthy subjects had a FeNO value over 37 ppb.

Discussion

Asthma is a heterogeneous, chronic, inflammatory disorder that is characterized by variabilities in disease expression and severity. An increasingly urgent need to classify asthma heterogeneity has coincided with the development of better tools for measuring disease characteristics, tools that highlight the disparities in clinical, physiologic and pathologic markers[11]. Patients with cough, mainly those with asthma/CVA, who showed a rapid response to asthma therapy were classified into five phenotypes, each with different clinical characteristics.

CVA is a form of asthma in which the main symptom is cough[9]. Some authors define CVA as a form of asthma without wheezes or rhonchi[19]. Differences in the diagnostic criteria between CVA and wheezing asthma are obscure: Despite exhibiting normal findings on lung auscultation in the clinic, patients might wheeze at night because asthma symptoms are worse during sleep. Wheezing was observed in about 24% of patients during deep expiration in our study, and its presence was useful in diagnosing asthma. Obase Y. *et al.* reported that CVA might be a very early precursor of bronchial asthma[19], thus we did not strictly stratify patients by wheezing.

Ten percent of patients exhibited a low $\dot{V}_{25}$ and they belonged to cluster 4, the distal airway narrowing group; however, only 5 of these 21 patients had an $FEV_{1.0\%}$ lower than 70%, suggesting that $\dot{V}_{25}$ might be a more sensitive biomarker than $FEV_{1.0\%}$. Forced expiratory flow between 25% and 75% of vital capacity (FEF25-75, MMF) is also widely used to assess distal airway narrowing. However, in some patients it is difficult to accurately measure FEF25 ($\dot{V}_{75}$) because this parameter is highly dependent on the validity of the FVC measurement and the level of expiratory effort[20]. In this study, though, we reviewed patients' medical records and flow-volume loops and found that in most cases $\dot{V}_{25}$ values were reliable, presumably because these are independent of subject effort due to the presence of an intrathoracic check valve[12]. Moreover, $\dot{V}_{25}$ was more sensitive than MMF for identifying a rapid response to ICS, as the former correlates well with forced expiratory flow at 50% (FEF50, $\dot{V}_{50}$), as shown in Table 2.

The expected $\dot{V}_{25}$ value can be calculated with the formulas published by the Japan Respiratory Society lung physiology expert committee.[14] In most normal subjects, $\dot{V}_{25}$ values were greater than 50% of predicted $\dot{V}_{25}$ values (Figure 1a, b).[12] Therefore, we consider that a measured $\dot{V}_{25}$ / expected $\dot{V}_{25}$ ($\%\dot{V}_{25}$) value less than 0.5 is likely to be an effective diagnostic biomarker of asthma/CVA. Subjects with asthma/CVA who demonstrated a

low $\dot{V}_{25}$ [$\dot{V}_{25} < 0.5$] belonged to cluster 4, the group with older participants, and thus these $\dot{V}_{25}$ values may reflect airway remodeling due to asthma.

Measurement of FeNO is a simple and noninvasive means of assessing airway inflammation. The ATS recommended the use of FeNO in the diagnosis of eosinophilic airway inflammation, a condition thought to be caused by TH2-type inflammation[14]. High blood eosinophil counts and total serum IgE level are also thought to result from this type of inflammation. However, these parameters are only weakly correlated with each other (Figure 7), suggesting that they are regulated by different mechanisms[21, 22].

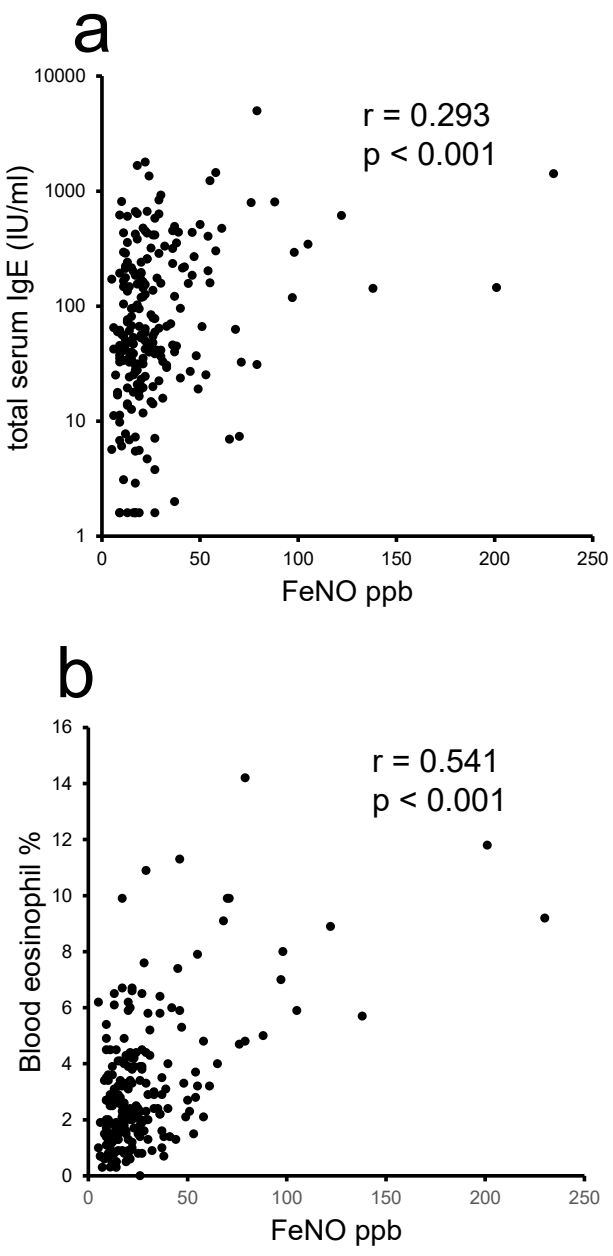

Figure 7.

FeNO correlates with total serum IgE (a) and blood eosinophil (b) percentage; however, the correlations are weak.

We found few abnormalities in blood eosinophil counts, presumably due to CVA being an early stage of bronchial asthma, a condition characterized by relatively mild eosinophilia. Patients with CVA may have milder eosinophilic inflammation and lower FeNO values[19]. One study found that the population attributable risk of asthma due to eosinophilic inflammation was approximately 50%[23]; therefore in up to 50% of cases, asthma cannot be attributed to eosinophilic inflammation. In our study, about half of the patients had eosinophilic bronchial inflammation based on FeNO values.

Patients with prolonged symptoms require long-term treatment and may progress to classical asthma. We hypothesized that those in cluster 5 would require longer treatment because this group is considered to have the most severe disease. However, based on our Kaplan-Meier analysis, the treatment periods did not differ significantly among the clusters (Figure 6).

According to the GINA2019 guidelines[9], making the initial diagnosis of asthma is based on identifying both a characteristic pattern of respiratory symptoms and variable expiratory airflow limitation[6]. Generally, in adults with respiratory symptoms typical of asthma, an increase or decrease in FEV_1 of >12% and >200mL from baseline, or a change in peak expiratory flow (PEF) of at least 10%, is accepted as being consistent with asthma[9]. For patients without airflow limitation, one option is to refer the patients for bronchial provocation testing to assess airway hyperresposiveness[9]. The diagnosis of CVA is especially difficult. The patients with CVA showed normal auscultation findings, normal chest X-ray, and usually normal FEV_1 values[6]. In this study, most of the patients exhibited normal FEV_1 values. Therefore, it was not applicable to consider bronchodilator reversibility tests, marked variations in lung function between visits or excessive variability in twice-daily peak expiratory flow. Airway hyper-responsiveness is one feature that can contribute to a diagnosis of asthma, and is assessed via methacholine challenge testing. However, as inhaled methacholine causes bronchoconstriction, patient safety should be considered when designing the testing facility[24]. In practice, this means that this test can be performed only in specialized hospitals and it is barely done in Japan because of burdens for both patients (it can cause asthma attack) and busy doctors (it takes about 1 hour). In some patients with CVA, cough is so severe that it causes serious symptoms such as insomnia, fatigue,

or rib fracture, as the commonly used antitussives are ineffective. Therefore, rapid diagnosis and rapid relief from symptoms by asthma treatment is the first priority for patients[8]. It was reported that after 1 week of inhaled β-agonist treatment, CVA significantly improved[25]. Diagnostic treatment using inhaled corticosteroids (ICS) is recommended for the diagnosis of CVA by Japanese guidelines[26].

In our study, patients with a FeNO value greater than 36.8 ppb constituted approximately 20%. In particular, 13% of patients had only FeNO abnormalities without auscultatory or PFTs abnormalities. Ten percent of patients demonstrated a low $\dot{V}_{25}$. We might be able to diagnose 43.0% of the patients with asthma/CVA immediately by the diagnostic criteria that at least one of the following findings exist: (1) wheezes, (2) FeNO value over 36.8 ppb, or (3) $\%\dot{V}_{25}$ lower than 50%. For the general physician who were difficult to diagnose asthma/CVA, $\dot{V}_{25}$ and FeNO can be useful diagnostic markers.

One limitation of this study is that the diagnosis of asthma/CVA was mainly based on patients' rapid response to asthma treatment and their overall clinical course. Patients with non-asthmatic eosinophilic bronchitis, atopic cough or post infectious cough were included in the study, but in clinical practice, it is of value to identify and classify patients who can be successfully treated with asthma therapy. Patients with post infectious cough would be categorized into Cluster 3, because they exhibited fewer wheezes, a lower rate of blood test and PFTs abnormalities, and tendency for short treatment periods.

In conclusion, we characterized patients with cough consisting mainly of asthma/CVA into five phenotypic groups, however, no significant differences in long-term prognosis were found among the phenotypes. We also found that FeNO and $\dot{V}_{25}$ could serve as diagnostic criteria.

Ethics approval

This investigation obtained ethics approval from Tokyo Medical and Dental University ethical committee (approval numbers 1931, 2047 and M2016-032).

Disclosure Statement

The authors declare no conflict of interest associated with this manuscript.

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