Trigger of bronchial hyperresponsiveness development may not always need eosinophilic airway inflammation in very early stage of asthma

Yasushi Obase, M.D., Ph.D.,[1] Terufumi Shimoda, M.D., Ph.D.,[2] Reiko Kishikawa, M.D., Ph.D.,[2] Shigeru Kohno, M.D., Ph.D.,[1] and Tomoaki Iwanaga, M.D., Ph.D.[2]

ABSTRACT

Background: *Cough variant asthma (CVA), a suggested precursor of standard bronchial asthma (SBA), is characterized by positive bronchial hyperresponsiveness (BHR) and a chronic cough response to bronchodilator that persists for >8 weeks.*

Objective: *Airway inflammation, BHR, and airway obstructive damage were analyzed to assess whether CVA represents early or mild-stage SBA.*

Methods: *Patients with newly diagnosed CVA (n = 72) and SBA (n = 84) naive to oral or inhaled corticosteroids and without exacerbated asthma were subjected to spirometry, impulse oscillometry, BHR tests, sputum induction, and fractional exhaled nitric oxide measurements.*

Results: *In the patients with CVA, spirometry demonstrated higher forced expiratory volume in 1 second (FEV_1) to forced vital capacity ratio, FEV_1 percent predicted, flow volume at 50% of vital capacity % predicted, and flow volume at 25% of vital capacity % predicted values, and impulse oscillometry demonstrated lower R_5–Z_{20}, AX, and Fres, and higher X_5 values. In addition, the fractional exhaled nitric oxide and sputum eosinophil numbers were lower and the PC_{20} was higher than in patients with moderate SBA. However, these factors were similar in the patients with CVA and in the patients with intermittent mild SBA. A significantly smaller proportion of the patients with CVA had increased sputum eosinophils than the patients with intermittent mild SBA ($p < 0.0001$). However, interestingly, among the patients with CVA, no significant differences in the PC_{20} values were found between the patients with and those without increased sputum eosinophils.*

Conclusions: *All measures of central and peripheral airway obstruction, eosinophilic inflammation, and airway hyperresponsiveness in patients with CVA were milder than in patients with moderate SBA but were similar to those of patients with intermittent mild SBA. In CVA, the BHR was not affected by airway eosinophilic inflammation, which indicated that the very early development of BHR may not always need airway eosinophilic inflammation.*

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Cough variant asthma (CVA) is characterized by chronic coughing, which is discontinued by bronchodilator therapy, that persists for at least 8 weeks and is not accompanied by wheezing. Although both patients with standard bronchial asthma (SBA) and patients with CVA have increased bronchial hyperresponsiveness (BHR) to methacholine,[1] SBA, unlike CVA, involves concurrent wheezing, dyspnea, and coughing. CVA has been reported to progress to bronchial asthma within 5 years in 30–40% of patients, and starting therapy with inhaled corticosteroid early can prevent this progression.[2–4]

From the [1]Department of Respiratory Medicine, Nagasaki University Graduate School, Nagasaki, Japan, and [2]Clinical Research Center, Fukuoka National Hospital, Fukuoka, Japan

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Address correspondence to Terufumi Shimoda, M.D., Ph.D., Clinical Research Center, Fukuoka National Hospital, 4-39-1 Yakatabaru, Fukuoka, 811-1394, Japan
E-mail address: t-shimoda@mfukuoka2.hosp.go.jp

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Which aspects of asthma pathogenesis (*e.g.*, airway inflammation, BHR, airway obstruction) are critical to distinguishing between CVA and SBA, particularly the intermittent mild form of asthma, remains unclear. Airway eosinophilic inflammation is believed to be a fundamental characteristic of asthma development,[5] and sputum eosinophil ratios and fractional exhaled nitric oxide (FeNO) are established biomarkers of airway inflammation.[6] Because the development of BHR might involve not only airway inflammation but also repeated airway contraction,[7] understanding the pathogenesis of CVA might help to clarify how BHR develops in asthma.

To clarify the physiologic and biologic differences between CVA and SBA, particularly the mild and moderate forms of SBA, we analyzed airway inflammation, BHR, and pulmonary functions, including the use of an impulse oscillometry system (IOS) in patients with these disorders. We also compared the relationship between BHR and airway eosinophilic inflammation in CVA and SBA.

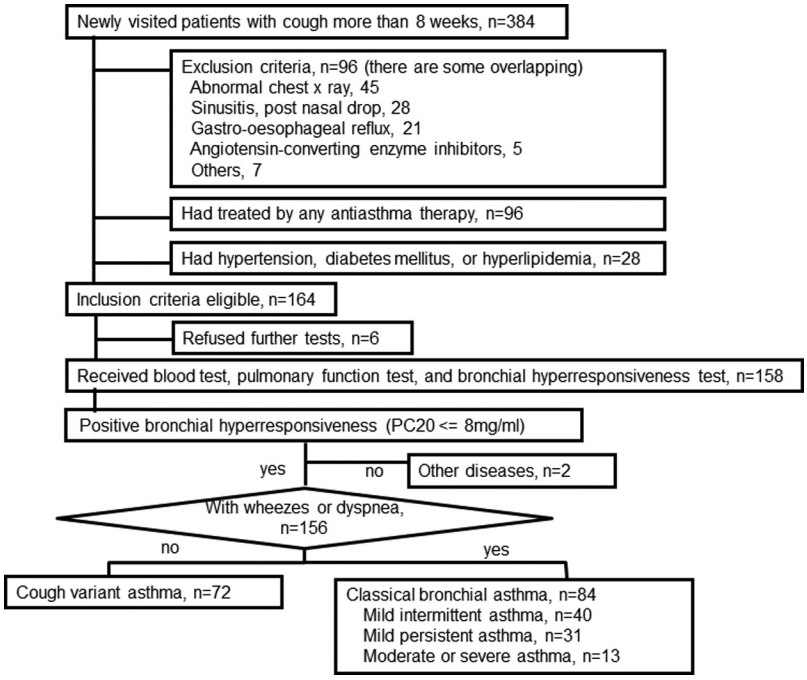

Figure 1. *Subject selection. From 384 candidate patients with a persistent cough for ≥8 weeks, a final total of 72 patients with cough variant asthma (CVA) and 84 patients with standard bronchial asthma (SBA) participated in the study.*

METHODS

Subjects

From 384 candidate patients with a persistent cough for 8 weeks or more, a total of 72 patients with CVA and 84 patients with mild or moderate SBA participated in this study (Fig. 1). Patients treated with any antiasthma medication within the 2 weeks before screening or who had concurrent hypertension, severe diabetes mellitus, or severe hyperlipidemia were excluded from the study because the treatments for these conditions could possibly affect the results. No patients had other apparent causes of the cough, including postnasal drip, gastroesophageal reflux, or angiotensin-converting enzyme inhibitors administration. Furthermore, all the subjects had normal chest radiograph results.

The diagnosis of CVA was based on the method reported by Corrao *et al.*[2,8–10] All the subjects were referred to our clinic for chronic coughing that had persisted for >8 weeks in the absence of wheezing or dyspnea. Wheezing or rhonchi were not audible on chest auscultation, even during forced expiration. None of the patients with CVA had a history of asthma or other respiratory diseases. The subjects with CVA had positive BHR to inhaled methacholine, and bronchodilators (inhaled β-2 agonists) were effective in treating their cough (Fig. 1).

The diagnosis and severity of SBA were determined according to the Global Initiative for Asthma guidelines.[1] All the patients with SBA had a history of episodic dyspnea, wheezing, and coughing, and had positive BHR to methacholine. Of the 84 patients with SBA, 40 had intermittent mild asthma, 31 had persistent mild asthma, and 13 had persistent moderate asthma. This study was approved by the institutional review board of Fukuoka National Hospital (approved number, 12–20). The experimental protocols and the purpose of the research were explained to all the study participants, and their written informed consent was obtained before inclusion in the study.

Measurements of IOS and Pulmonary Function

Impulse oscillation (IO) measurements were performed with the patient in a seated position. During the measurements, the subjects were advised to quietly breathe through a mouthpiece while wearing a nose clip. The subjects' cheeks were supported by the investigator's hands. Stable spontaneous volume and airflow were monitored, confirmed, and then recorded for ~40 seconds. Oscillatory mechanics were assessed by using a commercially available IO device (Master Screen-IOS; CareFusion, Wurmlingen, Germany). Continuous impulses (pyramidal-form pulses, 5 pulses/s) that contained sinusoidal waves of a broad-frequency spectrum were applied as the forced oscillation. The impulse pressure was produced with alternating changes in two directions (positive and negative). The respiratory system impedance (Z), the ratio of the mouth pressure to airflow during the impulses, the R value (real resistance), and the X value (imaginary reactance) were automatically calculated by using IOS program software, which included fast Fourier transform analysis (LAB Manager, version 4.65; Care-Fusion). The R_5 (R value at 5 Hz), R_{20} (R value at 20 Hz),

X_5 (X value at 5 Hz), and resonant frequency of X (Fres) values were provided as real-time data.[11]

After the IO measurement, spirometry was performed by using a rolling seal–type apparatus (CHESTAC-7800; CHEST, Tokyo, Japan). To avoid any negative effects of forced expiration on the airway, spirometry was never performed before the IO measurement. The predicted vital capacity, forced vital capacity (FVC), and forced expiratory volume in 1 second (FEV_1) were calculated by using equations reported by the Japanese Respiratory Society.[12]

Measurement of BHR to Acetylcholine

The challenge test was performed by using standardized methodology. After confirming that no antiasthma medications had been taken, the subjects inhaled an acetylcholine aerosol from a handheld nebulizer (PARI BOY 038; PARI GmbH, Starnberg, Germany) during tidal breathing for 2 minutes. The operating airflow rate was 5 L/min. Isotonic saline solution was inhaled first as a control; then, progressively doubled acetylcholine concentrations from 0.039 to 20 mg/mL were inhaled. The FEV_1 was measured after each inhalation with a spirometer (Chest Graph HI-701; CHEST) until the FEV_1 had fallen $>$ 20% from the post–saline solution FEV_1, expressed as provocative concentrations of acetylcholine that produced a 20% fall in FEV_1 (PC_{20}) or until the maximal concentration of acetylcholine had been administered. Subjects with a PC_{20} of $<$8000 μg/mL were considered to have airway hyperresponsiveness according to the American Thoracic Society criteria.[13]

Measurement of FeNO

FeNO was measured by the single-breath method (online measurement) by using a fast-response (0.02 second) chemiluminescence analyzer (NOA 280; Sievers Instruments Inc., Boulder, CO) according to the American Thoracic Society guidelines.[14] All measurements were recorded as the plateau during the last part of exhalation and were performed by using a mouth pressure of 16 cm of H_2O, which corresponds to an expiratory flow of 50 mL/s. FeNO was measured three times, and the differences in the measured values were within 5%. The NO concentrations were recorded as the average of these three values.

Sputum Induction and Processing

Sputum was induced for 20 minutes by the inhalation of 5 mL of 3% NaCl solution aerosolized by using a small ultrasonic nebulizer (Nescosonic nebulizer, UN-511; Nesco, Jakarta, Indonesia). The output of the instrument was ~5 L/min, and the mass-median aerodynamic diameter for the nebulized saline solution ranged from 1 to 5 μm. The portion induced during the first 10-minute interval was defined as central sputum, and the portion induced during the second 10-minute interval was defined as peripheral sputum.[8] Before coughing up sputum, each subject was asked to rinse his or her mouth and to blow his or her nose to minimize contamination with saliva and postnasal drip. Each subject was asked to cough during and after the inhalation exposures and to expectorate into empty containers.

The sputum was stored in a refrigerator, at 4°C, and processed within 30 minutes. The sputum samples were transferred to a Petri dish, and the more viscous parts were collected by using forceps. To collect cells for cytospin preparations, the samples were processed by the method described by Metso *et al.*[15] Cytospin slides were allowed to air-dry for 30 minutes and were then stained by using the Giemsa staining method. At least 400 nonsquamous cells, including eosinophils, neutrophils, lymphocytes, macrophages, and ciliated epithelial cells, were differentially counted. The results were expressed as percentages of total nonsquamous cell counts. If the examination of slides revealed macrophages and ciliated epithelial cells, then the sample was considered to be of bronchial origin and was included in the study.

High-Sensitivity C-Reactive Protein Measurements

Levels of high-sensitivity C-reaction protein were measured by latex nephelometry. Blood drawn from the cubital vein was centrifuged to obtain serum, which was frozen at −80°C until testing. The tests were conducted in accordance with the U.S. Food and Drug Administration requirements for high-sensitivity C-reactive protein assay reagents; therefore, the measurement sensitivity was ≤0.02 mg/dL and the coefficient of variation at a C-reactive protein concentration of 0.1 mg/dL did not exceed 3%.[16]

Statistical Analysis

The mean values were compared between patients with each severity of bronchial asthma and patients with CVA by using the Mann-Whitney *U*-test. The Scheffé test was used to compare means among the groups of patients with differing bronchial asthma severities. Statistical significance (p value) was set at 0.05. All statistical analyses were performed by using StatMate IV statistical analysis software (ATMS Co., Ltd., Tokyo, Japan).

RESULTS

Baseline Patient Characteristics

No statistically significant differences were detected in age, sex ratio, body mass index, smoking status, age of disease onset, atopic status, log immunoglobulin E, and family history of allergies among the groups (Ta-

Table 1 **Subject characteristics**

	CVA (n = 72)	Intermittent Mild Asthma (n = 40)	p Value vs CVA	Persistent Mild Asthma (n = 31)	p Value vs CVA	Persistent Moderate Asthma (n = 13)	p Value vs CVA
Age, y, 95% CI	41.7, 48.5	37.4, 45.3	0.48	37.5, 47.3	0.76	38.4, 56.2	0.98
Height, cm, 95% CI	161, 165	160, 166	0.99	160, 166	0.91	154, 167	0.99
Onset age, y, 95% CI	38.7, 45.4	31.9, 41.8	0.34	33.3, 44.2	0.74	21.0, 49.9	0.52
Duration, y, 95% CI	1.8, 4.3	2.6, 6.6	0.75	0.6, 6.6	0.09	3.4, 20.4	0.001
Body mass index, kg/m^2, 95% CI	21.7, 23.3	21.5, 23.7	0.99	22.2, 24.3	0.82	22.4, 25.1	0.72
No. men/women	26/46	15/25	0.99	15/16	0.34	7/6	0.37
Atopic/nonatopic	31/41	20/20	0.61	17/14	0.38	7/6	0.67
Childhood asthma, yes/no	0/72	6/34	0.0033	7/24	0.0002	4/9	<0.0001
Family history of allergy, yes/no	52/20	29/11	0.99	24/7	0.76	11/2	0.55
Nonsmoker/ex-smoker/current smoker	47/16/9	21/13/6	0.28	18/7/6	0.99	6/2/5	0.99
Exercise-induced asthma, yes/no	0/72	14/26	<0.0001	7/24	0.0002	4/9	<0.0001
Log IgE, 95% CI	1.77, 2.05	1.64, 2.11	0.99	1.84, 2.28	0.77	1.91, 2.76	0.22
Blood eosinophil ratio, 95% CI	2.7, 3.7	3.6, 5.9	0.15	4.6, 8.1	0.0005	4.9, 10.6	0.0003
Log PC_{20}, 95% CI	2.99, 3.28	2.98, 3.34	0.99	2.74, 3.14	0.46	2.35, 3.04	0.04
FeNO, ppb, 95% CI	20.2, 32.5	25.5, 52.4	0.71	53.7, 119.2	<0.0001	56.4, 161.8	<0.0001
Log hs-CRP, 95% CI	2.44, 2.66	2.34, 2.64	0.94	2.46, 2.76	0.95	2.34, 3.11	0.67
Sputum eosinophils > 3%/< 3%*	11/53	15/19	0.008	18/13	0.0001	12/1	<0.0001

CVA = cough variant asthma; CI = confidence interval; IgE = immunoglobulin E; PC_{20} = provocative concentrations of acetylcholine that produced a 20% fall in FEV_1; FeNO = fractional exhaled nitric oxide; hs-CRP = high-sensitivity C-reaction protein.

**Sputum samples were not obtained from all the subjects.*

ble 1). The proportion of patients with CVA and with high sputum eosinophil ratios was significantly lower than that of the patients with SBA with any degree of severity ($p < 0.01$).

Pulmonary Function and IOS Factors

The FEV_1:FVC, FEV_1 %predicted, V_{50} %predicted, and V_{25} %predicted values of the patients with CVA were similar to those of patients with intermittent mild SBA (Table 2). The impedance at 5 Hz (Z_5), resistance at 5 Hz minus resistance at 20 Hz (R_5-R_{20}), area of reactance (AX), resonant frequency (Fres), and reactance at 5 Hz (X_5) values in the patients with CVA were similar to those of patients with intermittent mild SBA (Table 2).

BHR and Airway Inflammation

All the patients had positive BHR ($PC_{20} < 8.0$ mg/mL), and the log PC_{20} values in patients with CVA were consistent with those of patients with intermittent or persistent mild SBA (Table 1). The central and peripheral sputum eosinophil percentages were significantly lower in patients with CVA than in patients with persistent SBA ($p < 0.01$ and $p < 0.05$, respectively) but were not different between patients with CVA and patients with mild intermittent SBA (Table 3). FeNO did not significantly differ between patients with intermittent mild SBA and patients with CVA ($p = 0.71$). The serum log high-sensitivity C-reaction protein values did not differ between the patients with CVA and patients with any severity of SBA ($p = 0.9$) (Table 3).

BHR in Patients with CVA and Patients with SBA with and without Sputum Eosinophilia

In the patients with CVA, increased sputum eosinophils (>3%) had no effect on BHR. However, in the patients with SBA, significantly lower PC_{20} values

Table 2 **Pulmonary function and IOS factors expressed by 95% confidence intervals**

	CVA (n = 72)	Intermittent Mild Asthma (n = 40)	p Value vs CVA	Persistent Mild Asthma (n = 31)	p Value vs CVA	Persistent Moderate Asthma (n = 13)	p Value vs CVA
%FVC	110, 116	107, 117	0.99	107, 116	0.99	92, 111	0.08
FEV_1:FVC, %	79, 82	77, 82	0.97	73, 80	0.10	65, 75	0.0002
FEV_1,% predicted	100, 106	95, 104	0.72	90, 101	0.14	74, 92	0.0003
V_{50}, % predicted	80, 90	73, 89	0.94	61, 80	0.06	38, 57	<0.0001
V_{25}, % predicted	60, 71	53, 70	0.93	47, 64	0.36	26, 43	0.0005
Z_5, kPa/(L/s)	0.27, 0.32	0.28, 0.38	0.63	0.30, 0.39	0.45	0.30, 0.45	0.22
X_5, kPa/(L/s)	−0.10, −0.08	−0.13, −0.09	0.38	−0.14, −0.10	0.04	−0.18, −0.11	0.007
R_5-R_{20}, kPa/(L/s)	0.014, 0.031	0.012, 0.048	0.94	0.023, 0.067	0.34	0.04, 0.148	0.0005
AX, kPa/L	0.19, 0.28	0.20, 0.56	0.53	0.28, 0.74	0.08	0.25, 1.12	0.02
Fres, times/second	10.2, 11.6	10.1, 13.0	0.92	11.3, 15.1	0.17	13.5, 17.6	0.003

IOS = impulse oscillometry; FVC = forced vital capacity; FEV_1 = forced expiratory volume in 1 second; V_{50} = flow volume at 50% of vital capacity; V_{25} = flow volume at 25% of vital capacity; Z_5 = impedance at 5 Hz; X_5 = reactance at 5 Hz; R_5-R_{20} = resistance at 5 Hz minus resistance at 20 Hz; AX = reactance area; Fres = resonant frequency.

Table 3 **Sputum-cell differentiation of central and peripheral airways expressed by 95% confidence intervals**

	CVA (n = 72)	Intermittent Mild Asthma (n = 40)	p Value vs CVA	Persistent Mild Asthma (n = 31)	p Value vs CVA	Persistent Moderate Asthma (n = 13)	p Value vs CVA
Central sputum, %							
Macrophage	44, 56	34, 52	0.55	29, 48	0.21	23, 50	0.32
Neutrophil	37, 49	41, 60	0.55	27, 47	0.21	22, 54	0.32
Eosinophil	1, 4	2, 7	0.94	10, 30	<0.0001	10, 36	0.0003
Lymphocyte	1.0, 1.6	0.4, 0.8	0.15	1.2, 2.9	0.94	0.4, 1.7	0.77
Peripheral sputum, %							
Macrophage	43, 57	39, 59	0.99	34, 52	0.66	23, 49	0.41
Neutrophil	36, 50	34, 55	0.65	26, 44	0.65	22, 56	0.99
Eosinophil	1, 5	1, 5	0.99	8, 27	0.0004	7, 38	0.0016
Lymphocyte	0.9, 1.3	0.8, 1.7	0.80	0.6, 1.5	0.99	0.3, 1.7	0.99

CVA = cough variant asthma.

were observed in the patients with increased numbers of sputum eosinophils ($p < 0.02$) (Fig. 2).

DISCUSSION

In this study, patients with CVA had nearly the same level of lung function as the patients with intermittent mild SBA, including airway reactance, airway resistance, airway inflammation, and BHR. However, increased eosinophilic airway inflammation was less common in patients with CVA than in patients with intermittent mild SBA (Table 1). Moreover, in contrast to the patients with SBA, BHR was not associated with bronchial eosinophilia in the patients with CVA (Fig. 2). Thus, CVA and mild SBA may be distinct disorders that can be differentiated based on the relationship level of eosinophilic airway inflammation to development of BHR.

In CVA, coughing is thought to be associated with mild airway spasms in the presence of positive BHR. Some researchers detected increased BHR in smaller numbers of patients with CVA than in patients with SBA,[17,18] whereas other studies showed that the degree of BHR did not differ between patients with CVA and patients with SBA.[19] Consistent with these latter studies, our present study demonstrated that patients with CVA had the same level of BHR as did patients with mild SBA. When defining CVA, CVA and SBA are often thought to represent the same pathogenesis of mild asthma. Our study revealed few indications of differences in airway inflammation and pulmonary

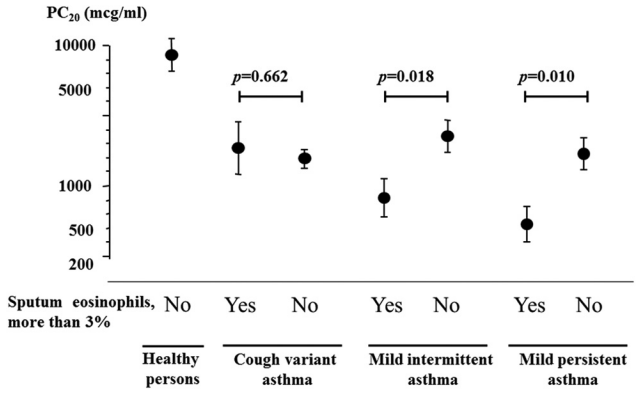

Figure 2. *The differences of PC_{20} values between the patients with and patients without sputum eosinophilia in each group. No significant difference was found between the bronchial hyperresponsiveness (BHR) in patients with CVA and with and without >3% sputum eosinophils. However, in patients with standard bronchial asthma (SBA), those with increased sputum eosinophils had significantly lower PC_{20} values than those without increased sputum eosinophils. PC_{20} = provocative concentrations of acetylcholine that produced a 20% fall in FEV_1.*

function between these two disorders. In the analysis of airway inflammation, our data indicated that only subtle inflammation may be present in the central and peripheral airways in both patients with CVA and patients with intermittent mild SBA. Moreover, Kim *et al.*[20] reported that sputum eosinophilia in CVA was associated with the subsequent development of classic asthma. Consistent with this report, our current study demonstrated that the proportion of patients in whom eosinophils accounted for at least 3% of the total nonsquamous cells in induced central or peripheral sputum was significantly higher in patients with intermittent mild SBA than in patients with CVA.

Some researchers stated that CVA is a precursor to asthma,[17] whereas other researchers indicated that CVA and SBA populations differ in many clinical characteristics.[21] Matsumoto *et al.*[22] reported that early treatment with inhaled corticosteroids may prevent the progression of CVA to SBA and that such treatment may also inhibit the development of wheezing. If CVA is simply a very early stage of asthma, then the progression of inflammation and the development of BHR during asthma development could be an interesting subject for future studies.

Is eosinophilic airway inflammation the sole determinant of BHR positivity? Surprisingly, airway eosinophilia was related to BHR only in patients with SBA, not in patients with CVA (Fig. 2), which indicates two possibilities. First, factors other than eosinophilic airway inflammation may induce a positive BHR. Unfortunately, we cannot provide examples of this pathogenesis at present but suggest repeated contraction as a candidate. Alternatively, eosinophilic inflammation may be the only factor that drives BHR, but this study was unable to detect eosinophilic airway inflammation due to the methodology used (*e.g.*, the samples were only sputum, not bronchial biopsy specimens). In this study, some limitations exist. First, the number of the subjects was small due to the difficulty in recruiting patients without exacerbations who still visit the hospital. Second, we used acetylcholine to evaluate BHR, whereas other studies used mannitol or another irritant. We believe that these limitations did not affect our main result because a significant relationship between eosinophilic inflammation and BHR was found in the mild SBA group, as expected.

CONCLUSION

A smaller number of patients with CVA have eosinophilic airway inflammation; however, BHR in these patients was similar to that of patients with mild intermittent SBA. Eosinophilic inflammation was not associated with BHR in patients with CVA. Evidently, CVA may be a very early precursor to SBA, which supports the concept that early treatment with inhaled corticosteroids; however any other factor concerned with little or no eosinophilic airway inflammation appeared to involve positive BHR development. Although most factors of the inflammation or respiratory function are overlapped between the CVA and intermittent mild SBA, we believe that understanding the mechanism of this unique pattern of pathogenesis will lead to significant insights into bronchial asthma treatment strategies in future studies.

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