

CONTROL

Association of Airway Inflammation with Asthma Control Level Evaluated by the Asthma Control Test

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Background. The association between airway inflammation and asthma control level is not clear at present. **Objective.** This study aimed to explore the association by using induced sputum and asthma control status as determined by the Asthma Control Test (ACT). We also evaluated the association between the scores for each ACT question item and eosinophilic or neutrophilic airway inflammation. **Methods.** The ACT and sputum induction were performed at the same time. Associations between total scores or scores for each question item and sputum eosinophil or neutrophil counts were examined. The study was approved by an Institutional Review Board. **Results.** Of the 101 patients with chronic asthma enrolled, data from 98 (controlled $n = 66$, uncontrolled $n = 32$) were analyzed [60.0 years (43.0–68.0) M:F = 34:64]. Current control status determined by the ACT was not significantly associated with eosinophilic or neutrophilic inflammation. Among the ACT items, only nocturnal symptoms were associated with sputum eosinophils: patients with a positive answer to the question had significantly higher eosinophil counts than patients with a negative answer [5.4 (2.2–17.6) versus 2.1 (0.7–7.3), respectively, $p = 0.08$]. Furthermore, significant correlation was found between eosinophil counts and the scores for nocturnal symptoms ($r_s = -0.218$ $p = 0.031$). On the other hand, patients with rescue use of a short-acting β_2 -agonist (SABA) had significantly higher sputum neutrophil counts than non-SABA users [73.4 (52.8–83.4) versus 61.0 (36.3–74.8), respectively, $p = 0.031$]. The other ACT items were not significantly associated with sputum neutrophils. The neutrophil count correlated significantly with the frequency of rescue SABA use ($r_s = -0.218$ $p = 0.031$). **Conclusions.** Asthma control level evaluated by the ACT was not associated with airway eosinophilic or neutrophilic inflammation. However, the frequency of nocturnal symptoms was associated with sputum eosinophilia, and the frequency of rescue SABA use was associated with sputum neutrophilia.

Keywords eosinophil, induced sputum, neutrophil, nocturnal symptoms, rescue use

INTRODUCTION

The primary goal of asthma management is to maintain clinical control by using a minimum of medications. To achieve this, recent guidelines recommend a treatment strategy based on control status (1). Several composite measures have been developed to assess the control status of asthma. The Asthma Control Test (ACT) is one of the most useful and simple composite questionnaires (2). This patient-completed instrument consists of five question items evaluating the frequency of rescue use of short-acting β_2 agonists (SABA), patient rating of asthma control, and three representative asthma symptoms, namely, shortness of breath, night-time waking, and interference with daily activities over the past month. The ACT has been proven to be valid and responsive to changes in asthma control (2–6).

The association between airway inflammation and current control status is not established at present. Bronchial asthma is typically recognized as an eosinophilic airway inflammatory disease. Corticosteroid reduction studies have consistently shown to be associated with both a loss

of clinical control and raised sputum eosinophils (7–10). The management strategies aimed at normalizing sputum eosinophil counts are associated with better control status and up to 60% reduction in severe asthma exacerbations (11–13). Thus, sputum eosinophil count is recognized as one of the most useful biomarkers to predict future risk of asthma exacerbation. However, eosinophils are not the sole cause of airway inflammation. Asthma consists of various inflammatory phenotypes (14), and neutrophils are also considered key players causing airway inflammation, especially in more severe asthma (15–18).

This study aimed to determine whether airway inflammation is associated with current control status determined by the ACT, and to identify the ACT question items associated with eosinophilic or neutrophilic airway inflammation. For this purpose, evaluation via the ACT was performed at the same time as sputum induction, since both patient symptoms and airway inflammation may vary over relatively short time intervals in response to allergens or infectious agents, or in response to treatment (19, 20).

METHODS

Subjects

Subjects comprised patients with chronic asthma diagnosed by specialists using the Global Initiative

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for Asthma guidelines (1). A total of 101 consecutive patients who provided informed consent to participate in this study were enrolled. We excluded patients <18 years old, women who were pregnant, and patients whose diagnosis was complicated by chronic obstructive lung disease (COPD). Patients with acute exacerbation were also excluded. All subjects were treated in the outpatient clinic at the Hiroshima University Hospital. Most patients had been followed at this clinic, but some patients were recently referred from primary care clinics. Sputum induction and the ACT were performed at the same time as the clinical research. Three patients failed to respond to sputum induction; therefore, data from 98 patients were analyzed (Table 1). Spirometry was performed in a subgroup of patients ($n = 64$) according to the guidelines of the American Thoracic Society (21). This study was approved by the Ethics Committee of Hiroshima University (No. 610).

ACT Questionnaire

The ACT questionnaire consists of five question items based on clinical symptoms and self-evaluation (for full sheet, see reference #4): restriction at work (item #1), shortness of breath (item #2), nocturnal symptoms (item #3), rescue SABA use (item #4), and self-evaluation of control (item #5). Each item is scored using a scale of 1–5 and then summed (total score, 5–25). “Total control” is defined as a full ACT total score of 25, “partial control” as an ACT total score of 24–20, and “uncontrolled” is defined as a total score of <20 points. The ACT was completed by patients on the same visit that sputum induction was performed. We obtained permission for the use of the ACT, including the analysis of correlations with individual sub-scores, from QualityMetric Incorporated via GlaxoSmithKline (GlaxoSmithKline plc, Brentford, Middlesex, UK).

Sputum Induction

Induced sputum was performed using a previously described method (22). Subjects were asked to rinse their mouths before coughing, discard saliva, and cough sputum into a clean container. The hypertonic saline solution was nebulized for 5 min with an ultrasonic nebulizer (Alfreda Pharma Corporation, Osaka, Japan) with 3 mL/min output. The concentration of NaCl solution was increased at 10 min intervals to 3, 4, and 5%. Sputum samples were collected every 10 min, and each sample was processed separately. Sputum samples were diluted with an equal volume of 0.1% dithiothreitol (Sigma-Aldrich, St. Louis, MO, USA) in phosphate-buffered saline solution. The percentage of inflammatory cells was calculated from the percentage of inflammatory cells, the total cell count, and the volume of each sample.

Using a hemocytometer, total cell counts were determined and the cytopsin preparation was stained with Diff Quick (International Medical Equipment Inc, San Marcos, CA, USA). The differential cell counts of each sputum sample were obtained by counting >300 non-squamous cells. Cell counting was performed by an

experienced observer blind to the clinical characteristics of the subjects.

Statistical Analysis

Statistical analyses were performed using SPSS Statistics version 17 (International Business Machines Corp. Armonk, NY, USA). Results are presented as median (interquartile range) values, unless otherwise indicated. Associations between the data were determined using the Spearman rank correlation. A comparison of airway inflammation markers among the totally controlled, partially controlled, and uncontrolled patients was performed using the Kruskal–Wallis test. A comparison of airway inflammation markers in the presence and absence of symptoms was performed using the Mann–Whitney test. In all statistical analysis, $p < 0.05$ was considered significant.

RESULTS

Patient Characteristics

The characteristics of the 98 subjects are shown in Table 1. There were 66 (67.3%) controlled and 32 (32.7%) uncontrolled patients with asthma. Ninety patients (91.8%) were treated with inhaled corticosteroids (ICS) equivalent to 100–1600 μ g/day of budesonide. The most frequent positive answer (score 1–4) among the ACT question items was “self-evaluation” (item #5) followed by “shortness of breath” (item #2) in both partially controlled (total ACT scores: 20–24) and uncontrolled (total ACT scores: <20) patients.

Association between Control Status, Spirometry, and Airway Inflammation

The frequency of patients with eosinophilic (>2%) or neutrophilic (>61%) sputum was not significantly different between the totally controlled, partially controlled, and uncontrolled patient groups (Table 1). There were no significant differences in eosinophil counts (Figure 1A) or neutrophil counts (Figure 1B) between the groups, or between controlled and uncontrolled asthma [eosinophils: 3.2 (0.9–9.8) vs. 4.9 (1.3–4.6), respectively, $p = 0.34$; neutrophils: 63.3 (36.9–78.8) vs. 68.6 (53.7–83.3), respectively, $p = 0.20$]. Furthermore, there was no correlation found between ACT total scores and eosinophil or neutrophil counts (Table 2).

In a subgroup analysis, there was a significant correlation between the percent predicted FEV₁ value and ACT score ($r_s = 0.36$, $p = 0.004$). In addition, the percent predicted FEV₁ value significantly correlated with sputum neutrophil ($r_s = -0.25$, $p = 0.045$), but not eosinophil counts ($r_s = -0.13$, $p = 0.32$).

Association of Each ACT Question Item with Sputum Inflammatory Cells

Among the ACT question items, only item #3, “nocturnal symptoms,” was associated with sputum eosinophils.

TABLE 1.—Background characteristics of the 98 subjects.

	Controlled			<i>P</i>
	Total	Partial	Uncontrolled	
Patient No. (%)	66 (67.3)		32 (32.7)	
Sex (M:F)	4:17	18:27	12:20	0.23 ^a
Age ^b	60.0 (45.3–68.3)	60.0 (44.8–65.0)	58.0 (38.0–70.0)	0.95 ^c
Severity ^d	(5, 13, 3, 0)	(1, 12, 19, 13)	(0, 2, 6, 24)	<0.001 ^a
Smoking history (C:E:N) ^e	4:2:15	8:13:24	9:6:17	0.34 ^a
Treatment with ICS ^f	17 (81.0)	43 (95.6)	30 (93.8)	0.12 ^a
ICS dose (μ g/day) ^{b,f}	800 (200–800)	400 (400–800)	800 (800–800)	0.007 ^c
Treatment with LABA ^g	2 (9.5)	11 (24.4)	21 (65.6)	<0.001 ^a
Treatment with LTRA ^h	6 (28.6)	14 (31.1)	14 (43.8)	0.42 ^a
Treatment with theophylline	7 (33.3)	13 (28.9)	11 (34.4)	0.86 ^a
Each item of the ACT				
Restriction at work: + (%) ^g	0 (0)	12 (26.7)	27 (84.3)	<0.001 ^a
Shortness of breath: + (%)	0 (0)	24 (53.3)	30 (93.8)	<0.001 ^a
Nocturnal symptoms: + (%)	0 (0)	12 (26.7)	26 (81.3)	<0.001 ^a
Rescue use of SABA: + (%)	0 (0)	19 (42.2)	27 (84.4)	<0.001 ^a
Self-evaluation: NC (%) ^h	0 (0)	39 (86.7)	32 (100)	<0.001 ^a
Sputum inflammatory cells				
Eosinophils >2%	12 (57.1)	30 (66.7)	22 (68.8)	0.44 ^a
Neutrophils >61%	11 (52.4)	24 (53.3)	19 (59.4)	0.26 ^a
Lymphocytes >2%	9 (42.8)	18 (40.0)	12 (37.5)	0.93 ^a
Eosinophils (%) ^b	2.1 (0.9–6.0)	3.8 (0.9–15.8)	4.9 (1.3–14.6)	0.44 ^c
Neutrophils (%) ^b	63.0 (37.9–71.2)	67.6 (33.8–82.4)	68.6 (53.7–83.3)	0.26 ^c
Lymphocytes (%) ^b	1.8 (0.9–3.5)	1.7 (0.6–3.0)	1.4 (0.7–2.5)	0.51 ^c

Note: ICS, inhaled corticosteroids; LABA, long-acting β_2 agonist; LTRA, leukotriene receptor antagonist; SABA: short-acting β_2 agonist.

^aChi-square test performed to compare groups.

^bResults are represented as median (interquartile) values.

^cKruskal–Wallis test performed to compare groups.

^dSeverity is presented by number of patient with intermittent, mild persistent, moderate persistent and severe persistent, respectively.

^eC: Current smoker, E: Ex-smoker, N: Never smoker.

^fEquivalent dose of budesonide.

^g+: score 1–4.

^hNC: Not completely controlled (score 1–4).

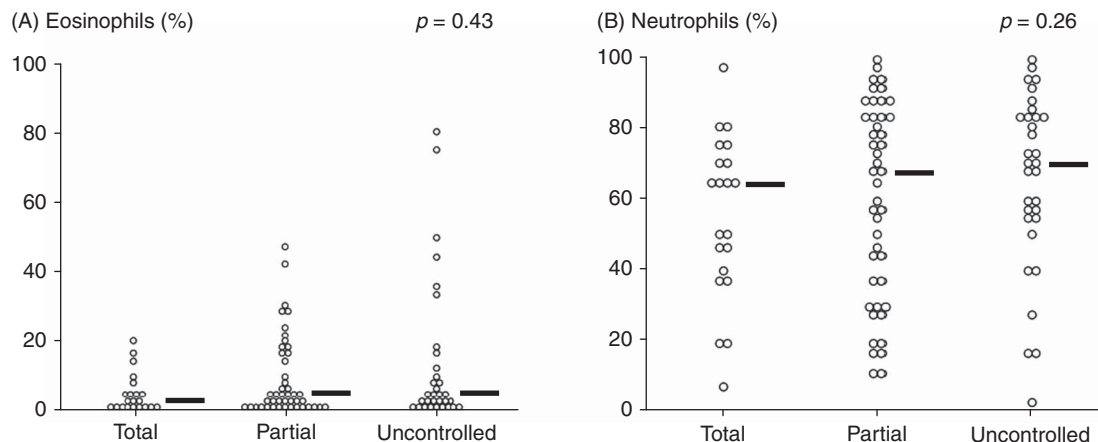


FIGURE 1.—Association between control status and eosinophils (A) or neutrophils (B) in induced sputum. There were no significant differences between patients with totally controlled, partially controlled, and uncontrolled asthma. Bar denotes median value.

Patients with a positive answer to the question (scores 1–4) had significantly higher eosinophils than patients with a negative answer (score 5) (Figure 2; 5.4 (2.2–17.6) vs. 2.1 (0.7–7.3), respectively, $p = 0.008$). Item #3 was not significantly associated with age, gender, smoking habit, or ICS use. Sputum eosinophil count was not significantly associated with age, gender, smoking, or ICS use. Furthermore, eosinophil counts correlated significantly

with the scores for item #3 only (Table 2, Figure 4). The correlation between eosinophil counts and scores for nocturnal symptoms were more prominent in partially controlled patients (partially controlled: $r_s = -0.34$, $p = 0.020$, $n = 45$; uncontrolled: $r_s = -0.03$, $p = 0.87$, $n = 32$). In partially controlled patients, 12 out of 45 had complicated nocturnal symptoms (Table 1), and more than 80% of these patients had eosinophilic sputum.

TABLE 2.—Correlations between airway inflammation and scores for each item on the Asthma Control Test (ACT).

	Eosinophil counts ^a		Neutrophil counts ^a	
	<i>rs</i>	<i>P</i>	<i>rs</i>	<i>P</i>
Total ACT score	−0.16	0.12	−0.02	0.26
Scores for restriction at work	−0.07	0.50	−0.02	0.82
Scores for shortness of breath	−0.08	0.43	−0.119	0.05
Scores for nocturnal symptoms	−0.218	0.031	−0.03	0.72
Scores for SABA rescue use	0.01	0.92	−0.218	0.031
Scores for self-evaluation	−0.13	0.21	−0.09	0.40

Note: SABA, short-acting β_2 agonist.

^aAssociations between the data were determined using the Spearman rank correlation.

On the other hand, patients with a positive answer (scores 1–4) to item #4 “rescue SABA use” had significantly higher sputum neutrophil counts than patients with a negative answer (score 5) (Figure 3; 73.4 (52.8–83.4) vs. 61.0 (36.3–74.8), respectively, $p = 0.031$). The other ACT items were not significantly associated with sputum neutrophils (Figure 3). A positive answer to item #4 (rescue use of SABA) was also significantly associated with gender (Chi-square test, $p = 0.027$), but not age, smoking, or ICS use. However, gender was not significantly associated with sputum neutrophils ($p = 0.099$). The neutrophil count correlated significantly with the scores for item #4 only (Table 2, Figure 4).

This correlation was not significant among the partially controlled or uncontrolled groups, presumably due to low statistical power (partially controlled: $rs = 0.23$, $p = 0.12$, $n = 45$; uncontrolled: $rs = 0.02$, $p = 0.93$, $n = 32$).

DISCUSSION

This study demonstrated that current asthma control status determined by the ACT was not associated with airway inflammation estimated by sputum inflammatory cells such as eosinophils or neutrophils. However, some items of the ACT questionnaire were associated with eosinophilic or neutrophilic airway inflammation. Patients with nocturnal symptoms had significantly higher sputum eosinophil counts than patients without these symptoms, and the scores relating to the symptoms correlated significantly with the number of eosinophils. On the other hand, patients with rescue SABA use had significantly higher neutrophil counts than those without SABA use, and the frequency of rescue use correlated significantly with the number of sputum neutrophils.

Control status determined by the ACT was not associated with eosinophilic airway inflammation. The number of patients with eosinophilic sputum and the number of eosinophils in sputum were not different among totally controlled, partially controlled, and uncontrolled asthma. Since the presence of eosinophilic sputum suggests high

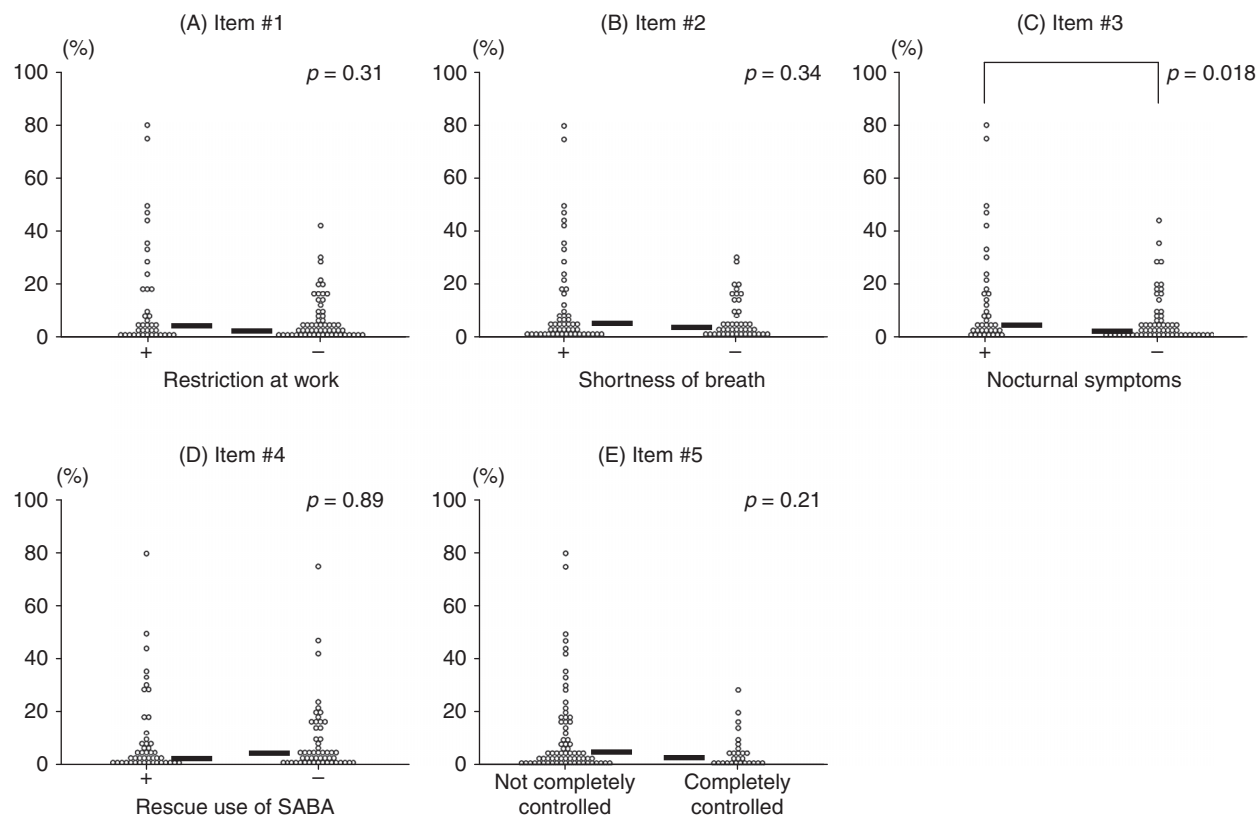


FIGURE 2.—Association of each item of the ACT questionnaire (A–E) with sputum eosinophils. Presence (+, not completely controlled) represented scores 1–4, and absence (–, completely controlled) represented score 5. Sputum eosinophils in patients with a positive answer (score 1–4) to item #3 “nocturnal symptoms” (C), but not the other items (A, B, D, E), were significantly higher than those in patients with a negative answer (score 5). A comparison was performed using the Mann–Whitney test. Bar denotes median value.

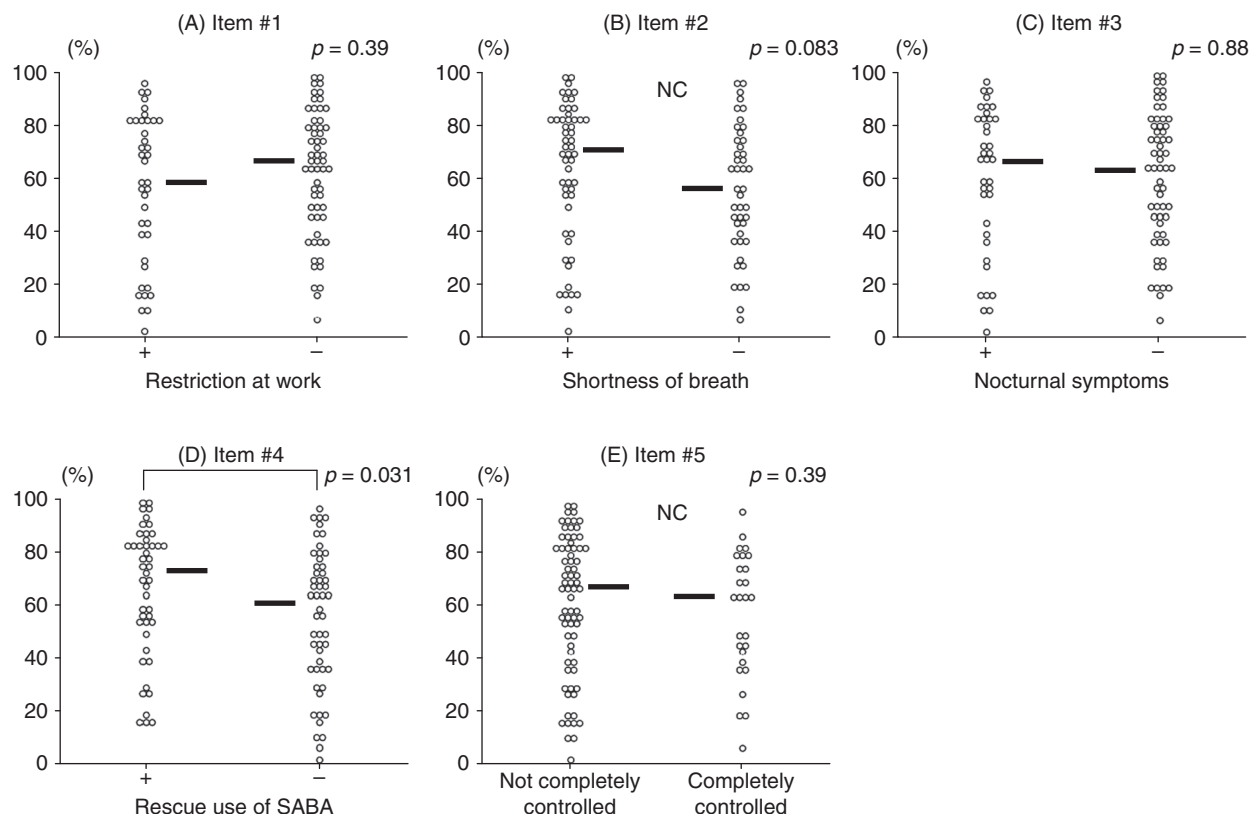


FIGURE 3.—Association of each item of the ACT questionnaire (A–E) with sputum neutrophils. Presence (+, not completely controlled) represented scores 1–4 and absence (–, completely controlled) represented score 5. Sputum neutrophils in patients with a positive answer (score 1–4) to item #4 “rescue SABA use” (D) were significantly higher than those in patients with a negative answer (score 5). There tended to be differences in item #2 “shortness of breath” (B), but these were not significant. The other items (A, C, E) showed no differences. A comparison was performed using the Mann–Whitney test. Bar denotes median value.

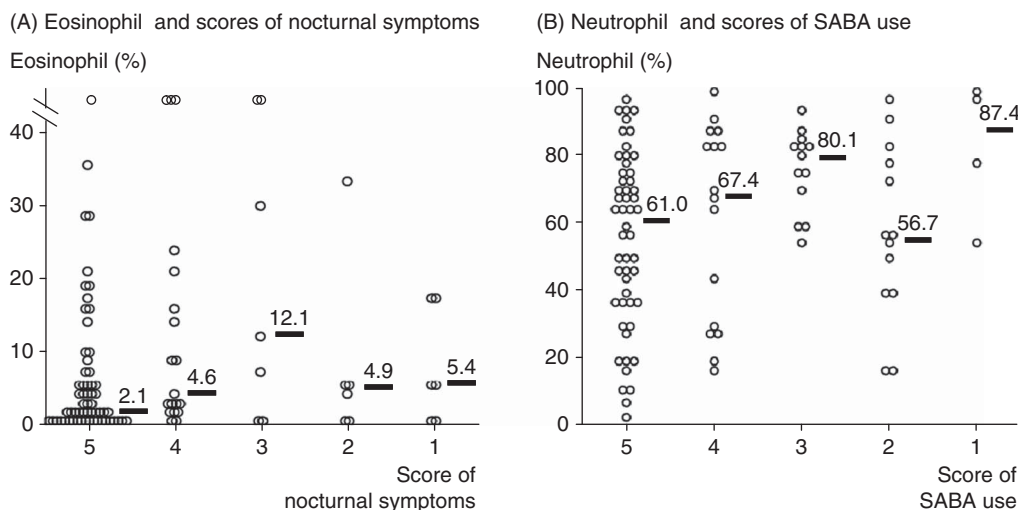


FIGURE 4.—Correlations between eosinophil counts and nocturnal symptoms scores (A), and between neutrophil counts and rescue SABA use scores (B). Weak but significant correlations were observed. Bar denotes median value.

risk of exacerbation (11–13), this finding supports the previous observation that exacerbations could also occur in patients with totally or well-controlled asthma (20, 23), although they are more common in patients with poorly controlled asthma (24). This study underscores the fact that eosinophilic airway inflammation is not the only factor in determining the control status of asthma defined

by the ACT. Thirty-two percent of uncontrolled patients showed non-eosinophilic sputum. Despite treatment with a high dose of ICS, their symptoms remained uncontrolled, and they were classified as non-eosinophilic asthma phenotypes (25). Such patients with “discordant symptoms” have also been described as “early symptom predominant” and “obese non-eosinophilic” phenotypes

by cluster analysis (26). It should be mentioned, however, that neither neutrophilic sputum nor both eosinophilic and neutrophilic sputum were associated with control status in this study population.

An association was found between eosinophilic airway inflammation and frequency of nocturnal symptoms. Most of our study population was under treatment with ICS, which suggests that eosinophilic inflammation in large airways could be controlled, whereas eosinophils may remain in small airways. This suggests that eosinophilic sputum in our population could derive from distal airways, although this notion was not proven in this study. It is known that peripheral dominant eosinophilic airway inflammation is one of the characteristics of nocturnal asthma (19, 27). This may be the reason for the association. It should be noted that nocturnal symptoms are useful markers in clinical settings, since more than 80% of patients with these symptoms had eosinophilic sputum in partially controlled asthma. In this population, the frequency of nocturnal symptoms could provide a clue to estimate eosinophilic airway inflammation. In such cases, small particle ICS would be beneficial (27).

This study also demonstrated, for the first time, that sputum neutrophilia correlated significantly with rescue SABA use. Besides rescue inhaler, shortness of breath tended to correlate with neutrophil counts. Accumulating evidence suggests that airway neutrophilia is predominant in more severe asthma, and there is an association between airway neutrophils and chronic airway narrowing (15–17, 28). We did not evaluate lung function with spirometry in the uniform way, which is one of several limitations in this study. However, the relationship between sputum neutrophilia and rescue SABA use could be interpreted as a result of symptomatic airflow limitation in more severe asthma.

This study included patients with possible coexistence of COPD, which is another limitation of the study. Smokers are usually excluded in asthma studies to prevent potential distortion of the results due to COPD. In this study, about half of the subjects were current or ex-smokers. Smoking could reduce responsiveness to ICS and increase the number of mast cells and neutrophils in the bronchial walls and sputum (29, 30). However, smokers are obviously frequent in real clinical settings (31). In addition, a strict discrimination between asthma and COPD is difficult because overlap syndrome has been described in more severe asthma, and is especially predominant in older asthma patients (32, 33).

CONCLUSIONS/KEY FINDINGS

Current asthma control was not associated with airway inflammation estimated by inflammatory cells in sputum. However, there were ACT items that correlated with eosinophilic or neutrophilic airway inflammation. The degree of nocturnal symptoms was associated with eosinophilic airway inflammation, whereas the frequency of rescue SABA use was associated with neutrophilic

airway inflammation. In clinical settings, especially in partially controlled asthma, nocturnal symptoms would be suggestive of remaining eosinophilic inflammation.

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DECLARATION OF INTEREST

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