



Contents lists available at ScienceDirect



Pathophysiological characteristics of asthma in the elderly: a comprehensive study

Hideki Inoue, MD^{*}; Akio Niimi, MD, PhD[†]; Tomoshi Takeda, MD, PhD^{*}; Hisako Matsumoto, MD, PhD^{*}; Isao Ito, MD, PhD^{*}; Hirofumi Matsuoka, MD, PhD^{*}; Makiko Jinnai, MD, PhD^{*}; Kojiro Otsuka, MD, PhD^{*}; Tsuyoshi Oguma, MD^{*}; Hitoshi Nakaji, MD, PhD^{*}; Tomoko Tajiri, MD^{*}; Toshiyuki Iwata, MD^{*}; Tadao Nagasaki, MD^{*}; Yoshihiro Kanemitsu, MD^{*}; Kazuo Chin, MD, PhD[‡]; and Michiaki Mishima, MD, PhD^{*}

^{*} Department of Respiratory Medicine, Graduate School of Medicine, Kyoto University, Kyoto, Japan

[†] Department of Respiratory Medicine, Allergy and Clinical Immunology, Graduate School of Medicine, Nagoya City University, Nagoya, Aichi, Japan

[‡] Department of Respiratory Care and Sleep Control Medicine, Graduate School of Medicine, Kyoto University, Kyoto, Japan

ARTICLE INFO

Article history:

Received for publication March 6, 2014.

Received in revised form August 4, 2014.

Accepted for publication August 5, 2014.

ABSTRACT

Background: Comprehensive studies of the pathophysiologic characteristics of elderly asthma, including predominant site of disease, airway inflammation profiles, and airway hyperresponsiveness, are scarce despite their clinical importance.

Objective: To clarify the pathophysiologic characteristics of elderly patients with asthma.

Methods: Patients older than 65 years (elderly; n = 45) vs those no older than 65 years (nonelderly; n = 67) were retrospectively analyzed by spirometry, computed tomographic indices of large airway wall thickness and small airway involvement (air trapping), impulse oscillation measurements, exhaled nitric oxide levels, blood and induced sputum cell differentials, methacholine airway responsiveness, and total and specific serum IgE levels.

Results: Elderly patients with asthma had significantly lower values for forced expiration volume in 1 second, mid-forced expiratory flow (percentage predicted), and ratio of forced expiration volume in 1 second to forced vital capacity than nonelderly patients with asthma (median 81.2% vs 88.8%, $P = .02$; 50.9% vs 78.6%, $P = .03$; 0.72 vs 0.78, $P = .001$, respectively). In computed tomographic measurements, elderly patients with asthma had significantly greater airway wall thickening and air trapping than nonelderly patients. Impulse oscillation measurements indicated that elderly patients with asthma showed significantly greater resistance at 5 Hz (used as an index of total airway resistance), greater decrease in resistance from 5 to 20 Hz, a higher ratio of decrease in resistance from 5 to 20 Hz to resistance at 5 Hz, higher integrated area between 5 Hz and frequency of resonance, greater frequency of resonance, and lower reactance at a frequency of 5 Hz (potential markers of small airway disease) than nonelderly patients. There were no significant differences in blood or sputum cell differentials, exhaled nitric oxide, or methacholine airway responsiveness between the 2 groups. Total serum IgE levels and positive rates of specific IgE antibodies against several allergens were significantly lower in elderly than in nonelderly patients with asthma.

Conclusion: Based on spirometric, computed tomographic, and impulse oscillation analyses, elderly patients with asthma have greater involvement of small and large airways than nonelderly patients with asthma.

© 2014 American College of Allergy, Asthma & Immunology. Published by Elsevier Inc. All rights reserved.

Introduction

The number of elderly people, defined by the World Health Organization as those with a chronologic age of 65 years,¹ is expected

Reprints: Akio Niimi, MD, PhD, Department of Respiratory Medicine, Allergy and Clinical Immunology, Graduate School of Medicine, Nagoya City University, 1 Kawasumi, Mizuho-cho, Mizuho-ku, Nagoya, Aichi 467-8602, Japan; E-mail: a.niimi@med.nagoya-cu.ac.jp.

Disclosure: Authors have no conflicts of interest to disclose.

<http://dx.doi.org/10.1016/j.anai.2014.08.002>

1081-1206/© 2014 American College of Allergy, Asthma & Immunology. Published by Elsevier Inc. All rights reserved.

to increase from 546 million in 2011 to 1.6 billion in 2050.² The incidence of elderly asthma also can be expected to increase owing to the aging of society. The prevalence of asthma in the elderly is 6% to 10% in developed countries,³ and the proportions of elderly patients among asthma deaths are approximately two thirds in Australia⁴ and more than 85% in Japan.⁵ Moreover, there are specific issues associated with the management of elderly asthma, such as the several differential diagnoses (eg, chronic obstructive pulmonary disease), multiple comorbidities, poor inhaler device use, poor

adherence to therapy, increased side effects, and decreased responsiveness to medication.³

Different clinical measurements have been used to evaluate the pathophysiology of asthma. Computed tomography (CT) has been used to assess large airway wall remodeling^{6,7} and small airway involvement (ie, air trapping)^{8,9} in patients with asthma. Impulse oscillation (IOS) is a noninvasive method of measuring respiratory resistance (R) and reactance (X), which could differentiate large from small airway disease.^{10–13} Further, induced sputum cell differentials¹⁴ and exhaled nitric oxide (FeNO)¹⁵ levels have been used to assess airway inflammation profiles.

The process of aging is normally associated with various age-related structural changes in the respiratory system. With advancing age, elastic fibers in the lung parenchyma decrease. These changes can alter the elastic properties of the airways, resulting in a loss of elastic recoil.¹⁶ Thus, in elderly patients, small airways can tend to collapse during expiration, possibly leading to air trapping and an increase in residual volume (RV). Elderly patients with asthma are assumed to have more prominent small airway disease, although evidence for this is lacking. Moreover, aging may affect immunologic and inflammatory profiles in patients with asthma. Airway neutrophilia may be more predominant in elderly patients with asthma than in the nonelderly,^{17,18} although conflicting evidence exists showing that sputum cellular profiles are similar in young and elderly patients with asthma.¹⁹

Because comprehensive studies on elderly asthma, addressing its physiologic, radiologic, and immunologic features, are scarce, these pathophysiologic characteristics of elderly asthma were investigated using spirometry, CT, IOS, induced sputum, FeNO, and IgE measurements and compared with results in nonelderly patients with asthma.

Methods

Subjects

Study subjects were retrospectively selected from 136 patients with stable asthma who underwent chest multidetector raw CT for research purposes^{20,21} at the outpatient clinic at Kyoto University Hospital from February 2006 through October 2009. The inclusion criteria of this study were as follows: (1) diagnosis of asthma according to American Thoracic Society criteria²²; (2) clinically stable disease that had been fully controlled for at least 1 month²³ at the time of examinations; (3) never smoker or ex-smoker who had smoked for less than 5 pack-years but had stopped smoking more than 12 months before study entry; (4) treatment with inhaled corticosteroids (ICSs) for at least 3 months; and (5) absence of other respiratory diseases, including evidence of emphysema, on CT images. According to the inclusion criteria, 112 patients were eligible for this study (Table 1). Elderly patients were defined as those older than 65 years, based on the World Health Organization statement.¹ In this study, the subject's age was determined at the time of CT examination. The following clinical examinations were performed in each subject during follow-up: spirometry (n = 112, 100%), IOS (n = 111, 99.1%), induced sputum (n = 76, 67.9%), airway responsiveness test (n = 79, 70.5%), FeNO (n = 110, 98.2%), peripheral blood cell differentials (n = 112, 100%), and serum total IgE and allergen specific IgE (n = 112, 100%). However, to maintain the integrity of the clinical data to be analyzed in this retrospective study, only the data obtained within 4 weeks of the date of CT measurement were used. As a result, the number and percentage of subjects for whom these data were available were decreased in each group as specified in Tables 2 to 6. The frequency of disease exacerbation, classified as that requiring systemic corticosteroids or hospitalization,²⁴ was counted for the 12 months before and after the CT examination. This study was approved by the ethics committee of Kyoto University (approval number E-189 and C-147).

Table 1

Subject characteristics^a

	Elderly patients with asthma (>65 y)	Nonelderly patients with asthma (≤65 y)	P value
Patients	45	67	
Men/women	11/34	21/46	NS
Age (y)	73.1 ± 5.3	48.6 ± 12.9	<.001
Disease duration (y)	12.7 ± 16.2	8.0 ± 10.5	NS
Exacerbations per year	0 (0–2.5)	0 (0–3)	NS
Severity (step 1/2/3/4) ^b	0/12/16/17	0/23/31/13	NS
Smoking, ex/never	1/44	12/55	.01
Pack-years	0.11 ± 0.75	0.34 ± 1.1	NS
Dose of ICS (μg/d; equivalent to CFC-BDP)	800 (400–3,200)	800 (200–2,400)	NS
BMI (kg/m ²)	23.0 ± 3.5	23.5 ± 4.1	NS
Allergic rhinitis, present	11 (24)	36 (54)	.002
Atopic dermatitis, present	1 (2)	6 (9)	NS

Abbreviations: BMI, body mass index; CFC-BDP, chlorofluorocarbon-11/12-beclomethasone dipropionate; ICS, inhaled corticosteroid; NS, not significant.

^aData are expressed as number (percentage) or median (range), except for age, disease duration, pack-years, and BMI, which are presented as mean ± SD.

^bThe clinical severity of asthma was defined by patient symptoms and lung function on current therapy as step 1 (intermittent), step 2 (mild persistent), step 3 (moderate persistent), or step 4 (severe persistent), according to the 2005 criteria of the Global Initiative for Asthma.

Written informed consent was obtained from all subjects for participation in this study.

Outcome Measurements

Pulmonary function tests

Pre-bronchodilator values of forced vital capacity (FVC), force expiration volume in 1 second (FEV₁), and mid-forced expiratory flow (FEF_{25–75}) were examined using a ChestGraph HI-701 spirometer (Chest MI, Inc, Tokyo, Japan). The ratio of RV to total lung capacity (TLC), which is considered to reflect air trapping, also was measured using a CHESTAC-8800 (Chest MI, Inc). To exclude the effects of age and physique on pulmonary function tests, the predicted values of FVC and FEV₁, which were quoted from the publication of the Japanese Respiratory Society,²⁵ were used for comparisons between elderly and nonelderly patients with asthma. The predicted values of FEF_{25–75} and RV/TLC were calculated from other published equations.²⁶

CT measurements

Each subject underwent multidetector raw CT (Aquilion 64; Toshiba Medical Systems, Tokyo, Japan) as described previously.^{7,27} To evaluate large airway wall dimensions, 3 parameters were analyzed: airway wall area (WA) corrected as a percentage of total wall area (WA%), WA normalized for body surface area (WA/BSA; square millimeters per square meter), and normalized absolute

Table 2

Comparison of pulmonary function tests between elderly and nonelderly patients with asthma^a

	Elderly patients with asthma (>65 y)	Nonelderly patients with asthma (≤65 y)	P value
Spirometry	41 (91)	63 (94)	
FVC (%pred)	91.0 (46.4–135)	97.6 (58.7–141)	NS
FEV ₁ (%pred)	81.2 (40.8–133)	88.8 (34.7–112)	.02
FEV ₁ /FVC	0.718 (0.440–0.896)	0.784 (0.409–0.934)	.001
FEF _{25–75} (%pred)	50.9 (14.2–148)	78.6 (9.6–152)	.03
Lung volume measurement	37 (82)	57 (85)	
RV/TLC (%pred)	110 (81.3–187)	109 (67.1–258)	NS

Abbreviations: FEF_{25–75}, mid-forced expiratory flow; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; NS, not significant; %pred, percentage of predicted value; RV/TLC, ratio of residual volume to total lung capacity.

^aData are presented as number (percentage) or median (range).

Table 3

Comparison of computed tomographic measurements between elderly and nonelderly patients with asthma^a

	Elderly patients with asthma (>65 y)	Nonelderly patients with asthma (≤65 y)	P value
Central airway wall thickness	45 (100)	67 (100)	
WA%	61.7 (52.9–70.9)	57.6 (49.0–70.3)	<.001
WA/BSA (mm ² /m ²)	16.1 (10.3–22.4)	14.7 (9.2–19.8)	.01
T/BSA (mm/m)	1.10 (0.90–1.40)	1.01 (0.75–1.21)	<.001
Small airway involvement	41 (91)	60 (90)	
Full inspiration			
LAA%	16.5 (3.8–28.8)	16.7 (5.1–27.6)	NS
MLD (HU)	–853 (–881 to –722)	–853 (–901 to –777)	NS
Full expiration			
LAA%	7.0 (1.5–21.0)	5.1 (0.4–17.9)	.002
MLD (HU)	–771 (–861 to –658)	–748 (–847 to –607)	.003
E/I ratio			
LAA% E/I	0.46 (0.18–0.91)	0.33 (0.03–0.67)	<.001
MLD E/I	0.91 (0.84–0.99)	0.88 (0.72–0.95)	<.001

Abbreviations: BSA, body surface area; E, expiration; I, inspiration; LAA%, percentage of low-attenuation area; MLD, mean lung density; NS, not significant; T, airway wall thickness; WA, airway wall area; WA%, airway wall area corrected as a percentage of total wall area.

^aData are presented as number (percentage) or median (range).

wall thickness (T/BSA; millimeters per meter)⁷ at the right apical segmental bronchus and right posterior basal segmental bronchus, from which tangential views of the bronchus were available. At full inspiration, consecutive slices of the 2 bronchi were automatically measured and averaged. To assess air trapping, the percentage of low-attenuation areas (LAA%; <–960 HU) and mean lung density (MLD) at full inspiration and full expiration were analyzed.⁹ The ratios of full expiration to full inspiration (E/I ratio) for LAA% and MLD also were evaluated. A higher E/I ratio indicates more prominent small airway involvement.⁹ Spirometrically gated CT,²⁸ which analyzes full-inspiratory and full-expiratory lung fields by monitoring a patient's spirometric status, was performed in 47 subjects. The other subjects were carefully instructed by technicians to breathe in deeply for a full inspiration and to breathe out completely for a full expiration.⁹

IOS measurements

Respiratory impedance was measured using a Jaeger Master-Screen IOS (Jaeger/Toennies, Hochberg, Germany) according to standard recommendations.²⁹ Rectangular mechanical pulses, including the entire frequency spectrum, were generated and applied to the subject's airway through a mouthpiece with a cheek support. Impedance measurements included resistances at frequencies from 5 to 35 Hz (R5 to R35), reactance at frequencies from

Table 4

Comparison of IOS measurements between elderly and nonelderly patients with asthma^a

	Elderly patients with asthma (>65 y)	Nonelderly patients with asthma (≤65 y)	P value
IOS	25 (56)	53 (79)	
R5 (kPa · s · L ⁻¹)	0.48 ± 0.20	0.35 ± 0.12	<.001
R20 (kPa · s · L ⁻¹)	0.34 ± 0.10	0.31 ± 0.09	NS
R5–R20 (kPa · s · L ⁻¹)	0.14 ± 0.12	0.05 ± 0.05	<.001
X5 (kPa · s · L ⁻¹)	–0.23 ± 0.15	–0.12 ± 0.06	<.001
AX (kPa/L)	1.62 ± 1.8	0.44 ± 0.44	<.001
Fres (L/s)	19.6 ± 7.9	12.8 ± 4.1	<.001
(R5–R20)/R5	0.25 ± 0.16	0.12 ± 0.10	<.001

Abbreviations: AX, integrated area between 5 Hz and frequency of resonance; Fres, frequency of resonance; IOS, impulse oscillation; NS, not significant; R5, resistance at 5 Hz; R20, resistance at 20 Hz; X5, reactance at 5 Hz.

^aData are presented as number (percentage) or mean ± SD.

Table 5

Comparisons of FeNO levels, peripheral blood cell differentials, induced sputum cell differentials, and AHR between elderly and nonelderly patients with asthma^a

	Elderly patients with asthma (>65 y)	Nonelderly patients with asthma (≤65 y)	P value
FeNO	32 (71)	53 (79)	
FeNO (ppb)	24.6 (5.9–98.6)	26.9 (10.3–110)	NS
Induced sputum cell differentials	24 (53)	35 (52)	
Eosinophils (%)	0.5 (0–32.5)	1.5 (0–54.8)	NS
Neutrophils (%)	67.0 (32.8–98.5)	59.5 (4–94.3)	NS
Inflammatory subtypes ^b			
E/N/P/M	4/8/5/7	13/9/5/8	NS
Blood cell differentials	45 (100)	67 (100)	
Eosinophils (%)	3.6 (0.4–25.9)	3.6 (0.1–25.9)	NS
Neutrophils (%)	59.9 (37.4–80.8)	56.2 (36.3–82.9)	NS
AHR measurements	26 (58)	40 (60)	
Dmin (U)	3.6 (0.09–50)	8.2 (0.15–50)	NS
SRs (cmH ₂ O · L ⁻¹ · s ⁻¹ · min ⁻¹)	1.38 (0.28–5.19)	1.49 (0.39–13.6)	NS

Abbreviations: AHR, airway hyperresponsiveness; Dmin, cumulative dose of methacholine at inflection point at which respiratory resistance began to increase; E, eosinophilic; FeNO, exhaled nitric oxide; M, mixed granulocytic; N, neutrophilic; NS, not significant; P, pauci-granulocytic; ppb, parts per billion; SRs, slope of dose–response curve for methacholine and respiratory resistance.

^aData are presented as number (percentage) or median (range). For eosinophils and neutrophils, median percentages (range) are listed.

^bSubjects were classified as having 1 of 4 inflammatory subtypes by induced sputum cell differentials: eosinophilic (eosinophils ≥1.0%, neutrophils <61%), neutrophilic (eosinophils <1.0%, neutrophils ≥61%), pauci-granulocytic (eosinophils <1.0%, neutrophils <61%), or mixed granulocytic (eosinophils ≥1.0%, neutrophils ≥61%).

5 to 35 Hz (X5 to X35), and frequency of resonance (Fres), which represents the point at which the usually negative reactance reaches 0. AX was defined as the integrated area between 5 Hz and Fres. It is assumed that respiratory resistances at 5 Hz (R5) and 20 Hz (R20) reflect total airway resistance and large airway resistance, respectively.¹⁰

Several previous studies adopted the decrease in resistance from 5 to 20 Hz as representing frequency dependency (R5–R20) and X5, AX, and Fres as indices of small airway abnormalities.^{10–13} The authors previously reported that R5–R20 and AX correlated with the conventional parameters of small airway obstruction, namely FEF_{25–75} and RV/TLC.¹¹ Hence, they used R5–R20, X5, AX, and Fres as indices of small airway disease. To exclude the potential effects of age or physique on IOS measurements,^{30,31} they also evaluated the ratio of R5–R20 to R5 [(R5–R20)/R5] as an alternative index of small airway disease.

Table 6

Comparisons of IgE and atopic status between elderly and nonelderly patients with asthma^a

	Elderly patients with asthma (>65 y)	Nonelderly patients with asthma (≤65 y)	P value
Patients	45 (100)	67 (100)	
Serum total IgE (IU/mL)	91 (5–2,100)	210 (5–8,700)	.006
≥1 positive specific IgE	22 (49)	50 (75)	.005
Positive rates of individual specific IgE			
Cat dander	2 (4.4)	12 (17.9)	.03
Dog dander	1 (2.3)	14 (20.9)	.005
House dust	12 (27.3)	34 (50.8)	.01
Mites (<i>Dermatophagoides pteronyssinus</i>)	13 (28.9)	34 (50.8)	.02
Japanese cedar pollen	9 (20.0)	40 (59.7)	<.001
Mixed Gramineae pollens	4 (8.9)	16 (23.9)	.04
Mixed weed pollens	0 (0.0)	5 (7.5)	NS
Mixed molds	1 (2.2)	7 (10.5)	NS
Trichophyton	5 (11.4)	7 (10.5)	NS

Abbreviation: NS, not significant.

^aData are presented as number (percentage) or median (range).

Exhaled nitric oxide

Exhaled nitric oxide was measured before spirometry by the online method using a chemiluminescence analyzer (NOA 280; Sievers Instruments, Boulder, Colorado).³² The average of 3 measurements at an expiratory flow rate of 50 mL/s was used for analyses.³³

Sputum analysis

Sputum induction tests were performed as described previously.^{14,34} Briefly, after inhaling 200 µg of salbutamol, patients inhaled 3% saline solution through an ultrasonic nebulizer for 15 minutes. Sputum plugs were dispersed with 0.1% dithiothreitol and phosphate buffered saline. Slides were prepared by cytospin and were stained with Diff-Quick for differential cell counts. On each slide, 400 nonsquamous cells were counted and identified as eosinophils, neutrophils, macrophages, lymphocytes, or epithelial cells. Sputum samples that had squamous cell contamination in at least approximately 50% of the fields were eliminated. Cell differentials of eosinophils and neutrophils were used to classify patients into 4 inflammatory subtypes: eosinophilic (eosinophils $\geq 1.0\%$, neutrophils $< 61\%$), neutrophilic (eosinophils $< 1.0\%$, neutrophils $\geq 61\%$), pauci-granulocytic (eosinophils $< 1.0\%$, neutrophils $< 61\%$), and mixed granulocytic (eosinophils $\geq 1.0\%$, neutrophils $\geq 61\%$).^{35,36}

Airway responsiveness to methacholine

Airway responsiveness to methacholine was measured by the continuous inhalation of methacholine during tidal breathing, with simultaneous measurement of respiratory resistance (Astograph; Chest MI, Inc).^{37,38} There were 10 nebulizers that contained 2-fold increasing concentrations of methacholine (49–25,000 µg/mL). Each concentration of the methacholine solution was inhaled for 1 minute. Salbutamol was inhaled for a period of 2 minutes at the following instances: when respiratory resistance (Rrs) reached twice the initial Rrs, when the inhalation of methacholine was performed to its maximum concentration, or when subjects indicated signs of dyspnea. Dmin was the minimal cumulative dose of methacholine at the inflection point at which respiratory resistance began to increase. Dmin was represented in units, where 1 U equals 1 minute of inhalation of a 1.0-mg/mL aerosol solution of methacholine. Subjects with a Dmin lower than 12.5 U were considered to have a positive response to methacholine.³⁹ SRs was the slope of the dose–response curve for methacholine and respiratory resistance. Dmin and SRs were used as parameters of airway sensitivity and airway reactivity, respectively.³⁸

IgE measurements

Allergen specific IgE antibodies to cat dander, dog dander, house dust, mites (*Dermatophagoides pteronyssinus*), Japanese cedar pollen, mixed Gramineae pollens, mixed weed pollens, mixed molds, and Trichophyton were detected with a radioallergosorbent test fluoro-enzyme immunoassay (Phadia, Uppsala, Sweden).⁴⁰ Specific IgE antibody levels higher than 0.7 UA/mL were considered positive results.⁴⁰ Subjects who had at least 1 positive allergen specific IgE antibody were regarded as “atopic.”

Statistical Analysis

Spirometry, CT measurements, Dmin, SRs, FeNO, induced sputum cell differentials, and serum total IgE levels are presented as median (range). IOS results are expressed as mean (SD). Elderly and nonelderly patients with asthma were compared using unpaired *t* test, Mann-Whitney *U* test, and χ^2 test, as appropriate. *P* values less than .05 were considered statistically significant. All statistical analyses were performed using JMP 8 (SAS Institute, Cary, North Carolina).

Results

Subject Characteristics

Table 1 lists the baseline characteristics of the subjects in this study. There were 45 elderly subjects with asthma (> 65 years old) and 67 nonelderly patients with asthma (≤ 65 years old). The mean ages of the elderly and nonelderly patients with asthma were 73.1 ± 5.3 and 48.6 ± 12.9 years, respectively. There were fewer ex-smokers among the elderly vs nonelderly patients with asthma, although there was no difference in pack-years between the 2 groups. Disease duration, frequency of exacerbations, disease severity, body mass index, and dose of ICS did not differ between the 2 groups. There were no correlations between subject age and disease duration in an analysis of all 112 subjects (Spearman correlation, $\rho = 0.14$; $P = .13$). Prevalence of allergic rhinitis was significantly higher in the nonelderly patients with asthma than in the elderly patients (Table 1). None of the patients had comorbid diseases such as chronic obstructive pulmonary disease, heart failure, or healed pulmonary tuberculosis.

Differences between the 2 groups for the prevalence of patients for whom each clinical datum was available were statistically significant only for the IOS measurement (56% vs 79%, $P = .0079$; Table 4).

Pulmonary Function Tests

Table 2 presents the results of pulmonary function tests, most of which were evaluated as a percentage of normal predicted values. FEV₁, FEV₁/FVC, and FEF_{25–75} were significantly lower in elderly compared with nonelderly patients with asthma (Table 2). RV/TLC was similar between the 2 groups.

CT Measurements

For indices of airway wall thickness, elderly patients with asthma had significantly higher values of WA%, WA/BSA, and T/ $\sqrt{BSA}$ than nonelderly patients with asthma (Table 3). For indices of air trapping, there were no differences in the inspiratory index of LAA% or MLD between the 2 groups. The full-expiration values of LAA% were significantly higher, whereas those of MLD were significantly lower, in elderly than in nonelderly patients with asthma (Table 3). Moreover, the E/I ratios of LAA% and MLD were significantly higher in the elderly asthmatic than in the nonelderly asthmatic group (Table 3).

IOS Measurements

Elderly patients with asthma had significantly higher R5 values than nonelderly patients with asthma (Table 4). There was no difference in R20 between the 2 groups. R5–R20 was significantly higher in elderly patients with asthma compared with nonelderly patients with asthma. Elderly patients with asthma also had lower X5 and higher AX and Fres than nonelderly patients with asthma. In addition, (R5–R20)/R5, which was calculated to exclude the effect of age or physique, was higher in elderly than in nonelderly patients with asthma (Table 4).

Other Clinical Measurements

Table 5 presents the comparisons of inflammatory and airway responsiveness markers between elderly and nonelderly patients with asthma. FeNO was similar between the 2 groups. Further, no significant differences were observed in induced sputum cell differentials or proportions of each inflammatory subtype between elderly and nonelderly patients with asthma, although sputum neutrophils were marginally ($P = .08$) increased in elderly patients with asthma. There also were no significant differences in blood eosinophils, neutrophils, and the parameters of airway sensitivity

(Dmin) or airway reactivity (SRrs) between elderly and nonelderly patients with asthma. Blood neutrophils were only marginally ($P = .05$) increased in elderly patients with asthma.

The differences in IgE and atopic status between elderly and nonelderly patients with asthma are presented in Table 6. Serum total IgE levels were significantly lower in elderly patients with asthma compared with nonelderly patients with asthma. There were fewer subjects who exhibited positivity for at least 1 specific IgE antibody (“atopic”) in the elderly vs nonelderly asthmatic group. Elderly patients with asthma had lower positive rates of specific IgE against cat dander, dog dander, house dust, mites, Japanese cedar pollen, and mixed Gramineae pollens than nonelderly patients with asthma.

Discussion

Despite its considerable impact on the clinical management of elderly patients with asthma, the effects of aging on the pathophysiology of asthma have rarely been investigated. The authors comprehensively studied the pathophysiologic characteristics of elderly patients with asthma, demonstrating prominent large and small airway involvement but a less atopic status compared with nonelderly patients with asthma.

Although the predominant site of airway disease (large or small airways) can vary from patient to patient,⁴¹ the determinants of such variations are poorly known.⁴² In this study, spirometric values of FEF_{25–75} and FEV₁ were significantly lower in elderly than in nonelderly patients with asthma. Because the percentages of predicted values, which were corrected for age and height, were used for the analyses of spirometric results and duration of asthma did not differ between elderly and nonelderly patients with asthma, these results demonstrate that elderly asthma more prominently involves obstruction of small and large airways, independent of aging per se or duration of disease.

The CT indices of large airway dimensions reflect the histologic changes in airway walls in patients with asthma.⁴³ The present results indicated that elderly patients with asthma had thicker large airway walls than nonelderly patients with asthma. Bai et al⁴⁴ examined postmortem lungs of young (19.1 ± 0.5 years old; $n = 14$) and middle-aged (42.6 ± 1.0 years old; $n = 13$) patients with fatal asthma. The middle-aged group, with longer disease duration (24.0 ± 13.4 years), showed significantly thicker total airway walls and smooth muscle layers than the younger group, whose disease duration was 7.6 ± 4.8 years. That study provided pathologic evidence of the progressive effects of aging and/or long-standing disease on airway remodeling, although further evidence supporting that pioneering study has been scarce. The present results confirm these findings radiologically and suggest that elderly disease, independent of disease duration, may contribute to remodeling of large airway walls. Elevated sputum levels of tissue inhibitor of matrix metalloproteinase-1 over those of matrix metalloproteinase-9 are associated with airway wall thickening, as assessed by CT.²³ Activity of matrix metalloproteinases decreases and that of tissue inhibitors of matrix metalloproteinases increases with aging in normal rat lungs, leading to collagen deposition and fibrosis in the peribronchial region.⁴⁵ In an aging model of asthma, 6-month-old mice had more prominent collagen accumulation and airway smooth muscle hypertrophy in their airways than younger mice.⁴⁶ Age-related alteration in collagen synthesis and degradation and asthma-specific airway inflammation can synergistically contribute to the progression of airway remodeling in elderly patients with asthma.

To evaluate small airway involvement, high-resolution CT indices, represented as decreased lung attenuation (measured by LAA% and MLD), have been quantified in patients with asthma.^{8,9} The E/I ratios of LAA% or MLD also are regarded as CT indices of air trapping,⁴⁷ with these ratios correlating more closely with

clinical measurements, such as severity score, airflow obstruction, and airway sensitivity, than absolute inspiratory or expiratory values.⁹ In this study, by using full-expiratory scans, the authors demonstrated that elderly subjects with asthma had higher LAA% and lower MLD than nonelderly subjects. The differences in E/I ratios between the 2 groups were more significant than those of full-expiration values. The authors believe that the E/I ratios reflect a dynamic change in airway collapse during expiration and can detect the degree of air trapping more accurately than absolute expiratory values. Normally, aging has effects on airway structure, such as airspace enlargement, which can result in decreased lung attenuation at inspiration.⁴⁸ However, because inspiratory LAA% and MLD were almost equivalent between elderly and nonelderly patients with asthma in this study, these effects of aging per se were not likely to have affected the results.

For the IOS results, asthma in the elderly was more prominently associated with small airway abnormalities, as reflected by R5–R20, X5, AX, and Fres, compared with that in the nonelderly. These findings were consistent with the results of spirometry and lung density measurements on CT discussed earlier. RV/TLC, a conventional parameter of air trapping, did not differ between elderly and nonelderly patients with asthma. The authors previously reported that IOS, but not RV/TLC, could detect the improvement of small airway abnormalities in patients with asthma treated with ultrafine-particle ICSs.¹¹ The present study confirms that IOS is a sensitive and useful measurement to detect small airway abnormalities. However, each IOS parameter may be influenced by subject age, in addition to height, according to 2 normative population studies.^{30,31} Moreover, the predictive equations of IOS parameters in the Japanese population have not been well established. To exclude the effect of age, the authors calculated the corrected R5–R20 by dividing it by R5. The difference between the groups in (R5–R20)/R5 values remained prominent (significantly higher in elderly than in nonelderly patients with asthma; $P < .001$). However, further evaluation is needed to validate the utility of IOS measurements for assessing small airway involvement.

In some studies, the neutrophil count in induced sputum was reportedly greater in elderly than in nonelderly subjects with^{17,18} and without⁴⁹ asthma, despite the existence of conflicting reports.¹⁹ In the present study, there were no differences in induced sputum cell differentials between elderly and nonelderly subjects with asthma, although elderly subjects had marginally increased neutrophils in their sputum and blood compared with nonelderly patients with asthma. The authors speculate that the limited number of subjects in this study may have resulted in a lack of statistical power. Further, a higher prevalence of ex-smokers in the nonelderly asthma group than in the elderly asthma group may have affected the results, because smoking has been linked to sputum neutrophilia.⁵⁰ Irrespective of group (elderly or nonelderly patients with asthma), this study cohort showed relatively low eosinophil and high neutrophil counts in induced sputum. This finding might be due to ICS treatment attenuating eosinophilia and inducing sputum neutrophilia,⁵¹ because all the study subjects were taking ICS (median 800 $\mu\text{g}/\text{d}$), whereas less than half the subjects in previous studies received ICS therapy.^{17,18} Moreover, the present cohort predominantly was comprised of patients with moderate to severe asthma, who are known to have sputum neutrophilia.⁵² The present FeNO results are consistent with the literature that FeNO levels are unrelated to age.⁵³

For airway responsiveness, it is controversial whether elderly patients with asthma are more responsive than nonelderly patients with asthma.^{54,55} In the present study, neither airway sensitivity nor airway reactivity differed significantly between elderly and nonelderly patients with asthma.

Elderly patients with asthma had lower total IgE levels, a lower prevalence of at least 1 positive allergen specific IgE antibody, and a

lower positive response to many of the allergen specific antibodies than nonelderly patients with asthma in this study. Owing to the decreased production of IgE with aging,⁵⁶ a feature of “immunosenescence,”⁵⁷ the proportion of patients with nonatopic (or “intrinsic”) asthma becomes dominant in elderly populations, reaching up to more than 50%. This also was confirmed by the present study.

Elderly patients with asthma are often assumed to include 2 subgroups: those who have had asthma from childhood (long-standing asthma) and those who developed asthma at an older age.⁵⁸ In a previous study, subjects with long-standing asthma had more irreversible airflow obstruction and hyperinflation compared with those with short-duration asthma.⁵⁹ In this study, more than half ($n = 57$) the study subjects were recruited from an early intervention study of mild-to-moderate asthma,²⁰ and their duration of asthma was a median of 0.68 years (range 0.25–40.35 years).²⁰ Moreover, the number of subjects with long-standing asthma was limited even in the elderly asthma group. Therefore, the authors did not address the characteristics of long-standing asthma. There was no correlation between disease duration and subject age, and there was no difference in disease duration between elderly and nonelderly patients with asthma. Therefore, the authors consider that the effect of disease duration on the study results was limited.

There are several limitations to this study. First, age per se could be a confounding factor in the investigation of elderly patients with asthma. The authors could not recruit elderly control patients without asthma owing to the difficulties in performing CT and induced sputum and airway responsiveness tests in normal subjects. In the authors' previous clinical studies that involved healthy controls,^{60,61} control subjects were recruited from the hospital staff, whose mean ages were much younger than the definition of elderly (51 years⁶⁰ and 33 years,⁶¹ respectively). It was actually impossible for the authors to recruit healthy elderly subjects older than 65 years from their hospital staff; almost all ordinary university employees in Japan retire at 60 or 65 years of age. Moreover, the authors do not have a system or custom to recruit healthy volunteers for research purpose from the public using public information such as the Internet by providing them a reward. The lack of controls may preclude a determination of whether the results observed are due to age or an interaction between age and asthma. To eliminate the effect of aging, the authors used percentages of predicted values for spirometry^{25,26} and $(R5-R20)/R5$ for IOS measurements, which yielded significant results indicating greater large and small airway involvement in elderly patients with asthma. The authors believe that the synergistic effects of age and asthma contribute to the progression of large and small airway involvement. Second, this study was retrospective in nature, and smoking status was not matched between the 2 groups. As a result, the prevalence of ex-smokers was higher in the nonelderly patients with asthma (12 of 67, 18%) than in the elderly patients with asthma (1 of 45, 2%). However, although the difference was statistically significant, the smoking index of all 67 nonelderly patients with asthma was as low as 0.34 ± 1.1 pack-years, which did not differ significantly from that of the 45 elderly patients with asthma (0.11 ± 0.75 pack-year). Indeed, additional analyses excluding the 13 ex-smokers showed similar results for CT and IOS measurements (data not shown). Even if the difference in smoking exposure between the 2 groups had been clinically relevant, the results that airway abnormalities as examined by spirometry, IOS, and CT were more pronounced in the elderly patients with asthma than in the nonelderly patients with asthma, who had smoked more than the former group, may be strengthened even further. Third, a subset of subjects did not undergo spirometrically gated inspiratory and expiratory CT. In such cases, the full-inspiratory and full-expiratory procedures were carefully explained and performed.⁹ Orlandi et al.⁶² reported that

airway wall area and lung attenuation assessed by inspiratory CT without spirometric gating were comparable to those assessed by spirometrically gated CT in patients with chronic obstructive pulmonary disease. Fourth, only subsets of patients were analyzed for each measurement. This is partly because only data of all clinical measurements that were performed within 4 weeks of the date of CT measurements were collected to maintain the integrity of the data. With respect to sputum induction, the authors previously reported that the success rate of sputum induction in 407 consecutive subjects with asthma was 73.0% (not very high), and that unsuccessful sputum induction was significantly associated with long-standing disease and the lack of smoking history.⁶³ The latter point represents a characteristic of the present patients.

In conclusion, elderly patients with asthma have more prominent large and small airway involvement and less atopy than nonelderly patients with asthma. Despite some limitations, these results may provide a better understanding of the pathophysiology and future therapeutic strategies for asthma in the elderly.

References

- [1] World Health Organization. Definition of an older or elderly person. Health Statistics and Health Information Systems. <http://www.who.int/healthinfo/survey/ageingdefolder/en/>. Published 2012. Accessed March 4, 2014.
- [2] US Census Bureau. World population information. International Data Base (IDB). <http://www.census.gov/population/international/>. Published 2014. Accessed March 4, 2014.
- [3] Gibson PG, McDonald VM, Marks GB. Asthma in older adults. *Lancet*. 2010; 376:803–813.
- [4] Australian Centre for Asthma Monitoring. *Asthma in Australia 2008*. Canberra: Australian Institute of Health and Welfare, <http://www.asthamonitoring.org/>. Accessed March 4, 2014.
- [5] *Vital Statistics of Japan. Volumes 1–3*. Tokyo, Japan: Ministry of Health, Labour and Welfare; 2011. <http://www.mhlw.go.jp/english/database/db-hw/vs01.html/>. Accessed March 4, 2014.
- [6] Niimi A, Matsumoto H, Takemura M, Ueda T, Nakano Y, Mishima M. Clinical assessment of airway remodeling in asthma: utility of computed tomography. *Clin Rev Allergy Immunol*. 2004;27:45–58.
- [7] Niimi A, Matsumoto H, Amitani R, et al. Airway wall thickness in asthma assessed by computed tomography. Relation to clinical indices. *Am J Respir Crit Care Med*. 2000;162:1518–1523.
- [8] Mitsunobu F, Ashida K, Hosaki Y, et al. Complexity of terminal airspace geometry assessed by computed tomography in asthma. *Am J Respir Crit Care Med*. 2003;167:411–417.
- [9] Ueda T, Niimi A, Matsumoto H, et al. Role of small airways in asthma: investigation using high-resolution computed tomography. *J Allergy Clin Immunol*. 2006;118:1019–1025.
- [10] Goldman MD, Saadeh C, Ross D. Clinical applications of forced oscillation to assess peripheral airway function. *Respir Physiol Neurobiol*. 2005;148: 179–194.
- [11] Yamaguchi M, Niimi A, Ueda T, et al. Effect of inhaled corticosteroids on small airways in asthma: investigation using impulse oscillometry. *Pulm Pharmacol Ther*. 2009;22:326–332.
- [12] Takeda T, Oga T, Niimi A, et al. Relationship between small airway function and health status, dyspnea and disease control in asthma. *Respiration*. 2010; 80:120–126.
- [13] Shi Y, Aledia AS, Tatavosian AV, Vijayalakshmi S, Galant SP, George SC. Relating small airways to asthma control by using impulse oscillometry in children. *J Allergy Clin Immunol*. 2012;129:671–678.
- [14] Pin I, Gibson PG, Kolendowicz R, et al. Use of induced sputum cell counts to investigate airway inflammation in asthma. *Thorax*. 1992;47:25–29.
- [15] Smith AD, Cowan JO, Filsell S, et al. Diagnosing asthma: comparisons between exhaled nitric oxide measurements and conventional tests. *Am J Respir Crit Care Med*. 2004;169:473–478.
- [16] Bellia V, Scichilone N, Battaglia S. Asthma in the elderly. *Eur Respir Soc Monogr*. 2009;43:56–76.
- [17] Mathur SK, Schwantes EA, Jarjour NN, Busse WW. Age-related changes in eosinophil function in human subjects. *Chest*. 2008;133:412–419.
- [18] Nyenhuis SM, Schwantes EA, Evans MD, Mathur SK. Airway neutrophil inflammatory phenotype in older subjects with asthma. *J Allergy Clin Immunol*. 2010;125:1163–1165.
- [19] Vignola AM, Bonanno A, Profita M, et al. Effect of age and asthma duration upon elastase and alpha1-antitrypsin levels in adult asthmatics. *Eur Respir J*. 2003;22:795–801.
- [20] Takeda T, Niimi A, Matsumoto H, et al. Effect of hydrofluoroalkane-134a beclomethasone (HFA-BDP) and fluticasone propionate dry powder inhaler (FP-DPI) on large and small airway indices in asthma: a randomized comprehensive study [abstract]. *Am J Respir Crit Care Med*. 2011;183(suppl 1): A1290.

- [21] Niimi A, Jinnai M, Fujimura M, et al. Risk factors associated with frequent exacerbations (FE) in severe asthma: a multicentre study in Japan [abstract]. *Eur Respir J Suppl.* 2009;1:E1860.
- [22] Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, November 1986. *Am Rev Respir Dis.* 1987;136:225–244.
- [23] Matsumoto H, Niimi A, Takemura M, et al. Relationship of airway wall thickening to an imbalance between matrix metalloproteinase-9 and its inhibitor in asthma. *Thorax.* 2005;60:277–281.
- [24] Bateman ED, Boushey HA, Bousquet J, et al. Can guideline-defined asthma control be achieved? The Gaining Optimal Asthma Control study. *Am J Respir Crit Care Med.* 2004;170:836–844.
- [25] Japanese Respiratory Society. *Reference Values of Spirogram and Arterial Blood Gas Levels in Japanese.* Tokyo, Japan: Japanese Respiratory Society; 2001 [in Japanese].
- [26] Cherniack RM, Raber MB. Normal standards for ventilatory function using an automated wedge spirometer. *Am Rev Respir Dis.* 1972;106:38–46.
- [27] Ohara T, Hirai T, Muro S, et al. Relationship between pulmonary emphysema and osteoporosis assessed by CT in patients with COPD. *Chest.* 2008;134:1244–1249.
- [28] Kalender WA, Rienmuller R, Seissler W, Behr J, Welke M, Fichte H. Measurement of pulmonary parenchymal attenuation: use of spirometric gating with quantitative CT. *Radiology.* 1990;175:265–268.
- [29] Oostveen E, MacLeod D, Lorino H, et al. The forced oscillation technique in clinical practice: methodology, recommendations and future developments. *Eur Respir J.* 2003;22:1026–1041.
- [30] Shiota S, Katoh M, Fujii M, Aoki S, Matsuoka R, Fukuchi Y. Predictive equations and the reliability of the impulse oscillometry system in Japanese adult subjects. *Respirology.* 2005;10:310–315.
- [31] Newbury W, Crockett A, Newbury J. A pilot study to evaluate Australian predictive equations for the impulse oscillometry system. *Respirology.* 2008;13:1070–1075.
- [32] ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. *Am J Respir Crit Care Med.* 2005;171:912–930.
- [33] Matsumoto H, Niimi A, Jinnai M, et al. Association of alveolar nitric oxide levels with pulmonary function and its reversibility in stable asthma. *Respiration.* 2011;81:311–317.
- [34] Takemura M, Niimi A, Minakuchi M, et al. Bronchial dilatation in asthma: relation to clinical and sputum indices. *Chest.* 2004;125:1352–1358.
- [35] Simpson JL, Scott R, Boyle MJ, Gibson PG. Inflammatory subtypes in asthma: assessment and identification using induced sputum. *Respirology.* 2006;11:54–61.
- [36] Matsuoka H, Niimi A, Matsumoto H, et al. Inflammatory subtypes in cough variant asthma: association with maintenance doses of inhaled corticosteroids. *Chest.* 2010;138:1418–1425.
- [37] Takishima T, Hida W, Sasaki H, Suzuki S, Sasaki T. Direct-writing recorder of the dose–response curves of the airway to methacholine. Clinical application. *Chest.* 1981;80:600–606.
- [38] Niimi A, Matsumoto H, Takemura M, Ueda T, Chin K, Mishima M. Relationship of airway wall thickness to airway sensitivity and airway reactivity in asthma. *Am J Respir Crit Care Med.* 2003;168:983–988.
- [39] Niimi A, Amitani R, Suzuki K, Tanaka E, Murayama T, Kuze F. Eosinophilic inflammation in cough variant asthma. *Eur Respir J.* 1998;11:1064–1069.
- [40] Matsuoka H, Niimi A, Matsumoto H, et al. Specific IgE response to Trichophyton and asthma severity. *Chest.* 2009;135:898–903.
- [41] Takishima T, Yanai M, Sasaki H. Site of airway hyperreactivity. *Am Rev Respir Dis.* 1991;143:S49–S51.
- [42] Busacker A, Newell JD Jr, Keefe T, et al. A multivariate analysis of risk factors for the air-trapping asthmatic phenotype as measured by quantitative CT analysis. *Chest.* 2009;135:48–56.
- [43] Lederlin M, Laurent F, Portron Y, et al. CT attenuation of the bronchial wall in patients with asthma: comparison with geometric parameters and correlation with function and histologic characteristics. *AJR Am J Roentgenol.* 2012;199:1226–1233.
- [44] Bai TR, Cooper J, Koelmeyer T, Pare PD, Weir TD. The effect of age and duration of disease on airway structure in fatal asthma. *Am J Respir Crit Care Med.* 2000;162:663–669.
- [45] Calabresi C, Arosio B, Galimberti L, et al. Natural aging, expression of fibrosis-related genes and collagen deposition in rat lung. *Exp Gerontol.* 2007;42:1003–1011.
- [46] Kang JY, Lee SY, Rhee CK, Kim SJ, Kwon SS, Kim YK. Effect of aging on airway remodeling and muscarinic receptors in a murine acute asthma model. *Clin Interv Aging.* 2013;8:1393–1403.
- [47] Gono H, Fujimoto K, Kawakami S, Kubo K. Evaluation of airway wall thickness and air trapping by HRCT in asymptomatic asthma. *Eur Respir J.* 2003;22:965–971.
- [48] Gillyool M, Lamb D. Airspace size in lungs of lifelong non-smokers: effect of age and sex. *Thorax.* 1993;48:39–43.
- [49] Thomas RA, Green RH, Brightling CE, et al. The influence of age on induced sputum differential cell counts in normal subjects. *Chest.* 2004;126:1811–1814.
- [50] Chalmers GW, MacLeod KJ, Thomson L, Little SA, McSharry C, Thomson NC. Smoking and airway inflammation in patients with mild asthma. *Chest.* 2001;120:1917–1922.
- [51] Cowan DC, Cowan JO, Palmay R, Williamson A, Taylor DR. Effects of steroid therapy on inflammatory cell subtypes in asthma. *Thorax.* 2010;65:384–390.
- [52] Wenzel S. Severe asthma: from characteristics to phenotypes to endotypes. *Clin Exp Allergy.* 2012;42:650–658.
- [53] Sippel JM, Holden WE, Tilles SA, et al. Exhaled nitric oxide levels correlate with measures of disease control in asthma. *J Allergy Clin Immunol.* 2000;106:645–650.
- [54] Cuttitta G, Cibella F, Bellia V, et al. Changes in FVC during methacholine-induced bronchoconstriction in elderly patients with asthma: bronchial hyperresponsiveness and aging. *Chest.* 2001;119:1685–1690.
- [55] Mitsuta K, Shimoda T, Kawano T, et al. Airway hyperresponsiveness and pulmonary function in adult asthma. *Respiration.* 2001;68:460–464.
- [56] Agache I, Akdis C, Jutel M, Virchow JC. Untangling asthma phenotypes and endotypes. *Allergy.* 2012;67:835–846.
- [57] Busse PJ, Mathur SK. Age-related changes in immune function: effect on airway inflammation. *J Allergy Clin Immunol.* 2010;126:690–699.
- [58] Diaz-Guzman E, Mannino DM. Airway obstructive diseases in older adults: From detection to treatment. *J Allergy Clin Immunol.* 2010;126:702–709.
- [59] Cassino C, Berger KI, Goldring RM, et al. Duration of asthma and physiologic outcomes in elderly nonsmokers. *Am J Respir Crit Care Med.* 2000;162:1423–1428.
- [60] Otsuka K, Matsumoto H, Niimi A, et al. Sputum YKL-40 levels and pathophysiology of asthma and chronic obstructive pulmonary disease. *Respiration.* 2012;83:507–519.
- [61] Yamaguchi M, Niimi A, Matsumoto H, et al. Sputum levels of transforming growth factor- β 1 in asthma: Relation to clinoradiological findings. *J Invest Allergol Clin Immunol.* 2008;18:202–206.
- [62] Orlandi I, Moroni C, Camiciottoli G, et al. Chronic obstructive pulmonary disease: thin-section CT measurement of airway wall thickness and lung attenuation. *Radiology.* 2005;234:604–610.
- [63] Matsuoka H, Niimi A, Matsumoto H, et al. Patients' characteristics associated with unsuccessful sputum induction in asthma. *J Allergy Clin Immunol.* 2008;121:774–776.