Annals of Allergy, Asthma and Immunology, 2014

Pathophysiological Characteristics of Asthma in the Elderly: A Comprehensive Study

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The authors have no conflicts of interest to disclose.

Declaration of all sources of funding: None

Word count for the manuscript: 4122 words

Number of tables: 6

Number of figures: 0

Each author's role in the study and manuscript

Hideki Inoue performed pulmonary function tests, contributed to the acquisition and interpretation of data, and drafted the manuscript.

Akio Niimi proposed the study, contributed to its design, recruited patients, participated in the acquisition and interpretation of data, and revised the manuscript.

Tomoshi Takeda performed CT analyses.

Hisako Matsumoto recruited patients and contributed to disease diagnosis and management.

Isao Ito recruited patients and contributed to disease diagnosis and management.

Hirofumi Matsuoka performed CT analyses.

Makiko Jinnai performed CT analyses and measured airway responsiveness to methacholine.

Kojiro Otsuka performed pulmonary function tests and measured airway responsiveness to methacholine.

Tsuyoshi Oguma performed IOS measurements.

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Toshiyuki Iwata performed IOS measurements.

Yoshihiro Kanemitsu contributed to acquisition of data.

Kazuo Chin contributed to data interpretation.

Michiaki Mishima supervised the study.

ABBREVIATION LIST

AX = the integrated area between 5 Hz and Fres

Dmin = cumulative dose of methacholine at the inflection point at which respiratory resistance begins to increase

E/I ratio = ratio of percentage of lung field occupied by low attenuation areas or mean lung density in full-expiratory scans to the respective values in full-inspiratory scans

FeNO = exhaled nitric oxide

FEF_{25-75} = mid-forced expiratory flow

Fres = frequency of resonance

HU = Hounsfield unit

IOS = impulse oscillation

LAA% = percentage of lung field occupied by low attenuation area <960 Hounsfield units

MLD = mean lung density

RV/TLC = residual volume/total lung capacity

Rrs, R = respiratory resistance

SRrs = the slope of the methacholine–respiratory resistance dose-response curve

X: respiratory reactance

KEYWORDS

Air trapping, airway remodeling, airway wall thickness, atopy, CT, elderly asthma, exhaled nitric oxide, impulse oscillation, small airway, large airway, induced sputum, spirometry

ABSTRACT

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

Objectives: To clarify the pathophysiological characteristics of elderly asthmatics.

Methods: We retrospectively analyzed subjects aged >65 years [elderly asthmatics; n = 45] and those aged ≤65 years [non-elderly asthmatics; n = 67], comparing them for spirometry, CT indices of large airway wall thickness and small airway involvement (air trapping), impulse oscillation (IOS) measurements, exhaled nitric oxide (FeNO) levels, blood and induced sputum cell differentials, methacholine airway responsiveness and total and specific serum IgE levels.

Results: Elderly asthmatics had significantly lower FEV_1 and FEF_{25-75} (% of predicted) than non-elderly asthmatics (median, 81.2% vs. 88.8%, $P = 0.02$, and 50.9% vs. 78.6%, $P = 0.03$, respectively). In CT measurements, elderly asthmatics had significantly greater airway wall thickening and air trapping than non-elderly asthmatics. IOS measurements indicated that elderly asthmatics showed significantly higher R5 (used as an index of total airway resistance), R5-R20, (R5-R20)/R5, AX and Fres, and lower X5 (potential markers of small airway disease), than non-elderly asthmatics. There were no significant differences in blood or sputum cell differentials, FeNO, or methacholine airway responsiveness between the two groups. Total serum IgE levels and positive rates of specific IgE antibodies against several allergens were significantly lower in elderly than non-elderly asthmatics.

Conclusion: Based on analyses of spirometry, CT and IOS, elderly asthmatics have greater involvement of small as well as large airways than non-elderly asthmatics.

Word count of abstract: 249 words

INTRODUCTION

The number of elderly people, defined by the World Health Organization as those with a chronological age of 65 years[1], is expected to increase from 546 million in 2011 to 1.6 billion in 2050.[2] The incidence of elderly asthma can also be expected to increase due to the aging of society. The prevalence of asthma among the elderly is between 6% and 10% in developed countries,[3] and the proportions of elderly patients among asthma deaths are about two-thirds in Australia[4] 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 (e.g. COPD), multiple comorbidities, poor inhaler device use, poor adherence to therapy, and increased side effects and decreased responsiveness to medication.[3]

A number of clinical measurements have been utilized to evaluate the pathophysiology of asthma. CT has been used to assess large airway wall remodeling[6, 7] and small airway involvement (i.e. air trapping)[8, 9] among patients with asthma. Impulse oscillation (IOS) is a noninvasive method of measuring respiratory resistance (R) and reactance (X), which may potentially differentiate large from small airway disease.[10-13] Further, induced sputum cell differentials[14] and exhaled nitric oxide levels (FeNO)[15] 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 may alter the elastic properties of the airways, resulting in a loss of elastic recoil.[16] Thus, in elderly subjects, small airways may tend to collapse during expiration, possibly leading to air trapping and an increase in residual volume. Elderly patients with asthma are assumed to have more prominent small airway disease, although evidence for this is lacking. Moreover, aging may also affect immunological and inflammatory profiles among asthmatics. Airway neutrophilia may be more predominant in elderly subjects with asthma

than the non-elderly[17, 18], although conflicting evidence exists showing that sputum cellular profiles are similar between young and elderly asthmatics.[19]

Since comprehensive studies on elderly asthma, addressing its physiological, radiological and immunological features are scarce, we investigated these pathophysiological characteristics of elderly asthma using spirometry, CT, IOS, induced sputum, FeNO and IgE measurements, and compared the results with those of non-elderly asthma.

METHODS

Subjects

Study subjects were retrospectively selected from 136 patients with stable asthma who underwent chest multidetector raw computed tomography (MDCT) scans for research purposes[20, 21] at our 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 the American Thoracic Society criteria;[22] (2) clinically stable disease that had been fully controlled for at least 1 month[23] 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 prior to study entry; (4) treatment with inhaled corticosteroids (ICS) 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. Elderly subjects 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 on each subject during 49 our follow-up of patients: spirometry (n=112, 100%), IOS (n=111, 99.1%), induced sputum 50 (n=76, 67.9%), airway responsiveness test (n=79, 70.5%), FeNO (n=110, 98.2%), peripheral 51 blood cell differentials (n=112, 100%), and serum total IgE and allergen specific IgE (n=112, 52 100%). However, to maintain the integrity of clinical data to be analyzed in this retrospective 53 study, we utilized only the data obtained within 4 weeks of the date of CT measurement. As a 54 result, the number and percentage of subjects for whom each data were available were 55 reduced 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). Written informed consent was obtained from all subjects for participation in this study.

Outcome Measures

Pulmonary Function Tests

Pre-bronchodilator values of FVC, FEV_1 and mid-forced expiratory flow (FEF_{25-75}) were examined using a ChestGraph HI-701 spirometer (Chest M.I., Inc., Tokyo, Japan). Residual volume/total lung capacity (RV/TLC), which is considered to reflect air trapping, was also measured using a CHESTAC-8800 (Chest M.I., Inc., Tokyo, Japan). To exclude the effects of age and physique on pulmonary function tests, the predicted values of FVC and FEV_1, which were quoted from the publication of the Japanese Respiratory Society,[25] were used for comparisons between elderly and non-elderly asthmatics. The predicted values of FEF_{25-75} and RV/TLC were calculated from other published equations.[26]

CT Measurements

Each subject underwent an MDCT scan (Aquilion™ 64; Toshiba Medical Systems, Tokyo, Japan) as described previously.[7, 27] To evaluate large airway wall dimensions, we analyzed three parameters: airway wall area (WA) corrected as a percentage of total wall area (WA%, %), WA normalized for body surface area (WA/BSA, mm^2/m^2) and normalized absolute wall thickness ($T/\sqrt{BSA}$, mm/m)[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 two 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 both full-inspiration and full-expiration were analyzed.[9] The ratio of

full-expiration to full-inspiration values (E/I ratio) of LAA% and MLD were also evaluated. A higher E/I ratio indicates more prominent small airway involvement.[9] Spirometric-gated CT,[28] which analyzes full-inspiratory and full-expiratory lung fields by monitoring the subject'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 MasterScreen 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 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 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]

A number of previous studies adopted the fall 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] We 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, we 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] we also evaluated the ratio of R5 - R20 to R5 [(R5 - R20)/R5] as an alternative index of small airway resistance.

FeNO

FeNO was measured prior to spirometry by the on-line method using a chemiluminescence analyzer (NOA 280™; Sievers Instruments, Boulder, CO).[32] The average of three measurements at an expiratory flow rate of 50 ml/second 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 via an ultrasonic nebulizer for 15 minutes. Sputum plugs were dispersed with 0.1% dithiothreitol (DTT) and phosphate buffered saline (PBS). Slides were prepared by cytospin and were stained with Diff-Quick for differential cell counts. In each slide, 400 non-squamous cells were counted and identified as eosinophils, neutrophils, macrophages, lymphocytes or epithelial cells. We eliminated sputum samples that had squamous contamination in approximately 50% or more of the fields. We used cell differentials of eosinophils and neutrophils to classify patients into four inflammatory subtypes: eosinophilic (eosinophils ≥1.0%, neutrophils <61%), neutrophilic (eosinophils <1.0%, neutrophils ≥61%), paucigranulocytic (eosinophils <1.0%, neutrophils <61%), and mixed granulocytic (eosinophils ≥1.0%, neutrophils ≥61%).[35,36]

Airway responsiveness to methacholine

Airway responsiveness to methacholine was measured by continuous inhalation of methacholine during tidal breathing, with simultaneous measurement of respiratory resistance (Astograph™; Chest, Tokyo, Japan).[37, 38] There were ten nebulizers, which contained 2-fold increasing concentrations of methacholine (49 μg/ml to 25,000 μg/ml). Each concentration of the methacholine solution was inhaled for one minute. Salbutamol was inhaled for a period of two 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 one unit equals one minute of inhalation of a 1.0 mg/ml aerosol solution of methacholine. Subjects with a Dmin of < 12.5 units were considered to have a positive response to methacholine.[39] SRrs was the slope of the methacholine–respiratory resistance dose-response curve. Dmin and SRrs 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 graminea pollens, mixed weed pollens, mixed molds and Trichophyton were detected with a radioallergosorbent test fluoroenzyme immunoassay (Phadia, Uppsala, Sweden).[40] Specific IgE antibody levels >0.7 UA/ml were considered positive.[40] Subjects who had at least one positive allergen-specific IgE antibody were regarded as “atopic”.

Statistical Analysis

Spirometry, CT measurements, Dmin, SRrs, FeNO, induced sputum cell differentials and serum total IgE levels are presented as medians (ranges). IOS results are expressed as means (SD). Elderly and non-elderly asthmatics were compared using an unpaired t-test, Mann-Whitney U test and χ^2 test, as appropriate. *P* values <0.05 were considered statistically significant. All statistical analyses were performed using JMP® software (version 8; SAS Institute Inc., Cary, NC).

RESULTS

Subject Characteristics

Table 1 shows the baseline characteristics of the subjects in this study. There were 45 elderly subjects with asthma (> 65 years old) and 67 non-elderly asthmatics (≤ 65 years old). The mean ages of elderly and non-elderly asthmatics were 73.1 ± 5.3 years and 48.6 ± 12.9 years, respectively. There were fewer ex-smokers among elderly as compared to non-elderly asthmatics, although there was no difference in pack-years between the two groups. Disease duration, frequency of exacerbations, disease severity, BMI and the dose of ICS did not differ between the two groups. There were no correlations between subject age and disease duration in an analysis of all 112 subjects (Spearman correlation, $\rho = 0.14$; $P = 0.13$). Prevalence of 171 allergic rhinitis was significantly higher in the non-elderly asthmatics than in the elderly 172 patients (Table 1). None of the patients had co-morbid diseases such as COPD, heart failure, 173 or healed pulmonary tuberculosis.

Differences between the two groups in terms of the prevalence of patients for whom each clinical data were available were statistically significant only for the IOS measurement (56% vs 79%; p=0.0079)(Table 4).

Pulmonary Function Tests

Table 2 shows the results of pulmonary function tests, most of which were evaluated as a percentage of normal predicted values. FEV_1, FEV_1 / FVC, and FEF_{25-75} were significantly lower in elderly compared to non-elderly asthmatics (Table 2). RV/TLC was similar between the two groups.

CT Measurements

In terms of indices of airway wall thickness, elderly asthmatics had significantly higher values of WA%, WA/BSA and T/$\sqrt{BSA}$ than non-elderly asthmatics (Table 3). With regard to indices of air trapping, there were no differences in the inspiratory index of LAA% or MLD between the two groups. The full-expiration values of LAA% were significantly higher, while those of MLD were significantly lower, in elderly than in non-elderly asthmatics (Table 3). Moreover, the E/I ratios of LAA% and MLD were significantly higher in the elderly asthmatic than in the non-elderly asthmatic group (Table 3).

IOS Measurements

Elderly asthmatics had significantly higher R5 values than non-elderly asthmatics (Table 4). There was no difference in R20 between the two groups. R5 - R20 was significantly higher in elderly asthmatics compared to non-elderly asthmatics. Elderly asthmatics also had lower X5 and higher AX and Fres than non-elderly asthmatics. Additionally, (R5 – R20)/R5, which was calculated to exclude the effect of age or physique, was also higher in elderly than in non-elderly asthmatics (Table 4).

Other Clinical Measurements

Table 5 shows the comparisons of inflammatory and airway responsiveness markers between elderly and non-elderly asthmatics. FeNO was similar between the two groups. Further, no significant differences were observed in induced sputum cell differentials or proportions of each inflammatory subtype between elderly and non-elderly asthmatics, although sputum neutrophils were marginally (P = 0.08) increased in elderly asthmatics. There were also no significant differences in blood eosinophils, neutrophils, and the parameters of airway sensitivity (Dmin) or airway reactivity (SRrs) between elderly and non-

elderly asthmatics. Blood neutrophils were only marginally ($P = 0.05$) increased in elderly asthmatics.

The differences in IgE and atopic status between elderly and non-elderly asthmatics are shown in Table 6. Serum total IgE levels were significantly lower in elderly asthmatics compared to non-elderly asthmatics. There were fewer subjects who were positive for at least one specific IgE antibody (so called “atopic”) among elderly as compared to non-elderly asthmatics. Elderly asthmatics had lower positive rates of specific IgE against cat dander, dog dander, house dust, mites, Japanese cedar pollen and mixed graminea pollens than non-elderly asthmatics.

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. We comprehensively studied the pathophysiological characteristics of elderly asthmatics, demonstrating prominent large and small airway involvement, but less atopic status, compared with non-elderly asthmatics.

Although the predominant site of airway disease (large or small airways) may vary from patient to patient[41], the determinants of such variations are poorly known.[42] In this study, spirometric values of FEF_{25-75}, as well as FEV_1, were significantly lower in elderly than in non-elderly asthmatics. Since 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 non-elderly asthmatics, these results demonstrate that elderly asthma more prominently involves obstruction of both small and large airways, independent of aging *per se* or duration of disease.

CT indices of large airway dimensions reflect the histologic changes in airway walls in patients with asthma.[43] Our results indicated that elderly asthmatics had thicker large airway walls than non-elderly asthmatics. Bai et al. examined postmortem lungs of young (19.1 ± 0.5 yrs-old; n=14) and middle-aged (42.6 ± 1.0 yrs-old; n=13) fatal asthma patients. The middle-aged group who had longer disease duration (24.0 ± 13.4 yrs) showed significantly thicker total airway wall and smooth muscle layer than the younger group whose disease duration was 7.6 ± 4.8 yrs.[44] Bai's study provided pathological evidence of the progressive effects of aging and/or long-standing disease on airway remodeling, although further evidence supporting his pioneering study has been scarce. Our results confirm these findings radiologically, and additionally, also suggest that elderly disease, independent of disease duration, may contribute to remodeling of large airway walls. Elevated sputum levels of

TIMP-1 (tissue inhibitors of matrix metalloproteinases [MMP]) over those of MMP-9 are associated with airway wall thickening, as assessed by CT.[23] Activity of MMP decreases and that of TIMP 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] Both age-related alteration in collagen synthesis and degradation, and asthma-specific airway inflammation may synergistically contribute to the progression of airway remodeling in elderly asthmatics.

To evaluate small airway involvement, HRCT indices, represented as decreased lung attenuation (measured by LAA% and MLD), have been quantified among patients with asthma.[8, 9] The ratios of LAA% or MLD between expiration and inspiration (E/I ratio) are also 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, we demonstrated that elderly subjects with asthma had higher LAA% and lower MLD than non-elderly subjects. The differences in E/I ratios between the two groups were more significant than those of full-expiration values. We 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 may result in decreased lung attenuation at inspiration.[48] However, since both inspiratory LAA% and MLD were almost equivalent between elderly and non-elderly asthmatics in this study, these effects of aging, *per se,* were not likely to have affected our results.

In terms of the IOS results, asthma in the elderly was more prominently associated with small airway abnormalities, as reflected by R5 - R20, X5, AX and Fres, as compared with

that in the non-elderly. These findings were consistent with the results of spirometry and lung density measurements on CT discussed above. RV/TLC, a conventional parameter of air trapping, did not differ between elderly and non-elderly asthmatics. We previously reported that IOS, but not RV/TLC, could detect the improvement of small airway abnormalities in asthmatics treated with ultra-fine particle inhaled corticosteroids.[11] The present study confirms that IOS is a sensitive and useful measure to detect small airway abnormalities. However, each IOS parameter may be influenced by subject age, in addition to height, according to two normative population studies.[30, 31] Moreover, the predictive equations of IOS parameters among the Japanese population have not been well established. To exclude the effect of age, we calculated the corrected R5 - R20 by dividing it by R5. The difference between the groups in (R5 - R20)/R5 values was still prominent (significantly higher in elderly than in non-elderly asthmatics, with $P < 0.001$). However, further evaluation is needed to validate the utility of IOS measurements for assessing small airway involvement.

In a number of studies, the neutrophil count in induced sputum was reportedly greater in the elderly than the non-elderly, both in normal[49] and asthmatic subjects,[17, 18] despite the existence of conflicting reports.[19] In the present study, there were no differences in induced sputum cell differentials between elderly and non-elderly asthmatics, although elderly subjects had marginally increased neutrophils in their sputum and blood as compared with non-elderly asthmatics. We 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 non-elderly asthma group than in the elderly asthma group may also have affected the results, because smoking has been linked to sputum neutrophilia.[50] Irrespective of the group (elderly or non-elderly asthmatics), our study cohort showed relatively low eosinophil and high neutrophil counts in induced sputum. This could also probably be because ICS treatment attenuates eosinophilia and induces sputum neutrophilia,[51] since all our study subjects were

taking ICS (800 μg/day by median), while less than half of the subjects in previous studies received ICS therapy.[17, 18] Moreover, our cohort predominantly comprised moderate to severe asthma patients, who are known to have sputum neutrophilia.[52] Our FeNO results are consistent with the literature that FeNO levels are unrelated to age.[53]

In terms of airway responsiveness, it is controversial whether elderly asthmatics are more responsive than non-elderly asthmatics.[54, 55] In our study, neither airway sensitivity nor airway reactivity differed significantly between elderly and non-elderly asthmatics.

Elderly asthmatics had lower total IgE levels, a lower prevalence of subjects with at least one positive allergen-specific IgE antibody, and a lower positive response to many of the allergen specific antibodies, than non-elderly asthmatics in this study. Due to the decreased production of IgE with aging,[56] a feature of "immunosenescence",[57] the proportion of nonatopic (or "intrinsic") asthma becomes dominant among elderly populations, reaching up to more than 50%. This was also confirmed by our study.

Elderly asthmatics are often assumed to include two 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 as compared to those with short-duration asthma.[59] In this study, more than half (n=57) of 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 to 40.35 years).[20] Besides, the number of subjects with long-standing asthma was limited even in the elderly asthma group. Therefore, we 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 non-elderly asthmatics. Therefore, we consider that the effect of disease duration on our 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 subjects with asthma. We could not recruit non-asthmatic elderly controls due to the difficulties in performing CT and induced sputum and airway responsiveness tests in normal subjects. In our previous clinical studies that involved healthy controls[60,61], control subjects were recruited from our hospital staff, whose mean ages were much younger than the defined elderly (51 years[60] and 33 years[61], respectively). It was actually impossible for us to recruit healthy elderly subjects aged > 65 years from our hospital staff; almost all ordinary university employees in Japan retire at the age of 60 or 65. Moreover, we do not have a system or custom to recruit healthy volunteers for research purpose from the public utilizing public information such as the Internet by providing them a reward. The lack of controls may preclude determination of whether the results observed are due to age itself or an age-asthma interaction. To eliminate the effect of aging, we therefore used percentages of predicted values for spirometry,[25, 26] and (R5-R20)/R5 for IOS measurements, both of which yielded significant results indicating a greater amount of large and small airway involvement in elderly asthmatics. We 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 two groups. As a result, the prevalence of ex-smokers was higher in the non-elderly asthmatics (12 out of 67; 18%) than in the elderly asthmatics (1/ of 45; 2%). However, although the difference was statistically significant, smoking index of the whole 67 non-elderly asthmatics was as low as 0.34 ± 1.1 pack-year, which did not significantly differ from that of the 45 elderly asthmatics (0.11 ± 0.75 pack-year). Indeed, additional analyses excluding 13 ex-smokers showed similar results for CT and IOS measurements (data not shown). Even if the difference in smoking exposure between the two groups was clinically relevant, our results that airway abnormalities as examined by spirometry, IOS and CT was more pronounced in

the elderly asthmatics than in the non-elderly asthmatics, who had smoked more than the former group, may even be further strengthened. Third, a subset of subjects did not undergo spirometric-gated inspiratory and expiratory CT. In such cases, the full inspiratory and 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 was comparable with those assessed by spirometric-gated CT in patients with COPD. Fourth, only subsets of patients were analyzed for each measurement. This is partly because we only collected data of all clinical measurements that were performed within 4 weeks of the date of CT measurements, in order to maintain integrity of the data. With respect to sputum induction, we previously reported that the success rate of sputum induction in consecutive 407 asthmatic subjects 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 our present patients.

We conclude that elderly patients with asthma have more prominent large and small airway involvement, as well as less atopy, than non-elderly asthma patients. Despite a number of limitations, our results may provide a better understanding of the pathophysiology and future therapeutic strategies for asthma in the elderly.

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618 **TABLES**

619 **Table 1** ***Subject Characteristics***

	Elderly Asthmatics (>65 yrs)	Non-elderly Asthmatics (≤65 yrs)	*P* Value
Subjects, n	45	67	
Sex, male/female	11/34	21/46	NS
Age, yrs	73.1 ± 5.3	48.6 ± 12.9	<0.001
Disease duration, yrs	12.7 ± 16.2	8.0 ± 10.5	NS
Exacerbations, n/yr	0 (0 – 2.5)	0 (0 - 3)	NS
Severity (step 1/2/3/4), n[a]	0 / 12 / 16 / 17	0 / 23 / 31 / 13	NS
Smoking, ex/never	1/44	12/55	0.01
Pack-years	0.11 ± 0.75	0.34 ± 1.1	NS
Dose of ICS, μg/d (equivalent to CFC-BDP)	800 (400 - 3200)	800 (200 - 2400)	NS
BMI, kg/m^2	23.0 ± 3.5	23.5 ± 4.1	NS
Allergic rhinitis, present	11 (24%)	36 (54%)	0.002
Atopic dermatitis, present	1 (2%)	6 (9%)	0.15

620 Data are expressed as number or median (range), except for age, disease duration, pack-years
621 and BMI, which are presented as mean ± SD. BMI = body mass index; CFC-BDP =
622 chlorofluorocarbon-11/12-beclomethasone dipropionate; ICS = inhaled corticosteroid; NS =
623 not significant.
624 [a]The clinical severity of asthma was defined by patient symptoms and lung function on
625 current therapy as step 1 (intermittent), step 2 (mild persistent), step 3 (moderate persistent)
626 or step 4 (severe persistent), according to the criteria of the Global Initiative for Asthma 2005.
627

Table 2 ***Comparison of Pulmonary Function Tests between Elderly and Non-elderly Asthmatics***

	Elderly Asthmatics (>65 yrs)	Non-elderly Asthmatics (≤65 yrs)	*P* Value
Spirometry, n	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)	0.02
FEV_1/FVC	0.718 (0.440 – 0.896)	0.784 (0.409 – 0.934)	0.001
FEF_{25-75}, %pred	50.9 (14.2 – 148)	78.6 (9.6 – 152)	0.03
Lung volume measurement, n	37 (82%)	57 (85%)	
RV/TLC, %pred	110 (81.3 – 187)	109 (67.1 – 258)	NS

Data are presented as median (range). FEF_{25-75} = mid-forced expiratory flow; NS = not significant; RV/TLC = residual volume/total lung capacity; %pred = percentage of predicted value.

634 **Table 3** ***Comparison of CT Measurements between Elderly and Non-elderly Asthmatics***

	Elderly Asthmatics (>65 yrs)	Non-elderly Asthmatics (≤65 yrs)	*P* Value
Central airway wall thickness, n	45 (100%)	67 (100%)	
WA%, %	61.7 (52.9 - 70.9)	57.6 (49.0 - 70.3)	<0.001
WA/BSA, mm^2/m^2	16.1 (10.3 - 22.4)	14.7 (9.2 - 19.8)	0.01
$T/\sqrt{BSA}$, mm/m	1.10 (0.90 - 1.40)	1.01 (0.75 - 1.21)	<0.001
Small airway involvement, n	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)	0.002
MLD, HU	-771 (-861 to -658)	-748 (-847 to -607)	0.003
E/I ratio			
LAA% E/I	0.46 (0.18 - 0.91)	0.33 (0.03 - 0.67)	<0.001
MLD E/I	0.91 (0.84 - 0.99)	0.88 (0.72 - 0.95)	<0.001

635 Data are presented as median (range). BSA = body surface area; E = expiration; HU =
636 Hounsfield unit; I = inspiration; LAA% = percentage of low attenuation area; MLD = mean
637 lung density; NS = not significant; T = airway wall thickness; WA = wall area.

Table 4 ***Comparison of Impulse Oscillation (IOS) Measurements between Elderly and Non-elderly Asthmatics***

	Elderly Asthmatics (>65 yrs)	Non-elderly Asthmatics (≤65 yrs)	*P* Value
IOS, n	25 (56%)	53 (79%)	
R5, $kPa \cdot s \cdot l^{-1}$	0.48 ± 0.20	0.35 ± 0.12	<0.001
R20, $kPa \cdot s \cdot l^{-1}$	0.34 ± 0.10	0.31 ± 0.09	NS
R5-R20, $kPa \cdot s \cdot l^{-1}$	0.14 ± 0.12	0.05 ± 0.05	<0.001
X5, $kPa \cdot s \cdot l^{-1}$	-0.23 ± 0.15	-0.12 ± 0.06	<0.001
AX, $kPa \cdot l^{-1}$	1.62 ± 1.8	0.44 ± 0.44	<0.001
Fres, $1 \cdot s^{-1}$	19.6 ± 7.9	12.8 ± 4.1	<0.001
(R5-R20)/R5	0.25 ± 0.16	0.12 ± 0.10	<0.001

Data are presented as mean ± SD. AX = the integrated area between 5Hz and Fres; Fres = frequency of resonance; NS = not significant; R5 = resistance at 5 Hz; R20 = resistance at 20 Hz; X5 = reactance at 5 Hz.

643 **Table 5** ***Comparisons of Exhaled Nitric Oxide levels (FeNO), Peripheral Blood Cell***
644 ***Differentials, Induced Sputum Cell Differentials, and Airway Hyperresponsiveness (AHR)***
645 ***between Elderly and Non-elderly Asthmatics***

	Elderly Asthmatics (>65 yrs)	Non-elderly Asthmatics (≤65 yrs)	*P* Value
FeNO, n	32 (71%)	53 (79%)	
FeNO, ppb	24.6 (5.9 – 98.6)	26.9 (10.3 – 110)	NS
Induced sputum cell differentials, n	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[a]			
n, E/N/P/M	4/8/5/7	13/9/5/8	NS
Blood cell differentials, n	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, n	26 (58%)	40 (60%)	
Dmin, unit	3.6 (0.09 - 50)	8.2 (0.15 - 50)	NS
SRrs, $cmH_2O/L/sec/mi$	1.38 (0.28 - 5.19)	1.49 (0.39 – 13.6)	NS

646 Data are presented as number or median (range). For eosinophils and neutrophils, median
647 percentages (range) are shown. AHR = airway hyperresponsiveness; Dmin = cumulative dose
648 of methacholine at the inflection point at which respiratory resistance began to increase;
649 FeNO = exhaled nitric oxide; NS = not significant; ppb = parts per billion; SRrs = the slope
650 of the methacholine–respiratory resistance dose-response curve.

651 [a] Subjects were classified into four inflammatory subtypes by induced sputum cell
652 differentials: eosinophilic (E; eosinophils ≥1.0%, neutrophils <61%), neutrophilic (N;
653 eosinophils <1.0%, neutrophils ≥61%), paucigranulocytic (P; eosinophils <1.0%, neutrophils
654 <61%) and mixed granulocytic (M, eosinophils ≥1.0%, neutrophils ≥61%).
655

656 **Table 6** ***Comparisons of IgE and atopic status between Elderly and Non-elderly***

657 ***Asthmatics***

	Elderly Asthmatics (>65 yrs)	Non-elderly Asthmatics (≤65 yrs)	*P* Value
Subject, n	45 (100%)	67 (100%)	
Serum total IgE, IU/mL	91 (5 - 2100)	210 (5 - 8700)	0.006
At least one positive specific IgE, n (%)	22 (49)	50 (75)	0.005
Positive rates of individual specific IgE, n (%)			
Cat dander	2 (4.4)	12 (17.9)	0.03
Dog dander	1 (2.3)	14 (20.9)	0.005
House dust	12 (27.3)	34 (50.8)	0.01
Mites (*Dermatophagoides pteronyssinus*)	13 (28.9)	34 (50.8)	0.02
Japanese cedar pollen	9 (20.0)	40 (59.7)	<0.001
Mixed graminea pollens	4 (8.9)	16 (23.9)	0.04
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

658 Data are presented as number (%) or median (range). NS = not significant.