

Influence of long-term cigarette smoking on immunoglobulin E-mediated allergy, pulmonary function, and high-resolution computed tomography lung densitometry in elderly patients with asthma

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Summary

Background Smoking is the most important cause of chronic obstructive pulmonary disease (COPD). However, the influence of cigarette smoking on the pathogenesis of asthma in the elderly remains controversial. This study attempted to clarify the influence of cigarette smoking on elderly asthmatics.

Methods Forty-eight asthmatics over 70 years old (25 ex-smokers and 23 never-smokers) and 20 patients with COPD over 70 years old (all ex-smokers) were studied to determine the influence of cigarette smoking on IgE-mediated allergy (total IgE, IgE antibodies against inhalant allergens, bronchial hyper-responsiveness (BHR), generation of leukotriene (LT) B₄ and C₄), pulmonary function, and the relative area of lung showing attenuation values less than –950 Hounsfield units (RA₉₅₀) on high-resolution computed tomography scans.

Results The incidence of positive IgE antibodies against inhalant allergens, BHR, and the generation of leukotriene B₄ (LTB₄) by leucocytes were significantly increased in patients with a history of smoking compared with those without. Residual volume (%RV) was significantly increased, and diffusing capacity for carbon monoxide was significantly decreased in ex-smokers with asthma and COPD compared with never-smokers with asthma. Inspiratory RA₉₅₀ and ratio of expiratory RA₉₅₀ to inspiratory RA₉₅₀ were significantly larger in asthmatics with a smoking history than in those without, and in COPD patients than in asthmatics.

Conclusion Cigarette smoking enhances the production of IgE antibodies, BHR, and generation of LTB₄ by leucocytes in elderly asthmatics. Increased hyper-inflation or emphysematous changes of the lungs expressed by increased RA₉₅₀, closely related to %RV, was more frequently observed in ex-smokers compared with never-smokers.

Keywords cigarette smoking, elderly asthmatics, IgE-mediated allergy

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Introduction

Smoking is closely related to the onset mechanisms of chronic obstructive pulmonary disease (COPD) [1, 2]. It has been shown that 80–90% of all COPD patients have a history of smoking [3], and patients with mild COPD can reduce their symptoms by stopping smoking [4]. In childhood asthma, epidemiologic studies suggest that children raised in the home of a cigarette smoker display a higher incidence of asthma than children raised in the home of non-smokers. An association between smoke exposure and the induction [5]

or exacerbation of asthma has also been reported [6]. Cigarette smoke exposure with allergens was found not to induce allergic airway responses in non-susceptible animals. Exposure to cigarette smoke increased sensitization to food allergens in the first few years of life [7], but was not associated with sensitization to inhaled allergens [8]. However, cigarette smoke exposure enhanced existing symptoms of allergic airway disease (i.e. airway hyper-responsiveness (AHR)), independent of elevated Ig levels and eosinophilic inflammation [9].

With regard to adult asthma, a recent case-control study showed that adult onset of asthma was not associated with a history of smoking [10]. In contrast, current smoking has been shown to increase asthma severity [11], and higher incidences of asthma are found in current and former smokers, compared with never-smokers [12–14]. Our previous studies using relative areas of lung showing attenuation values less than –950 Hounsfield units (HU) (RA₉₅₀) on

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high-resolution computed tomography (HRCT) scans revealed that HRCT lung densitometry was influenced by ageing and smoking [15, 16]. However, the influence of long-term cigarette smoking on the pathophysiology of asthma among elderly patients remains unclear.

In the present study, we compared IgE-mediated allergy related to bronchial hyper-responsiveness (BHR), and generation of leukotriene (LT) B_4 and C_4 , pulmonary function, and CT lung density between ex- and never-smokers in elderly asthmatics, and between subjects with asthma and COPD.

Methods

Subjects

Forty-eight asthmatic subjects over 70 years old (15 women and 33 men; mean age, 74.3 years) and 20 subjects with COPD over 70 years old (all men; mean age, 75.7 years) were recruited from Misasa Medical Center. Asthma and COPD were diagnosed according to the definition proposed by the American Thoracic Society [17]. All subjects with asthma had episodic symptoms of wheezing and coughing and experienced symptomatic relief and reversible airway response with an accompanying increase exceeding 15% in forced expiratory volume in 1 s (FEV_1) upon treatment with β_2 -adrenergic agonist. Twenty-five of the asthmatics had a history of smoking >20 years (49.1 pack-years). All the 25 were ex-smokers (mean age, 74.3 years; age at onset of the disease, 58.1 years). The remaining 23 asthmatics were never-smokers (mean age, 74.4 years; age at onset, 55.7 years). All patients with COPD had a history of progressive symptoms such as dyspnoea and cough, airways obstruction with FEV_1 <80% predicted and FEV_1 /forced vital capacity (FEV_1 /FVC) ratio <70%, no spirometric response to β_2 -adrenergic agonist (FEV_1 increase lower than 200 mL and 15% of baseline values), and a history of smoking >20 years (52.0 pack-years). All of them were ex-smokers. Patients with a history of atopy, asthma or other respiratory diseases, and positive RAST score or positive skin test against inhalant allergens were excluded from the study. All ex-smokers with asthma and COPD had stopped smoking for at least 6 months. All subjects were stable with no changes in respiratory symptoms and medication for at least 1 month.

Patients with a history of smoking were classified into two groups according to the ratio of expiratory low attenuation area (LAA) < -950 HU of the lungs on HRCT to inspiratory LAA: ≤ 0.5 and > 0.5 as described later. Serum IgE was measured by radioimmunosorbent test (RIST), and IgE antibodies specific to 12 common aeroallergens including house dust mite, pollens, moulds, and animal danders were measured using the Pharmacia CAP system (Pharmacia Diagnostics AB, Uppsala, Sweden).

Informed consent was obtained from all subjects, and the study protocol was approved by the ethics committee of our institution.

Bronchial responsiveness

Bronchial responsiveness to methacholine was measured by an Astograph (TCK6100, Chest Co., Tokyo, Japan).

Different concentrations of methacholine (49, 98, 195, 390, 781, 1563, 3125, 6250, and 12 500 μ g/mL) were prepared for bronchial challenge according to the method used by Chai et al. [18].

The increase in total respiratory resistance (R_{rs}) after methacholine inhalation was measured by the oscillation technique. A cumulative dose of methacholine causing a significant increase in R_{rs} was assessed as the minimum dose (D_{min}). The units for D_{min} are equivalent to 1 min of aerosol inhalation at 1 mg/mL during quiet tidal breathing. All medications were stopped 12 h prior to examination.

Leukotriene (B_4 , C_4) generation

The amount of leukotrienes, LTB_4 , and LTC_4 , generated by peripheral leucocytes was assessed as described previously [19–21].

Five millilitres of 6% dextran (molecular weight 200 000 kDa) (Nacalai Tesque Inc., Kyoto, Japan) was added to 20 mL heparinized peripheral blood, and the resultant mixture was incubated for 1 h at room temperature. The leucocyte-rich plasma supernatant was then removed and used. The number of cells was adjusted to 5×10^6 cells/mL in Tris CM buffer, and the cells were then incubated with 1 μ g calcium ionophore A23187 (Sigma, St Louis, MO, USA) for 15 min at 37 °C. After a $4 \times$ volume of prechilled ethanol (final, 80% ethanol) was added, the mixture was centrifuged at $1870 \times g$ for 30 min. A syringe filter (Toyo Roshi Co., Tokyo, Japan) was used to draw the supernatant, and the filtrate was decompressed and dried to a solid. Quantification of LTB_4 and LTC_4 was performed by high-performance liquid chromatography (HPLC) and ultraviolet (UV) spectroscopy, following the method of Lam et al. [22]. Quantities of LTB_4 and LTC_4 were expressed as nanograms per 5×10^6 cells.

Pulmonary function tests

Spirometry was performed by means of a CHESTAC 33 (Chest Co.) linked to a computer. The following measurements were performed on all subjects: FVC, FEV_1 , and FEV_1 /FVC. Residual volume (RV) was measured using body plethysmography (Autobox 2800, Chest Co.). The diffusing capacity for carbon monoxide (DL_{CO}) was measured by the single-breath technique using a CHESTAC 33. FVC, FEV_1 , RV, and DL_{CO} measurements for each patient were expressed as percentages of predicted values.

CT

All subjects underwent non-contrast HRCT scan of the lungs using a Toshiba Xpeed scanner (Toshiba, Tokyo, Japan) with 2 mm collimation, scanning time 2.7 s, voltage 120 kVp, and current 200 mA.

Maximal inspiratory and maximal expiratory HRCT scans were obtained at the following three anatomic levels, as described by Miniati et al. [23]: (1) top of the aortic arch, (2) origin of the lower lobe bronchus, and (3) 3 cm above the top of the diaphragm. The RA_{950} from the three anatomic lung levels was obtained both at full inspiration (inspiratory RA_{950}) and full expiration (expiratory RA_{950}). LAA values were expressed as the mean of inspiratory RA_{950} at three

anatomic lung levels. The ratio of expiratory RA₉₅₀ to inspiratory RA₉₅₀ (E/I ratio) was also calculated. The ratio was divided into two degrees described above: ≤ 0.5 and > 0.5 . The ratio > 0.5 was shown only in asthmatics with a history of smoking.

Statistical analysis

All statistical analyses were carried out using Stat View software (SAS Institute Inc., Cary, NC, USA). The results were expressed as means $\pm$ SD. Comparison of multiple groups was performed using ANOVA with Bonferroni/Dunn correction. The differences between groups were judged with the unpaired *t*-test or the Mann–Whitney *U*-test for continuous data, and with χ^2 tests for categorical data. A *P*-value of < 0.05 was regarded as statistically significant.

Results

IgE-mediated allergy

Serum IgE levels did not differ significantly between ex- and never-smokers in elderly patients with asthma. The frequency of patients with a positive RAST score against inhalant allergens was significantly increased in asthmatics with a history of smoking compared with those without ($P < 0.05$), as shown in Table 1. With regard to BHR to methacholine, $\log(D_{\min})$ was significantly lower in asthmatics with a smoking history than in those without ($P < 0.05$) or patients with COPD ($P < 0.01$) (Fig. 1).

The generation of LTB₄ by peripheral leucocytes was significantly larger in ex-smokers with asthma ($P < 0.001$) and in subjects with COPD ($P < 0.001$) than in never-smokers with asthma. The amounts of LTB₄ generated by ex-smoker asthmatics and COPD patients did not differ significantly. The leucocytes of asthmatics with and without a smoking history generated significantly more LTC₄ than those of COPD patients ($P < 0.05$). However, no significant difference was found in the production of LTC₄ between ex- and never-smokers in elderly asthmatics (Fig. 2).

Pulmonary function

In patients with asthma, values of %FVC, %FEV₁, and FEV₁/FVC tended to be larger in never-smokers compared with ex-smokers; however, these differences were not significant. The %RV value was significantly larger in ex-smoker asthmatics and in COPD patients than in never-smoker asthmatics ($P < 0.01$). The %DL_{CO} was significantly decreased in ex-smokers with asthma and COPD compared with never-smokers with asthma ($P < 0.01$). The values of %RV and %DL_{CO} did not differ significantly between ex-smokers with asthma and COPD (Table 1).

LAA of the lungs on HRCT

The inspiratory RA₉₅₀ was significantly larger in ex-smoker asthmatics ($P < 0.001$) and in COPD patients ($P < 0.001$) than in never-smoker asthmatics, and larger in COPD patients than in ex-smoker asthmatics ($P < 0.001$) (Fig. 3).

Table 1. Characteristics of subjects studied

	Asthma		
	Smoking history (–)	Smoking history (+)	COPD
Number of subjects, <i>n</i>	23	25	20
Male/female, <i>n</i>	8/15	25/0	20/0
Age, years (SD)	74.4 (3.2)	74.3 (4.6)	75.7 (4.6)
Age at onset, years (SD)	55.7 (11.5)	58.1 (8.3)	67.9 (5.3)*†
Disease duration, years (SD)	18.7 (11.5)	16.2 (6.6)	7.8 (4.0)*†
Smoking, pack-years (SD)	0	49.1 (22.8)*	52.0 (27.1)*
Log ₁₀ (IgE, IU/mL) (SD)	2.34 (0.57)	2.35 (0.58)	1.85 (0.53)**††
Positive RAST, %	52.2	88.0**	0*†
FVC, %pred (SD)	84.0 (15.1)	80.1 (16.0)	73.9 (12.1)
FEV ₁ , %pred (SD)	77.2 (23.1)	68.7 (19.2)	53.6 (16.7)*††
FEV ₁ /FVC, % (SD)	65.8 (10.5)	59.1 (10.7)	43.4 (7.6)*†
RV, %pred (SD)	131.1 (26.5)	170.1 (40.9)*	193.1 (27.5)*
DL _{CO} , %pred (SD)	88.4 (17.2)	70.1 (17.6)*	64.0 (10.1)*

Values are presented as mean and standard deviation (SD) in parentheses. Positive RAST, frequency of subjects with a positive RAST score against inhalant allergens; FVC, forced vital capacity; %pred, percentage of the predicted value; FEV₁, forced expiratory volume in 1 s; RV, residual volume; DL_{CO}, diffusing capacity of the lung for carbon monoxide; COPD, chronic obstructive pulmonary disease. *, **, $P < 0.01$, $P < 0.05$ vs. asthma without a smoking history. †, ††, $P < 0.01$, $P < 0.05$ vs. asthma with a smoking history.

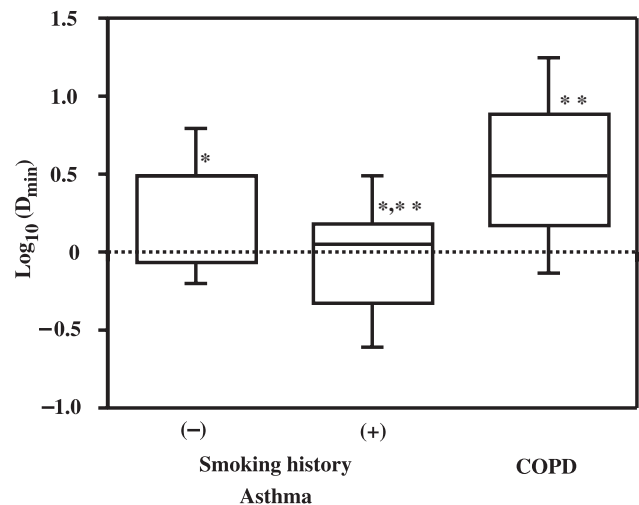


Fig. 1. Bronchial hyper-responsiveness to methacholine in patients with asthma and chronic obstructive pulmonary disease. * $P < 0.05$, ** $P < 0.01$.

The E/I ratio was also significantly higher in ex-smoker asthmatics ($P < 0.001$) and in COPD patients ($P < 0.001$) than in never-smoker asthmatics, and larger in COPD patients than in ex-smoker asthmatics ($P < 0.001$) (Fig. 4). Eleven of 25 asthmatics (44.0%) with a history of smoking showed a ratio > 0.5 ; in contrast, all asthmatic patients without a smoking history showed a ratio ≤ 0.5 .

Table 2 shows the comparison of clinical characteristics between subjects with a ratio > 0.5 and those with a ratio

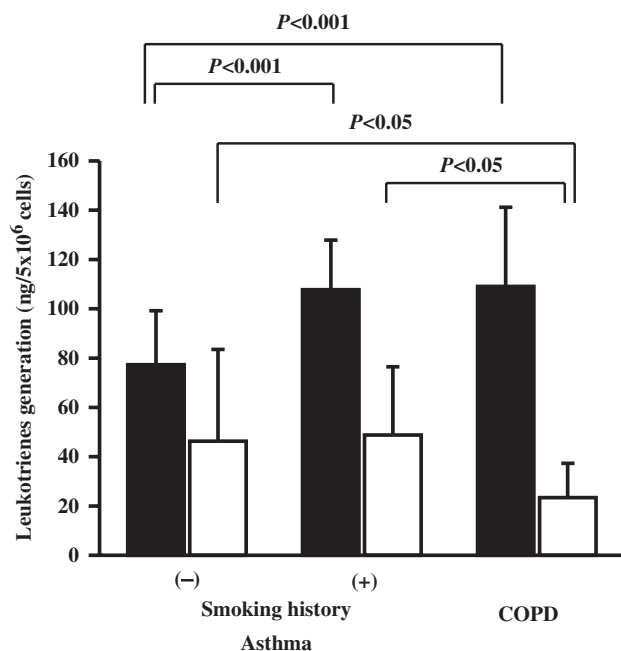


Fig. 2. Generation of leukotriene B₄ (LTB₄) (■) and C₄ (LTC₄) (□) by leucocytes in patients with asthma and chronic obstructive pulmonary disease.

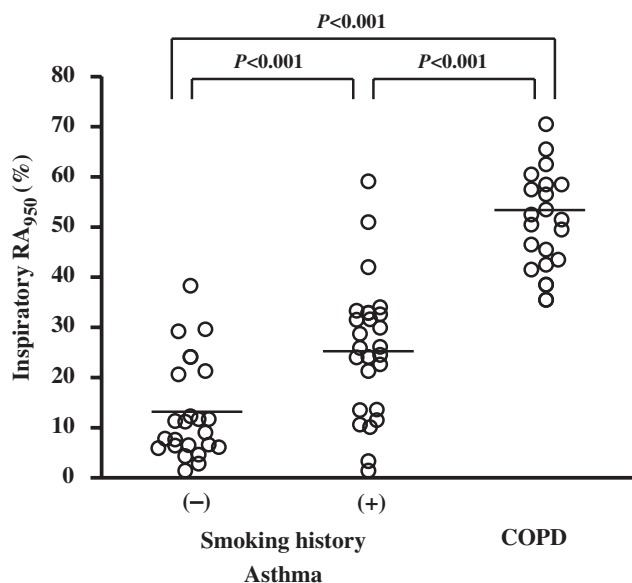


Fig. 3. Relative areas of lung showing attenuation values less than -950 Hounsfield units (RA₉₅₀) at full inspiration on high-resolution computed tomography in patients with asthma in relation to smoking.

≤0.5 in asthmatics with a history of smoking. No significant differences in serum IgE levels, frequency of patients with a positive RAST score, BHR, and generation of LTB₄ and LTC₄ by peripheral leucocytes were observed between groups. In subjects with a ratio >0.5, significantly larger %RV was observed compared with those with a ratio ≤0.5. The %DL_{CO} was decreased in patients with a ratio >0.5 compared with those ≤0.5 (Table 2).

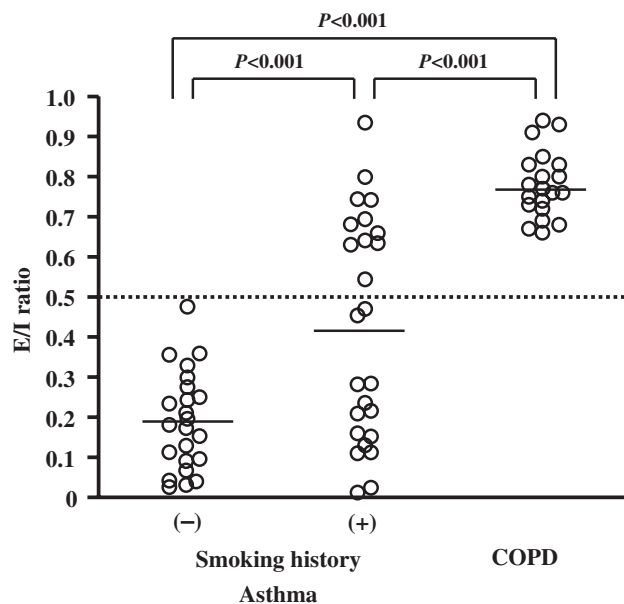


Fig. 4. Ratio of expiratory RA₉₅₀ to inspiratory RA₉₅₀ (E/I ratio) in patients with asthma in relation to smoking.

Table 2. Characteristics of asthmatics with a smoking history classified by E/I ratio of the lungs on HRCT

	E/I ratio		Differences
	≤0.5	>0.5	
Number of subjects, <i>n</i>	14	11	NA
Age, years (SD)	73.3 (3.7)	75.5 (5.5)	NS
Age at onset, years (SD)	56.8 (6.9)	59.7 (10.0)	NS
Disease duration, years (SD)	16.5 (6.1)	15.8 (7.4)	NS
Smoking, pack-years (SD)	47.3 (27.2)	51.5 (16.6)	NS
Log ₁₀ (IgE, IU/mL) (SD)	2.27 (0.62)	2.46 (0.54)	NS
Positive RAST, %	85.7	90.9	NS
Log ₁₀ (<i>D</i> _{min} , units) (SD)	0.10 (0.38)	-0.20 (0.42)	NS
LTB ₄ generation, ng/5 × 10 ⁶ cells (SD)	108.1 (18.1)	107.1 (23.4)	NS
LTC ₄ generation, ng/5 × 10 ⁶ cells (SD)	43.9 (23.6)	55.0 (32.4)	NS
FVC, %pred (SD)	86.6 (12.7)	71.4 (16.4)	<i>P</i> <0.05
FEV ₁ , %pred (SD)	73.1 (17.2)	63.2 (21.0)	NS
FEV ₁ /FVC, % (SD)	60.5 (11.8)	57.2 (9.4)	NS
RV, %pred (SD)	154.9 (36.5)	189.5 (38.1)	<i>P</i> <0.05
DL _{CO} , %pred (SD)	76.9 (15.2)	61.4 (17.0)	<i>P</i> <0.05

Values are presented as mean and SD in parentheses. RA₉₅₀, relative areas of lung showing attenuation values less than -950 Hounsfield units; E/I ratio, a ratio of expiratory RA₉₅₀ to inspiratory RA₉₅₀; positive RAST, frequency of subjects with a positive RAST score against inhalant allergens; *D*_{min}, minimum dose of methacholine causing bronchospasm; LTB₄, LTC₄, leukotrienes B₄ and C₄; FVC, forced vital capacity; %pred, percentage of the predicted value; FEV₁, forced expiratory volume in 1 s; RV, residual volume; DL_{CO}, diffusing capacity of the lung for carbon monoxide; NA, not available; NS, not significant; HRCT, high-resolution computed tomography.

Discussion

Whether cigarette smoke exposure and the development of asthma are directly related remains controversial. In general,

cigarette smoke exposure is associated with the development of asthma in children. Environmental tobacco smoke (ETS) exposure has been found to be associated with respiratory symptoms in children [8, 24]. The prevalence of physician-diagnosed asthma is significantly larger in never-smokers with childhood ETS exposure compared with that in non-exposed subjects [25]. Furthermore, higher incidences of asthma have been observed in current and former smokers, compared with never-smokers [12–14]. In these cases, what role does cigarette smoke play?

In utero exposure of rats to aged and diluted sidestream (SS) cigarette smoke induces AHR in the offspring [26].

Cigarette smoke-induced AHR is reportedly not associated with elevated Ig and eosinophilic inflammation [9]. Cigarette smoke has an adjuvant effect on eosinophils, allergen-specific antibodies, and Th 2 cytokines (IL-2 and IL-10) in adult mice previously sensitized by injection with ovalbumin and aluminium hydroxide [27].

In the present study, a significantly higher incidence of patients having IgE antibodies specific to inhalant allergens was found in asthmatics with a history of smoking compared with those without a smoking history. In ex-smoker asthmatics, BHR to methacholine was significantly increased compared with never-smoker asthmatics or patients with COPD. Furthermore, the generation of LTB₄ by leucocytes was significantly increased in ex-smoker asthmatics and COPD patients compared with never-smoker asthmatics. In previous studies, we have shown that the presence of specific IgE antibodies against inhalant allergens enhances LTC₄ generation, BHR, and the relationship between LTC₄ and airway obstruction [21], and further that increased generation of LTB₄ is closely related to BHR in patients with asthma [20]. These results suggest that cigarette smoke influences IgE-related allergy, including the production of IgE antibodies, BHR, and the generation of LTs in elderly patients with asthma.

Regarding the relationship between pulmonary function and cigarette smoking, although no significant correlation has been found between the frequency of lower respiratory illness (LRI) and decreased FEV₁ in sustained quitters, the frequency of LRI has an adverse effect on the 5-year averaged annual rate of decline of FEV₁ in intermittent and continuing smokers of patients with mild COPD [4]. In the present study, %RV value was significantly increased and %DL_{CO} was significantly decreased in asthmatics with a smoking history compared with those without. No significant differences in %RV and %DL_{CO} were observed between ex-smokers with asthma and COPD. The results demonstrate that cigarette smoking enhances hyper-inflation of the lungs, as expressed by an increase in RA₉₅₀ and %RV in elderly patients with asthma.

The RA₉₅₀ on HRCT scans obtained at full inspiration has been shown to offer an objective measure of the extent of pulmonary emphysema by comparison with the histopathologic data [28, 29]. Previous studies have demonstrated that RA₉₅₀ is significantly larger in asthmatics with a smoking history compared with those without a smoking history [15]. RA₉₅₀ is also influenced by ageing and disease severity in asthmatics without a smoking history [16]. Here, the influence of cigarette smoking on inspiratory RA₉₅₀ and the E/I ratio, which is closely correlated with increased %RV, was

examined in elderly patients with asthma. Both inspiratory RA₉₅₀ and the E/I ratio were significantly higher in asthmatics with a smoking history and patients with COPD than in asthmatics without a smoking history. A ratio >0.5 was observed only in patients with a history of smoking. Furthermore, %RV in patients with E/I ratio >0.5 was significantly higher compared with %RV in those with a ratio ≤0.5. These results suggest that cigarette smoking enhances the LAA of lungs, and the ratio of expiratory LAA to inspiratory LAA on HRCT. Previously, we demonstrated that RA₉₅₀ correlates with parameters of airflow limitation (%FEV₁, FEV₁/FVC, and the mean forced expiratory flow during the middle half of the FVC (%FEF_{25–75})) and lung volume (total lung capacity (%TLC), functional residual capacity (%FRC), and %RV), but not with lung transfer factor (%T_{LCO}/VA) [16]. The results of HRCT lung densitometry in the present study may indicate that increases in both LAA of the lungs and the ratio of expiratory LAA to inspiratory LAA by cigarette smoking lead to disease severity.

In conclusion, our study shows that cigarette smoking may influence IgE-related allergy, including the production of IgE antibodies, BHR, and generation of LTs in elderly patients with asthma. We also demonstrate that cigarette smoking may enhance hyper-inflation or emphysematous changes of the lungs expressed by increased RA₉₅₀, closely related to %RV.

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