Airway wall structure assessed by endobronchial ultrasonography and bronchial hyperresponsiveness in patients with asthma

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Airway wall thickness and mural structure assessed by endobronchial ultrasonography

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Abstract

Background: Endobronchial ultrasonography (EBUS) is useful to assess the laminar structure of the bronchial wall. The purpose of this study was to assess the comparability of EBUS with high resolution CT (HRCT) for measuring whole bronchial wall thickness and to validate the use of EBUS as a tool for the assessment of bronchial wall structures in asthma.

Methods: Ten patients with stable asthma and eleven patients without asthma were studied. EBUS was performed with a radial 20-MHz ultrasonic probe into the intermediate bronchus undergoing fiberoptic bronchoscopies to assess the each layers of airway wall. A cross-section of the apical bronchus of the right upper lobe was examined from HRCT. The percentage airway wall thickness (WT%; defined as [(ideal outer diameter – ideal luminal diameter)/ideal outer diameter] x 100) and the percentage airway wall area (WA%; defined as (wall area/total airway area) x 100) were determined from both EBUS and HRCT to assess whole airway wall thickness. Bronchial hypereresponsivness was measured by inhalation of increasing concentration of methacholine using tidal breathing method.

Results: WT% and WA% measured by EBUS image were significantly higher in the patients with asthma than without asthma, respectively ($p<0.01$, $p<0.01$). WT% and WA% of the intermediate bronchus measured by EBUS were positively correlated with those of the right apical bronchus measured by HRCT images, respectively ($r=0.72$, $p<0.01$ and $r=0.72$, $p<0.01$). The thickness of the second layer of the patients with asthma was grater than that of the patients without asthma when evaluating laminar structure by using EBUS ($p<0.05$). PC20-FEV1 was negatively correlated with the thickness of the second layer ($r= -0.67$, $p<0.05$).

Conclusion: EBUS is able to evaluate the five different layers of cartilaginous bronchi. Therefore, EBUS may be useful to assess airway wall remodeling by evaluating bronchial mural structure in vivo.

Introduction

Airway wall thickening in patients dying of asthmatic attacks results from inflammation with edema and inflammatory cell infiltration and from structural changes, such as subepithelial fibrosis, mucous gland and goblet cell hyperplasia, and smooth muscle hypertrophy and hyperplasia (1,2). This structural change is considered a feature of airway wall remodeling, which is resulted from chronic inflammation (3-5). This thicking could be as important as smooth muscle shortening in determining the airway responsiveness of asthmatic patients (6).

Remodelling of the airway wall has been assessed using bronchial biopsy specimens (3,7-13) as well as inflammatory changes of airway using high resolution computed tomography (HRCT) (14-19). The assesment using HRCT technique involved radiation risk and is not applicable for bronchial mural structure. The invasive diagnostic tools such as bronchial biopsy using fiberoptic bronchoscopy is risk for hemorrhage from biopsied bronchial wall. Furthermore, it is often difficult to analysis the laminar structure of bronchial wall because of insufficient and/or damaged specimens.

The endobronchial ultrasonography (EBUS) is successfully used for the assessment of mediastinal lymph nodes and other mediastinal structures (20,21). Recently, EBUS has been shown to be useful to assess the laminar structure of the bronchial wall (22-27). The needle puncture experimental studies showed that the correlation between the ultrasonographic images and the bronchial wall structure have been clarified (22,24). To our knowledge, it has not yet been studied on the sonographic layer structure in the patient with asthma.

The purpose of this study was to assess the comparability of EBUS with HRCT for measuring whole bronchial wall thickness and to validate the use of EBUS as a tool for the assessment of bronchial wall structures in patients with asthma. In addition, we investigated the relation between each layer's thickness and bronchial hyperresponsiveness.

Methods

Subjects

Ten patients with stable asthma diagnosed according to American Thoracic Society

criteria (28) and eleven non-asthmatic subjects without respiratory symptom were studied. The study was approved by the Ethics Committee of our hospital (National Hospital Organization Kanazawa Medical Center, Kanazawa, Japan) and written informed consent from the patients was obtained.

Fiberoptic Bronchoscopy

Subjects were given the intramuscular administration of 0.5 g of atropine sulfate, and 25 mg of hydroxyzine and the intravascular administration of 0.1 mg of midazolam. Topical anesthesia was obtained with the inhalation of 4 percent lidocaine and 2 percent lidocaine sprayed into the oral passage, directly instilled onto the vocal cords, and used as needed on the bronchial mucosa.

Endobronchial ultrasonography and assessment of bronchial wall thickness

For the endobronchial ultrasonography (EU-M 20 Endoscopic Ultrasound System; Olympus, Tokyo, Japan), a 2.6mm-diameter, 20-MHz frequency radial mechanical transducer type ultrasonic probe (UM-BS20-26R; Olympus; Tokyo, Japan) and a

flexible balloon sheath that was equipped with a balloon at the tip (MAJ-643R; Olympus; Tokyo) were utilized. We introduced them through the 2.8-mm-diameter channel of a flexible bronchoscope (model IT-260; Olympus; Tokyo, Japan). The balloon sheath was inflated three times into the intermediate bronchus, to the minimun amount saline required to cause contact with the airway wall and obtain a 360° image. The image by EBUS revealed the layered structure of the bronchial wall, which was recorded on paper (UP-880 Video Graphic Printer; Sony; Tokyo, Japan) and videotape (22).

We decided to take the cartilaginous portion of the bronchial wall into detailed consideration. From cartilage containing parts of bronchial wall, three EBUS images showing the most obvious and well-defined laminar structure was selected by a blinded. All airway measurements were conducted by a single observer in a blind fashion. Firstly, we measured the whole bronchial wall thickness (WT) and whole wall area (WA). WT was defined as (outer diameter - inner diameter). Ideal outer diameter (Do) and ideal luminal diameter (DL) were calculated as by tracing the external and internal perimeters, respectively. These parameters were measured directly using electric

calipers. Percentage wall thickness (WT%) was defined as [(Do-DL)/Do] x 100. Whole wall area is defined as [total airway area (Ao) - luminal area (Al)]. Percentage wall area (WA%) was defined as [(Ao-Al)/Ao] x 100. These parameters, including WT% and WA%, were calculated from a mean of three EBUS images (26).

Secondly, the thickness of each layer was measured (Fig.1). The cartilaginous portion of the extrapulmonary bronchi is visualized as five layers (21). The absolute values of the thickness of each layer were measured. The mean values of the three EBUS images were used.

High Resolution Computerd Tomography (HRCT) and the assesment of wall thickness

The thoracic HRCT scan system was performed at 120 KVp, 250 mA, 1 mm collimation, and pitch 1.0. To obtain one section through the apical segmental bronchus, 3.8 cm of lung were scanned in the helical section. The starting point of the scan was determined on scout film according to this value, assuming that scanning would be terminated at the origin of the right upper lobe bronchus. Images were reconstructed using the FC10 algorithm at 1-mm spacings. A targeted reconstruction of the right lung

was performed using a subject-specific field of view (FOV) (153 mm). Each image was composed of a 512 x 512 matrix of numeric data (CT numbers) in HU (18).

Image analysis was carried out using ExaVision from Zaiosoftwre. Ideal outer diameter was calculated as outer perimeter/π. Ideal luminal diameter was calculated as internal perimeter/π. Wall thickness (WT) was defined as outer diameter – luminal diameter. WT% was equal to WT/Do ratio in percentage. Wall area was defined as total airway area - luminal area. Total wall area and luminal area were traced and calculated on the personal computer. WA% was equal to WA/Ao ratio in percentage. These parameters were calculated from a mean of three HRCT images. These parameters were calculated from a mean of three HRCT images.

Methacholine Challenge

Methacholine was dissolved in physiological saline solution to produce doubling concentrations of 0.04, 0.08, 0.16, 0.31, 0.63, 1.25, 2.5, 5, 10, 20, 40, 80 and 160 mg/ml. Saline and each concentration of methacholine were inhaled from a DeVilbiss 646 nebulizer (DeVilbiss Co., Somerset, PA) operated by compressed air at 5 L/min. The

nebulizer output was 0.14 ml/min. Saline was inhaled first for 2 min and FEV1 was measured. If the change in FEV1 from the baseline value was less than 10%, inhalation of methacholine was started, and if the saline solution caused 10% or more change in FEV1, the test was stopped or postponed. Methacholine was inhaled for 2 min by tidal mouth breathing, and this was followed immediately by three measurements of flow-volume curves at 1-min intervals; the curve with the largest FVC was retained for analysis. Increasing concentrations of methacholine were inhaled until decrease of 20% or more in FEV1 occurred.

Statistical analysis

Data were expressed as means ± SD and analyzed with the StatView 5.0 program (SAS Institute, Cary, NC). An unpaired t *test*, the Mann-Whitney U test was used to compare groups. Spearman's rank correlation test or Pearson's correlation test was used to analyze relations between variables. P-values less than 0.05 were considered significant.

Results

Patient's characteristics

The characteristics of the patients with asthma and non-asthmatic subjects are summarized in Table1. Eleven non-asthmatic subjects included 5 patients with lung cancer, 1 granuloma, 1 mediastinal lymphadenopathy and 4 others (Table1). The mean (SD) FEV1, %predicted FEV1 and FEV1/FVC ratio were 1.70 (0.39) L, 67.2 (12.8) % and 57.4 (16.1) %, respectively, in the patients with asthma, and those values were significantly lower than those in the non-asthmatic subjects, respectively ($p<0.01$). The geometric mean (geometric standard error of the mean) PC20-FEV1 was 0.88 (1.27) in the patients with asthma, which was lower than that those in the non-asthmatic subjects ($p<0.01$).

Whole bronchial wall thickness measured by EBUS and HRCT

The mean (SD) WT% measured by EBUS images was 27.9 (4.2) % in the patients with asthma and 18.9 (2.6) % in the non-asthmatic subjects (Fig.2). The mean (SD) WA% measured by EBUS images was 47.9 (6.0) % in the patients with asthma and 34.2

(4.2) % in the non-asthmatics (Fig.3). WT% and WA% measured by EBUS images were significantly higher in the patients with asthma than those in the non-asthmatic subjects, respectively ($p<0.01$, $p<0.01$).

The mean (SD) WT% measured by HRCT was 35.3 (5.1) % in the patients with asthma and 28.1 (4.1) % in the patients without asthma. The mean (SD) WA% measured by HRCT was 58.0 (6.4) % in the asthmatic patients and 48.1(6.1) % in the non-asthmatic patients. The WT% and WA% measured by HRCT images were significantly greater in the asthmatics compared with those in the non-asthmatics, respectively ($p<0.05$, $p<0.05$).

Relationship between WT% and WA% measured by EBUS and HRCT

WT% and WA% measured by EBUS images were positively correlated with those measured by HRCT images, respectively ($r=0.721$, $p<0.01$ and $r=0.724$, $p<0.01$) (Fig.4 and Fig.5).

Detail of five layers of airway and bronchial responsiveness to methacholine

Thickness of the five layers (1: hyperechoic marginal echo, 2: hypoechoic submucosal tissue, 3: hyperechoic inner marginal echo of the cartilage, 4: hypoechoic cartilage, 5: hyperechoic outer marginal echo of the cartilage) was measured by EBUS in the asthmatic patients and non-asthmatic subjects (Figure 6). The mean (SD) values of thickness were 0.37 (0.12), 0.36 (0.09), 0.32 (0.06), 0.66 (0.12) and 0.41 (0.21) mm for the layers 1, 2, 3, 4 and 5 in the patients with asthma and 0.29 (0.08), 0.28 (0.06), 0.27 (0.08), 0.60 (0.25) and 0.37 (0.11) in the patient without asthma, respectively. The thickness of the 2nd layer in the patients with asthma was significantly grater than that in the patients without asthma ($P<0.05$) (Fig.7).

In the patient with asthma, PC20-FEV1 was significantly and negatively correlated with the thickness of the 2nd layer ($r= -0.67$, $p<0.05$). In contrast, there was no correlation between PC20-FEV1 and the thickness of any other layers (1, 3, 4 and 5th layer) (Table2).

Discussion

This is the first study, to our knowledge, to investigate the relationship between

airway wall thickness, as assessed by EBUS and HRCT scan, and bronchial responsiveness, measured in the patients with asthma. Airway wall thickeness (WT% and WA% in large airway) measured by EBUS image were significantly higher in the patients with asthma than those in the patients without asthma. Airway wall thickness (WT% and WA% in large airway, such as intermediate bronchus) measured by EBUS images were positively correlated with those of the apical segmental bronchus of the right upper lobe measured by HRCT images. Furthermore, we found that the thickness of the second layer in large airway was greater in the patient with asthma than in without asthma using EBUS images. The thickeness of the second layer assessed by EBUS examination was positively correlated with non-specific bronchial responsiveness in the patients with asthma.

Wall thickness results from inflammatory changes, such as edema and inflammatory cell infiltration, and from structual changes, such as mucous gland hyperplasia, reticular basement membrane thickening, vascular prolification, and airway smooth muscle hypertrophy and hyperplasia. These structural changes are features of airway remodeling associated with chronic inflammation (7). Airway wall thickening may thus

lead to airway hyperresponsiveness (AHR), an essential feature of asthma (29,30).

HRCT has been used to measure airway wall dimensions in patients with asthma (15-19). Patients with asthma have thicker airways on HRCT scan than healthy control subjects have (15-18), and the degree of thickening is related to the severity of disase (16,17,19), airflow obstruction (17,18) and AHR (31). In this study, the apical segment of the right upper lobe was chosen for its convenient orientaion, so that the CT images aviod tangential cuts through the airway (17).

EBUS is a noninvasive and safe method to assess mediastinal lymph node, other mediastinal structure and the laminar structure of the bronchial wall (20-26). The subjects did not feel the discomfort, such as dyspnea or coughing associated with the procedure in this study. It is proper that we should perform EBUS study at the apical segmental bronchus of the right lung, the same bronchus measured by HRCT. But, the apical segment of the right upper lobe cannot be accessed by using a 20-MHz ultrasonic probe since the transducer is too rigid to permit entry into this lung segment (26). Therefore, we performed EBUS at the large airway, intermediate bronchus, but not apical segmental bronchus of the right lung measured by HRCT study. Ultrasound

images obtained in this large airway were clear enough to assess not only each layer of bronchial wall but also whole bronchial wall.

Recently, Irani et al showed that EBUS was useful to identify and quantitatively assess bronchial wall structure in lung transplant recipients (27). We were able to discriminate the previously described multilayer structure of the airway wall at this localization (20,22-25). Kurimoto et al have reported that the first layer (hyperechoic layer) is a marginal echo extended from the inner margin of the mucosal epithelium to the inner part of the mucosal tissue, the second layer (hypoechoic) is the outer part of mucosal tissue, the third layer (hyperechoic) is marginal echo on the inside of cartilage, the fourth layer (hypoechoic) is cartilage, and the fifth layer (hyperechoic) is marginal echo on the outside of the cartilage in needle-puncture experiment (22). In this study, the thickness of the second layer was higher in the patient with asthma than without asthma. As the second layer is equal to the large part of submucosal tissue containing airway smooth muscle (22), we found that the thickness of submucosal layer in the patient with asthma was greater than that in non-asthmatic subjects using EBUS technique. The thickness of the second layer was positively corelated with bronchial

responsiveness in the patient with asthma. In contrast, the thickness of whole bronchial wall was not correlated with bronchial responsiveness in the patient with asthma. As the techinique of EBUS involved the inflation of a saline-filled baloon around the ultrasound probe, we were concerned that the saline-filled baloon may compress the airway and alter the whole wall thickness measures. Shaw et al reported that ultrasound images recorded from the sheep airways *in vitro* study, with the baloon inflated, demonstrated a slightly greater airway wall thickness (~0.5 mm) than when it was deflated, although this difference did not rearch statistical significance (26). When the latex baloon sheath is in contact with the airway, its thickness is included in the measured thickness of the first layer. As the thickness of the first layer of this ultrasound image contains at least the three componet, latex baloon, epithelium and a inner part of sumucosal tissue, we can not exactly evaluate the length of epitelium in the patients with asthma. The resolution of the 20-MHz ultrasound probe is limited, which explains why the borders of the particular layers appear blurred. A 30-MHz probe providing a higer image resolution has been developed (25). Future studies using this new equipment is required.

We conclude that the thickness of whole airway and submucosal layer measured by EBUS image are significantly higher in patients with asthma than in non-asthmatic subjects. Furthermore, we showed positive correlation of bronchial responsiveness with the thickness of the submucosal tissue measured by endobronchial ultrasound, but not with whole airway wall thickness determined by HRCT in patients with asthma. This study suggests that the thickness of the second layer (hypoechoic submucosal layer) might be partially related to the degree of bronchial responsiveness in patients with asthma. The endobronchial ultrasound is a useful technique to evaluate a distinct laminar airway structure, such as bronchial wall remodeling in patients with asthma.

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Legend for figures

Fig.1: Indices of airway wall thickness on EBUS and HRCT scan.

Ftg.2: WT% measured by EBUS in asthmatic patients and non-asthmatic subjects. WT% measured by EBUS image differed significantly between patients without asthma (open circle) and patients with asthma (shaded circle) ($p<0.01$). Error bars are expressed as mean (SD).

Fig.3: WA% measured by EBUS in patients with asthma (shaded circle) and subjects without asthma (open circle). WA% measured by EBUS image differed significantly between asthmatic patients (open circle) and non-asthmatic subjects (shaded circle) ($p<0.01$). Error bars are expressed as mean (SD).

Fig.4: Relationship between WT% measured by EBUS and HRCT.

WT% measured by EBUS images were positively correlated with those measured by HRCT images ($r=0.721$, $p<0.01$).

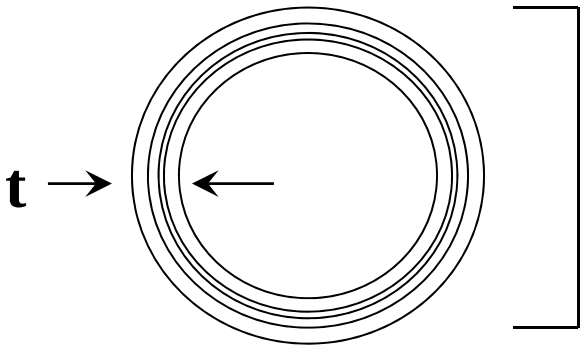
Fig.5: Relationship between WA% measured by EBUS and HRCT. WA% measured by EBUS images were positively correlated with those measured by HRCT images ($r=0.724$, $p<0.01$).

Fig.6: Analysis of each layer of bronchial wall (the right intermediate bronchus) using EBUS. The left, A: Definition of the cartilaginous portion to be measured; the sector starts in the center of the bronchus. The right, B: Measurement of the absolute thickness of each layer.

Fig.7: Thickness of the five layers (1: hyperechoic marginal echo, 2: hypoechoic submucosal tissue, 3: hyperechoic inner marginal echo of the cartilage, 4: hypoechoic cartilage, 5: hyperechoic outer marginal echo of the cartilage) measured in patient without asthma (open column) vs. patients with asthma (closed column). The thickness of the 2nd layer in patients with asthma was significantly greater than that in non-asthmatic subjects ($P<0.05$). Error bars are expressed as mean (SD).

Figure 1: Indices of airway wall thickness on EBUS and HRCT scan.

(1) WT (whole bronchial wall thickness)

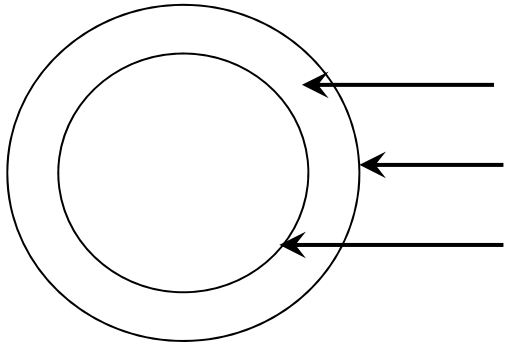


WT= t × 2

= Do (outer diameter)

WT% (percent wall thickness) = WT/Do x 100

(2) WA (whole bronchial wall area)



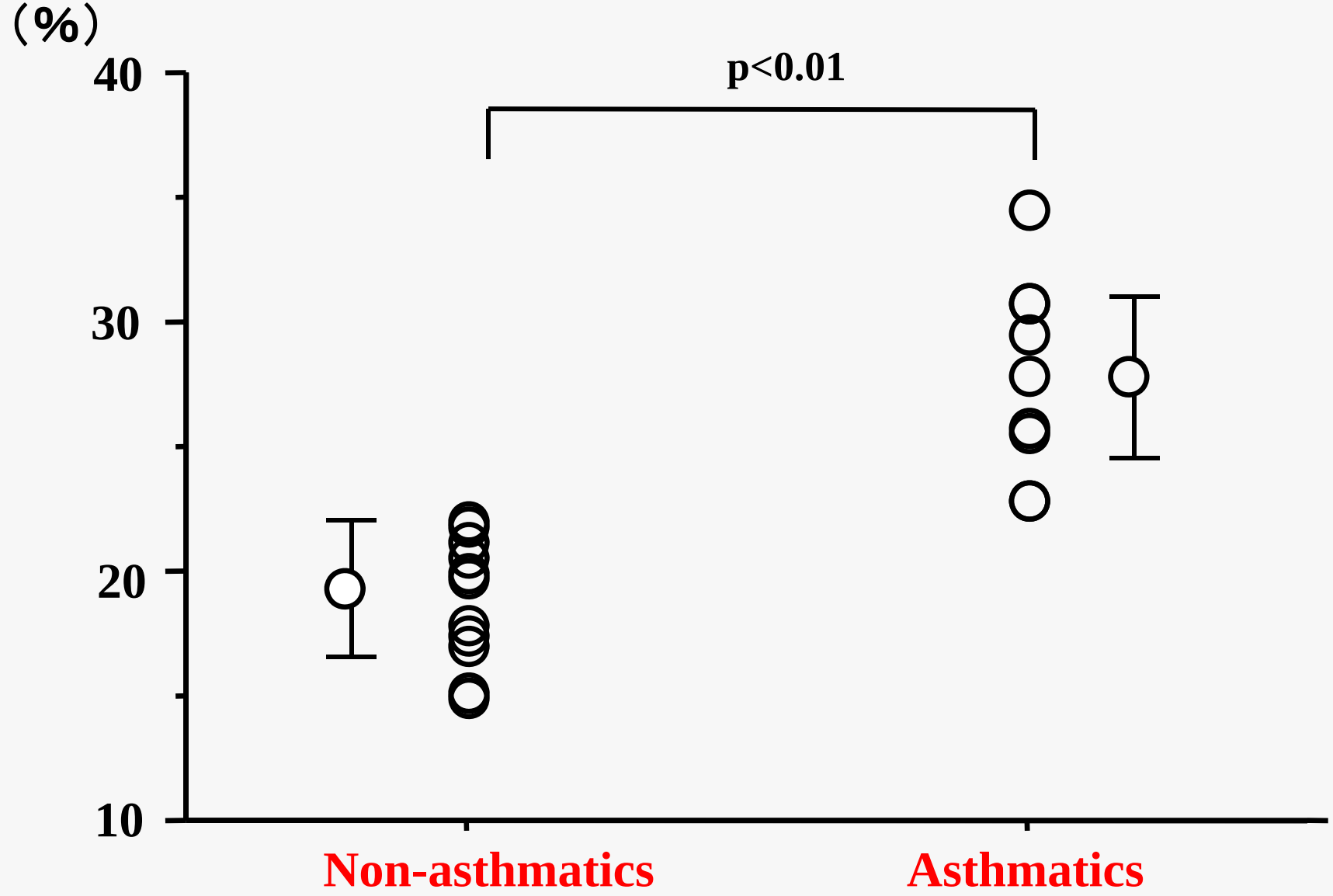
WA(airway wall area)

Ao (total wall area)

Al (luminal area)

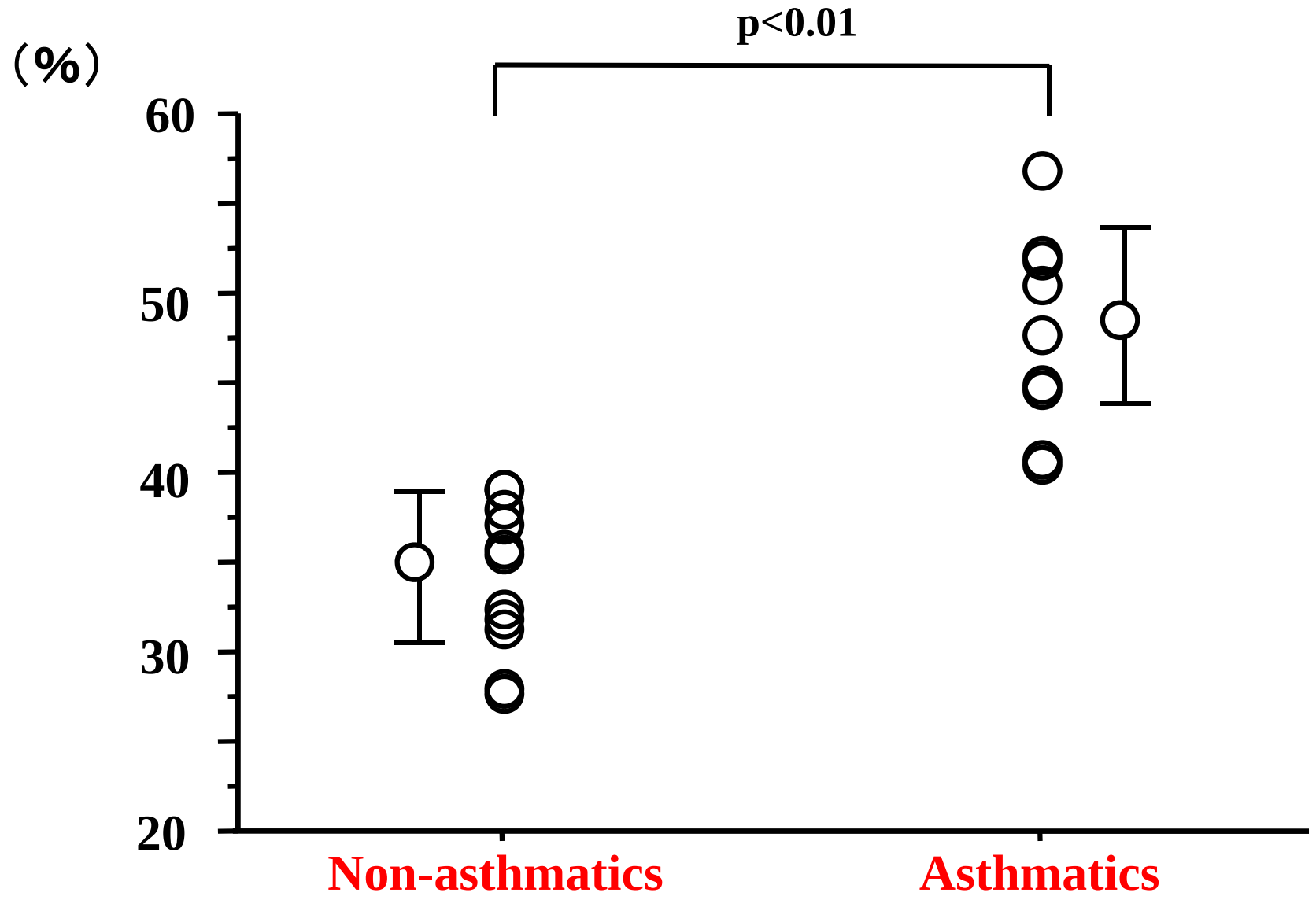
WA% (percent wall area) = WA/Ao x 100

Figure 2: WT% measured by EBUS in the patients with and without asthma



Error bars are expressed as mean (SD)

Figure 3 : WA% measured by EBUS in the patients with and without asthma



Error bars are expressed as mean (SD)

Figure 4 : Correlation of WT% measured by EBUS and HRCT

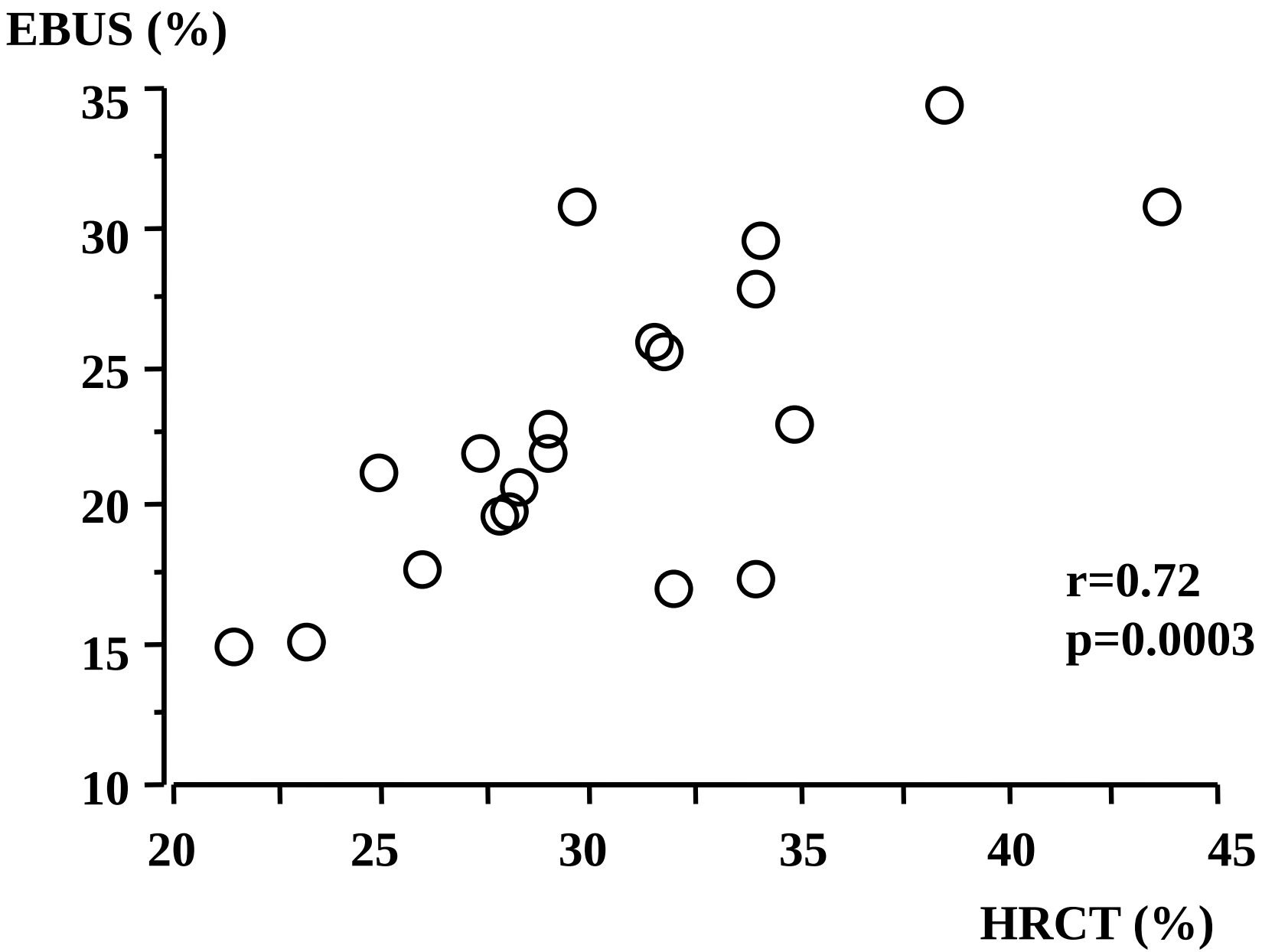


Figure 5 : Correlation of WA% measured by EBUS and HRCT

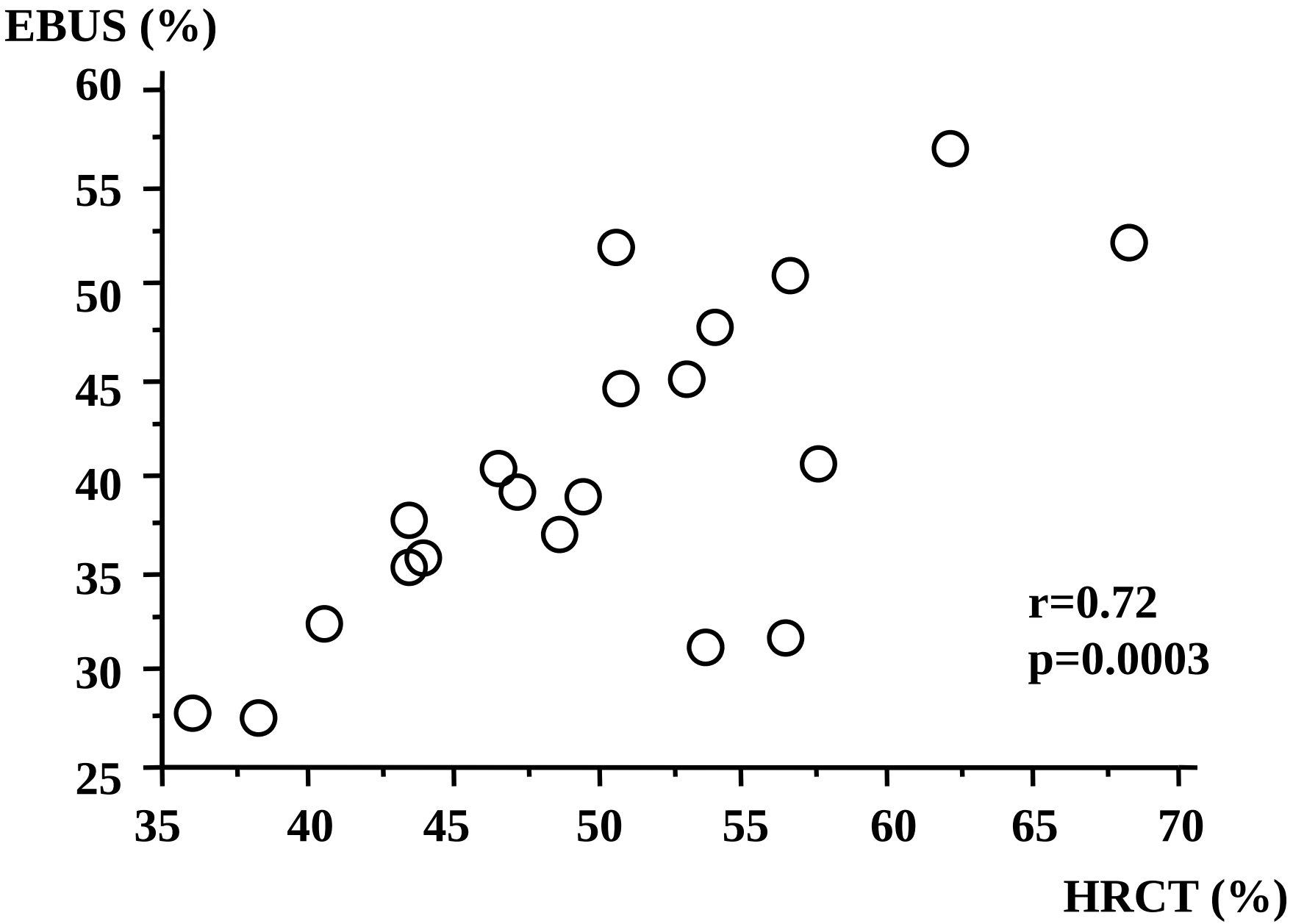


Table 1 : Patient's characteristics

	Non-asthmatics (N=11)	Asthmatics (N=10)
Sex (M/F)	8/3	7/3
Age (years)	62.2 ± 10.7	63.0 ± 13.3
Disease		
Asthma		10
Lung cancer	5	
Granuloma	1	
Mediastinal lymphadenopathy	1	
Others	4	
FVC, L	3.13 ± 0.67	3.10 ± 0.84
FVC, % pred.	114.9 ± 15.3	102.9 ± 18.0
FEV1, L	2.36 ± 0.44	1.70 ± 0.39**
FEV1, % pred.	123.6 ± 12.4	72.4 ± 17.0**
FEV1/FVC ratio, %	76.1 ± 6.5	53.3 ± 13.6**
PC20-FEV1, mg/ml	63.5 (1.26)	1.02 (1.29)**#

**: p<0.01 compared with non-asthmatic group. Data are shown as Mean±SD.
#: shown as geometric mean (geometric standard error of the mean).

Figue 6 : Layers of the bronchial wall by EBUS

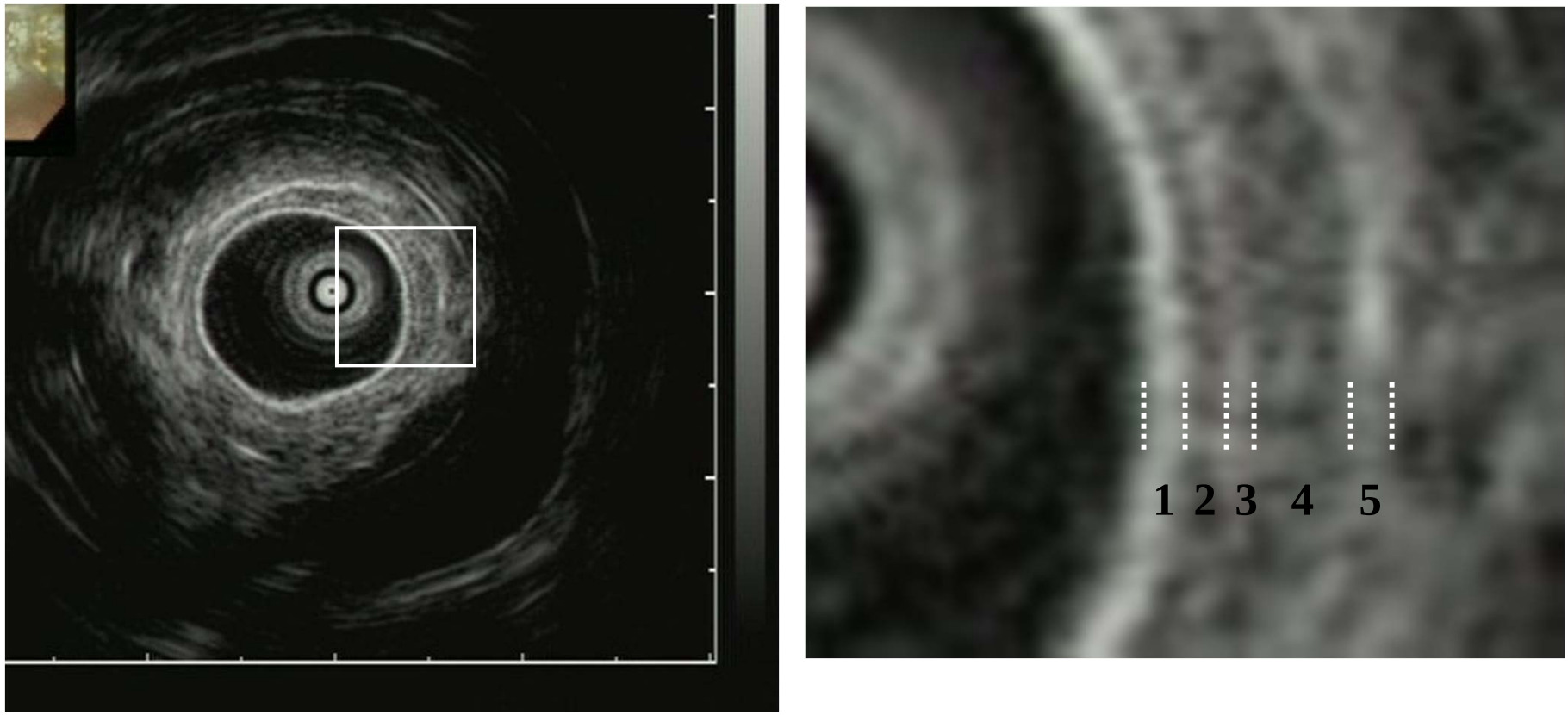
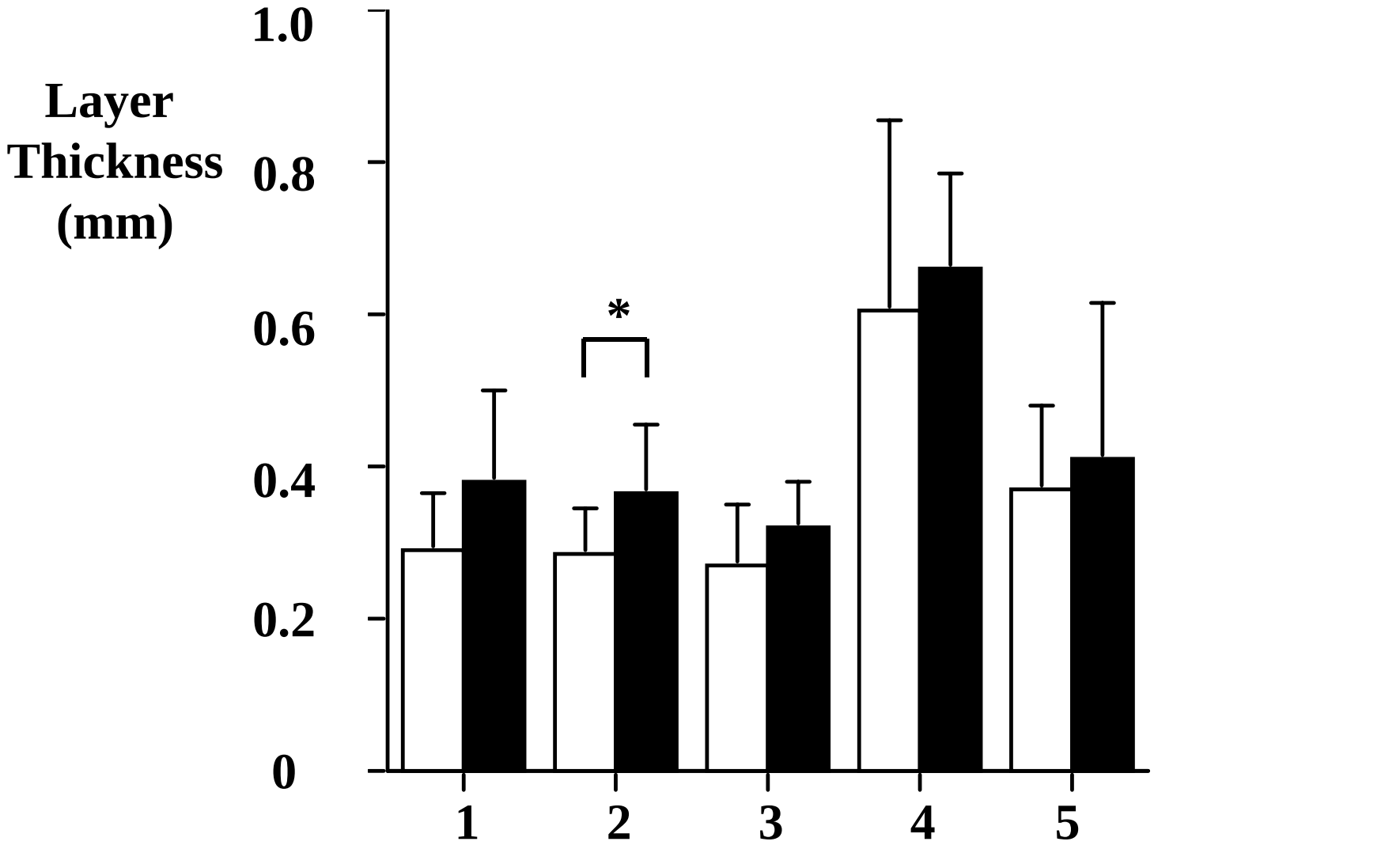


Figure 7 : The thickness of each layers of bronchial wall



Error bars are expressed as mean (SD)

Figure 8 : Correlation of the thickness of 2nd layer and Log (PC_{20}-FEV_1) in the patients with asthma