Correlation between the bronchial subepithelial layer and whole airway wall thickness in patients with asthma

K Kasahara, K Shiba, T Ozawa, K Okuda, M Adachi

Background: The epithelial reticular basement membrane (Rbm) of the airway wall thickens in patients with asthma. However, whether the thickening parallels whole airway wall thickening, which limits airflow, is unknown. The aim of this study was to examine the correlation between the bronchial Rbm thickening and whole airway wall thickening in asthma. In addition, the association of Rbm and whole wall thickening with airflow obstruction was examined.

Methods: Forty-nine patients with asthma and 18 healthy control subjects took part in the study. The Rbm thickness was measured in bronchial biopsy specimens and whole airway wall thickness was assessed with high resolution computed tomographic (HRCT) scanning after pretreatment with oral steroids for 2 weeks and inhaled β2 agonist to minimise reversible changes of the airway walls. The percentage airway wall area (WA%; defined as (wall area/total airway area) x 100) and percentage airway wall thickness (WT%; defined as (ideal outer diameter – ideal luminal diameter)/ideal outer diameter] x 100) were determined from HRCT scans to assess whole airway wall thickness. Spirometric tests were also performed.

Results: WA% and WT% were higher in patients with asthma than in healthy subjects. Both WA% and WT% were strongly correlated with Rbm thickness. Moreover, these three indices of airway wall thickness were inversely correlated with the percentage of predicted forced expiratory volume in 1 second in patients with asthma.

Conclusions: These findings indicate that Rbm thickening parallels whole airway wall thickening which can cause irreversible airflow obstruction in patients with asthma.
severe symptoms upon entry. We believed pretreatment with oral prednisolone and an inhaled β2 agonist was sufficient to suppress latent inflammation and airway spasms. HRCT was performed during the end inspiratory phase after the respiratory function tests. In addition, bronchial biopsy specimens were taken in 22 of the 49 patients who consented to bronchoscopic examination after HRCT scanning.

The study was approved by the Showa University Ethics Committee and all subjects gave written informed consent.

### Pulmonary function tests
Spirometric tests were performed with an Autospiro AS-300 spirometer (Minato Co, Osaka, Japan) to measure forced vital capacity, FEV1, and peak expiratory flow. Each measurement was repeated at least three times and the highest acceptable measurement was compared with normal predicted values.

### HRCT scanning
The thoracic HRCT scan system (Siemens Somatom Plus4, Erlangen, Germany) was used as described previously\(^{19-23}\) with the thin section (1 mm collimation) technique at 120 kVp, 250 mA, 1 second scan time, and 20 cm field of view at the end inspiratory phase. Scans were done at five selected levels: the superoinferior margin of the aortic arch, the tracheal carina, 1 cm below the carina, the inferior pulmonary veins, and 2 cm above the diaphragm.\(^{24}\) The images were viewed at a window level of −450 Hounsfield units (HU) and a window width of 1500 HU to analyse airway wall thickness.\(^{25,26}\)

Airway wall dimensions were calculated using a validated method described by Okazawa and Awadh.\(^{9,10}\) The CT images were printed at a magnification of ×10 that of standard prints and the outer and internal perimeters of bronchial cross sections were traced. The images of each bronchus examined were approximately perpendicular to the bronchial axis, at which the ratio of the diameter of the long axis to that of the short axis was less than 1.2. The traced images were scanned into a Macintosh personal computer (Apple Computer, Cupertino, CA, NIH image) and the outer perimeter (Po, mm), inner perimeter (Pi, mm), total airway area (Ao, mm\(^2\)), and luminal area (Al, mm\(^2\)) were measured. We also calculated ideal outer diameter (Do = Po/π) and ideal luminal diameter (Dl = Pl/π). Three or more bronchi with Dl of 3–5 mm in a single scanning slice were examined (a total 15 or more bronchi per subject). Because bronchial biopsies were taken from subsegmental bronchial bifurcations whose luminal diameters were also 3–5 mm, bronchi of this size were chosen.

Percentage wall area (WA%), defined as \(\frac{(Ao – Al)/Ao \times 100}{\}}\), and percentage wall thickness (WT%), defined as \(\frac{(Do – Dl)/Do \times 100}{\}}\), were also 3–5 mm, bronchi of this size were chosen. Percentages of percentage wall area (WA%) and percentage wall thickness (WT%) between patients with asthma and healthy controls. Differences with p values of <0.05 were considered significant.

### RESULTS

#### Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>Asthma patients (n=49)</th>
<th>Healthy controls (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>45.3 (15.4)</td>
<td>44.9 (14.5)</td>
</tr>
<tr>
<td>Sex (M:F)</td>
<td>25:24</td>
<td>9:9</td>
</tr>
<tr>
<td>Duration of asthma (years)</td>
<td>11.8 (13.8)</td>
<td>0</td>
</tr>
<tr>
<td>Atopy:non-atopy</td>
<td>29:20</td>
<td>10:8</td>
</tr>
<tr>
<td>Dose of BDP (µg/day)</td>
<td>757 (486)</td>
<td>105 (7)</td>
</tr>
<tr>
<td>%PEF=PEF/predicted PEF</td>
<td>90 (25)</td>
<td>105 (7)</td>
</tr>
<tr>
<td>%FEV1=FEV1/predicted FEV1</td>
<td>85 (14)</td>
<td>99 (6)</td>
</tr>
<tr>
<td>WT (bronchial wall thickness)</td>
<td>Dl – Do (\times 100)</td>
<td>Dl – Do (\times 100)</td>
</tr>
<tr>
<td>WT% (percent wall thickness)</td>
<td>(Al/Dl) (\times 100)</td>
<td>(Al/Dl) (\times 100)</td>
</tr>
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</table>

### Indices of airway wall thickness on HRCT scan.

\(Dl/Do \times 100\), were calculated (fig 1). The measurements were performed by two radiology technicians in a blinded fashion. Mean values from 15 or more measurements per subject were calculated and compared in patients with asthma and healthy controls.

### Bronchial biopsies

No patients had had an asthma attack before fiberoptic bronchoscopy (BF type 240, Olympus Co, Tokyo, Japan). Lidocaine (20 ml of 2% solution for local anaesthesia), atropine (0.5 mg intramuscularly), and midazolam (0.1 mg/kg by slow intravenous infusion) were used as premedication. At the start of bronchoscopy the subject’s percutaneous oxygen saturation and pulse were monitored by pulse oximeter (Pulsox, Teijin Co, Tokyo, Japan) and respiratory rate and blood pressure were observed. Four or five biopsy specimens were taken from subsegmental bronchial bifurcations in the right lower lung in each subject. The luminal surfaces of the biopsy specimens were stained with eosin to ensure proper orientation for cross sectioning. Each specimen was fixed in 10% buffered formalin solution at room temperature, embedded in paraffin, subsequently sectioned at 5 µm thick with a microtome, and stained with haematoxylin-eosin. One measurable stained section was chosen from each biopsy specimen (total 3–5 stained sections from each subject used for measurement); sections from incorrectly oriented biopsies were discarded.

Rbm thickness was quantified by measuring the area of the whole Rbm (from the base of the bronchial epithelium to the outer limit of the reticular lamina) and the length of true basement membrane of the Rbm area using a computerised image (Apple Computer, Cupertino, CA, NIH image). Rbm thickness was calculated as Rbm area/length of basement membrane and the mean values of 3–5 measurements in 3–5 stained sections per subject were used for analysis. All measurements were performed by a single pathologist in a blinded fashion.

### Statistical analysis

All statistical analyses were performed with StatView software, version 4.5 (Abacus Concepts, Berkeley, CA, USA) on a Macintosh computer. Values were expressed as mean (SD). Simple regression analysis was used to compare WA% or WT% with Rbm thickness or percentage predicted FEV1, (%FEV1). The Mann-Whitney U test was used to compare WA%, WT%, and %FEV1, between patients with asthma and healthy controls. Differences with p values of <0.05 were considered significant.
beclomethasone dipropionate, and respiratory function measurements, are shown in table 1. The mean %FEV1 (before pretreatment) differed significantly between the groups (p<0.05).

Differences in WA% and WT% between patients with asthma and healthy controls

The findings of HRCT scan measurements are shown in fig 2A and B. Both WA% and WT% were significantly higher in patients with asthma than in healthy controls (p<0.0001). Error bars are expressed as mean (SD).

DISCUSSION

Our study shows for the first time, to our knowledge, a strong positive correlation between Rbm thickness determined in biopsy specimens and whole airway wall thickness determined by HRCT scanning in patients with asthma. Furthermore, this thickening was correlated with a deterioration in respiratory function. Many investigators have reported a thicker Rbm in patients with asthma than in healthy subjects.1–4 However, whether an increase in Rbm thickness of only a few micrometres can cause narrowing of the airway lumen or airflow obstruction has been unclear. Furthermore, the thickening does not explain why whole airway wall thickness on HRCT scans was 20% greater in patients with asthma than in healthy subjects. Our findings suggest that Rbm thickening appears simultaneously with hypertrophy or hyperplasia of other components of the airway wall which may cause respiratory function deterioration in patients with asthma. Previous studies have used Rbm thickening as an.
index of remodelling of the airway wall in asthma. Our findings also support the results of these previous reports. Furthermore, we found no difference in mean airway wall thickness in five HRCT slices obtained at different lung levels, which suggests that irreversible airway wall thickening occurs uniformly throughout the lung in asthma.

Three indices of airway wall thickness—Rbm thickness in bronchial biopsy specimens and WA% and WT% in HRCT scans—were significantly and negatively correlated with %FEV₁. These findings suggest that the irreversible airway wall thickening induces airflow obstruction in patients with asthma. Previous studies have also shown that thickening of either the bronchial Rbm or the airway wall is correlated with disease severity, decreases in respiratory function, and airflow hyperresponsiveness in patients with asthma. Several recent studies have found that patients with asthma, even if they are receiving intensive treatment including inhaled steroids, had a greater decline in respiratory function with age than did healthy controls. Our findings and those of these earlier studies suggest that some patients with asthma have irreversible or partially reversible airway obstruction which may cause refractory airway obstruction despite treatment with corticosteroids, bronchodilators, and other anti-inflammatory drugs. Such airway obstruction can be induced by structural alterations of the bronchi.

In contrast, other studies have reported that airway wall thickening is not correlated with airflow obstruction. In asthma, many factors in addition to irreversible airway wall thickening may contribute to airflow obstruction including airway wall oedema, inflammation, mucus secretion, and bronchial smooth muscle spasms. We used doses of oral steroids and inhaled β₂ agonists before the trial which were sufficient to prevent the effects of reversible changes of the airway wall. For example, we rarely found eosinophils in bronchial biopsy specimens (data not shown). Pretreatment with anti-inflammatory agents and bronchodilators was needed to evaluate the irreversible airway wall thickening and to

Figure 4 HRCT scan representing the airway wall in (A) a healthy control subject and (B) a patient with asthma. Note that the airway wall is thicker (arrow) in the asthmatic patient than in the healthy control.
compare the thickness with respiratory function. Some previous studies may have measured both reversible and irreversible structural alterations when comparing airway wall thickness with respiratory function.

In conclusion, our findings show that whole airway wall thickening evaluated by HRCT scanning parallels bronchial Rbm thickening determined in biopsy specimens after pretreatment with oral steroids and inhaled β₂ agonists. Thickening of the airway wall assessed either in biopsy specimens or by HRCT scanning is also correlated with airflow obstruction. Our findings suggest that Rbm thickening appears with hypertrophy or hyperplasia of other components of the airway wall in patients with asthma and that these irreversible changes of the airway wall may cause respiratory function deterioration and refractory airflow obstruction.

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Informed consent was obtained from all subjects, and the human experimentation guidelines of the US Department of Health and Human Services and those of the Showa University Human Investigation Committee were followed.

REFERENCES