

ORIGINAL ARTICLE

Clinical Mechanisms in Allergic Disease

Respiratory impedance is correlated with airway narrowing in asthma using three-dimensional computed tomography

M. Karayama¹ | N. Inui^{1,2}  | K. Mori¹ | M. Kono¹ | H. Hozumi¹ |
Y. Suzuki¹  | K. Furuhashi¹ | D. Hashimoto¹ | N. Enomoto¹ | T. Fujisawa¹ |
Y. Nakamura¹ | H. Watanabe² | T. Suda¹

¹Second Division, Department of Internal Medicine, Hamamatsu University School of Medicine, Hamamatsu, Japan

²Department of Clinical Pharmacology and Therapeutics, Hamamatsu University School of Medicine, Hamamatsu, Japan

Correspondence

Naoki Inui, Second Division, Department of Internal Medicine, Hamamatsu University School of Medicine, Hamamatsu, Japan.
Email: inui@hama-med.ac.jp

Summary

Background: Respiratory impedance comprises the resistance and reactance of the respiratory system and can provide detailed information on respiratory function. However, details of the relationship between impedance and morphological airway changes in asthma are unknown.

Objective: We aimed to evaluate the correlation between imaging-based airway changes and respiratory impedance in patients with asthma.

Methods: Respiratory impedance and spirometric data were evaluated in 72 patients with asthma and 29 reference subjects. We measured the intraluminal area (Ai) and wall thickness (WT) of third- to sixth-generation bronchi using three-dimensional computed tomographic analyses, and values were adjusted by body surface area (BSA, Ai/BSA, and WT/ $\sqrt{\text{BSA}}$).

Results: Asthma patients had significantly increased respiratory impedance, decreased Ai/BSA, and increased WT/ $\sqrt{\text{BSA}}$, as was the case in those without airflow limitation as assessed by spirometry. Ai/BSA was inversely correlated with respiratory resistance at 5 Hz (R5) and 20 Hz (R20). R20 had a stronger correlation with Ai/BSA than did R5. Ai/BSA was positively correlated with forced expiratory volume in 1 second/forced vital capacity ratio, percentage predicted forced expiratory volume in 1 second, and percentage predicted mid-expiratory flow. WT/ $\sqrt{\text{BSA}}$ had no significant correlation with spirometry or respiratory impedance.

Conclusions & Clinical Relevance: Respiratory resistance is associated with airway narrowing.

KEYWORDS

asthma, forced oscillation technique, remodeling, respiratory impedance, three-dimensional computed tomography

1 | INTRODUCTION

Asthma is characterized by chronic airway inflammation and hyperresponsiveness to various inhaled antigens. The pathogenesis of asthma involves bronchoconstriction and airway remodelling, which

lead to airflow limitation.¹ Assessment of airflow limitation has an important role in asthma management.

Spirometry provides crucial information regarding the severity and variability of airflow limitation, as well as for the diagnosis of asthma.¹ Spirometry is simple, convenient, and widely used, but has

only weak correlations with symptoms and disease control, and is limited regarding its ability to detect functional airway changes.²⁻⁷ Assessment of airflow limitation remains challenging, and novel approaches are needed for better clinical evaluation of asthma.

Recently, the usefulness of respiratory impedance has been demonstrated in several respiratory diseases, including asthma.²⁻¹³ Respiratory impedance comprises the resistance and reactance of the respiratory system, which provides information regarding respiratory function.¹⁴⁻¹⁶ Respiratory resistance is the forward pressure of conducting airways and is considered an index of airway calibre. Respiratory reactance is thought to reflect the dynamic elastance and inertia of the respiratory system.¹⁴⁻¹⁶ Testing of respiratory impedance has broad applicability for patients who cannot tolerate spirometry because the testing requires only quiet tidal breathing (as opposed to the forced expiratory manoeuvre required for conventional spirometry).¹⁴ Respiratory impedance demonstrates good correlation with symptoms and can be used to detect subtle changes in airway function that cannot be detected by conventional spirometry.²⁻⁷ Respiratory impedance measurement is expected to be a useful approach for asthma management.

Computed tomography (CT) of the chest can provide information on airway structural changes in asthma.¹⁷⁻²⁰ Following recent advances in imaging technology, quantitative CT analyses can provide detailed airway information.²¹⁻²⁷ Airway changes based on quantitative CT analyses are associated with impaired spirometry in asthmatic patients^{17-19,21}; however, the correlation between respiratory impedance and CT-based airway changes is unknown. Understanding the association between respiratory impedance and structural airway changes may aid asthma management.

We aimed to evaluate functional and imaging-based airway changes and their correlation using respiratory impedance measured by the forced oscillation technique (FOT) and three-dimensional (3D) CT analyses in patients with asthma.

2 | METHODS

2.1 | Subjects

This was a prospective observational study conducted in accordance with the ethical standards of the Declaration of Helsinki. The study protocol was approved by the Institutional Review Board of Hamamatsu University School of Medicine (Hamamatsu, Japan, approval number: 25-182). Each patient provided written informed consent to be included in the study.

We enrolled patients satisfying the definition of asthma set by the Global Initiative for Asthma (GINA).¹ Exclusion criteria were as follows: uncontrolled asthma¹; requiring a treatment change, the presence of respiratory tract infection, or exacerbation within 4 weeks before study commencement; current or former smokers with smoking history of ≥ 10 pack-years; receiving long-term oxygen therapy; diffuse lung diseases; neuromuscular diseases; congenital anomalies of the bronchial tree; and history of thoracic surgery. "Exacerbation" was defined as episodes of progressively worsening dyspnoea, cough, wheeze, chest

tightness, or a combination of these symptoms requiring systemic corticosteroids.¹ Subjects not suffering from asthma were enrolled as a reference population and were required to have normal pulmonary function (assessed by spirometry) and to not meet the exclusion criteria.

2.2 | Multi-detector row computed tomography

Multi-detector row CT (MDCT) imaging was performed using a 64-slice MDCT machine (Aquilion-64; Toshiba Medical Systems, Tokyo, Japan) in the supine position at full inspiration breath-hold. MDCT parameters were as follows: collimation, 64×0.5 mm; tube voltage, 120 kV; tube current, 200 mA; rotation time, 0.5 seconds; and pitch, 0.83. These parameters were varied by the scanner system to achieve the optimum radiation dose and image quality. Images were reconstructed using a standard reconstruction algorithm (FC50) for the lung using a slice thickness of 0.5 mm and a reconstruction interval of 0.5 mm.

2.3 | 3D CT airway analyses

A 3D-reconstructed bronchial tree was obtained from MDCT data using Synapse Vincent (Fuji Film, Tokyo, Japan). The bronchial pathway was rendered automatically into multi-planar reconstruction images with a window width of 1600 Hounsfield units (HU) and a window level of -600 HU (Figure 1). Airway structural measurements were performed according to previous studies^{28,29} and comprised 3 main approaches. First, we selected 6 airways originating from 6 segmental bronchi (B1, B2, B3, B8, B9, and B10) in the right lung. The middle-lobe bronchi B6 and B7 were excluded from analyses because they are associated with atelectasis, airway collapse, and mucus hypersecretion. The left lung was also excluded for analyses to avoid transmitted cardiac motion artefacts. Second, in each selected airway, 4 levels of bronchi (third generation (segmental), fourth generation (subsegmental), fifth generation, and sixth generation) were identified by tracing airway trees peripherally on the same trunk. Third, at the mid-point for each bronchus level, we computed wall thickness (WT, mm) and intraluminal area (A_i , mm²) perpendicular to the long axis of the airway (Figure 1). WT was calculated as the mean distance from the outer to inner edge of the airway assuming the airway as a "true" circle. The inner and outer airway contours were automatically extracted by the image-analysing software. If an error occurred during automatic extraction of airway contours, it was corrected manually. As a result, 6 images at each level of the bronchus and a total of 24 images in each subject were evaluated. Results for each generation of bronchi were expressed separately as a mean of 6 airways, and to eliminate the potential influence of body size in each patient, A_i and WT were adjusted by body surface area (BSA): A_i/BSA and $WT/\sqrt{BSA}$, respectively, according to previous reports.^{21,22,30-32}

2.4 | Measurement of physiological function

Spirometry and FOT were performed on the same day as CT of the chest, and FOT was performed before spirometry to avoid the

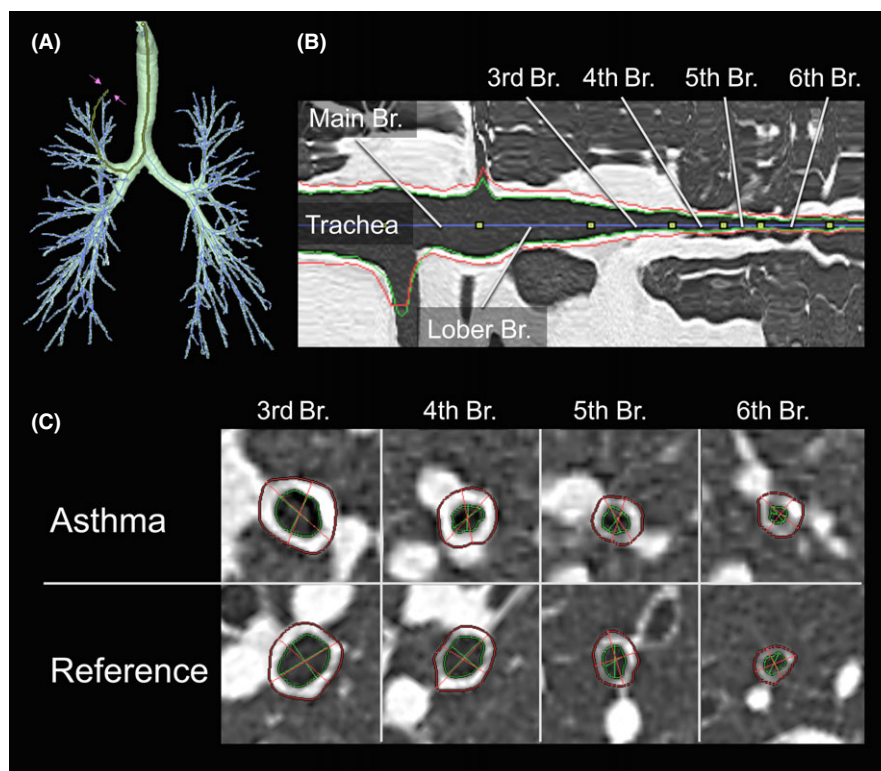


FIGURE 1 Airway three-dimensional computed tomographic analyses. A, Image of the bronchial tree reconstructed by three-dimensional computed tomography. B, Longitudinal section of the reformatted airway in the multi-planar reconstruction image. C, Short-axis images of the airway at third- to sixth-generation bronchi. Red and green lines denote the outer and inner edge of the airway, respectively. Main Br., main bronchi; Lower Br., lower bronchi; 3rd Br., third-generation bronchi; 4th Br., fourth-generation bronchi; 5th Br., fifth-generation bronchi; 6th Br., sixth-generation bronchi

influence of forced breathing. Short-acting β_2 -agonists were not used for >12 hours before these tests, and we used Autospirometer System 7 (Minato Medical Science, Osaka, Japan) according to standards set by the Japanese Respiratory Society.³³ Respiratory impedance was measured using an FOT device (Most-Graph 01; Chest MI, Tokyo, Japan) according to standard recommendations, as reported previously.^{9,15} Briefly, impulse oscillatory signals generated by a loudspeaker at intervals of 0.25 seconds were applied to the respiratory system through a mouthpiece during tidal breathing at rest. Mouth pressure and flow signals were measured and calculated to obtain resistance and reactance properties against an oscillatory frequency ranging from 4 to 36 Hz. We evaluated respiratory resistance at 5 Hz and 20 Hz (R5 and R20, respectively); the difference between R5 and R20 (R5-R20); reactance at 5 Hz (X5); resonant frequency (Fres; where reactance crosses zero and the elastic and inertial forces are equal in magnitude and opposite); and low-frequency reactance area (ALX; the reactance integral from 5 Hz to Fres). Each index was expressed as mean value for the entire breath cycle from beginning of inspiration to the end of expiration.

2.5 | Statistical analyses

We used Wilcoxon's test for continuous variables and Fisher's exact test for categorical groups. The correlation between Ai/BSA (or WT/ $\sqrt{BSA}$) and physiological function tests was assessed using

partial rank correlation analyses adjusted for age, duration of asthma, dose of inhaled corticosteroid, and WT/ $\sqrt{BSA}$ (or Ai/BSA), as the potential confounding factors.^{4,17,20,29,34-39} Differences between correlation coefficients were evaluated using the Meng-Rosenthal-Rubin method. Data are medians (range or interquartile range) unless indicated otherwise; $P < .05$ (two-sided) was considered significant. All values were analysed using JMP v 9.0.0 (SAS Institute Japan, Tokyo, Japan), except for partial rank correlation analyses and Meng-Rosenthal-Rubin method, for which we used R version 3.2.4 (The R Foundation for Statistical Computing, Vienna, Austria, 2016).

3 | RESULTS

3.1 | Patient characteristics

We enrolled 72 asthma patients and 29 reference subjects (Table 1). Asthma patients had a median age of 67 years and were predominantly female (66.7%). Asthma patients had a ratio of median (range) values for forced expiratory volume in 1 second/forced vital capacity (FEV₁/FVC) of 72.1% (40.9-98.0), percentage predicted FEV₁ of 83.6 (21.1-128.4)%, and percentage predicted mid-expiratory flow (%predicted MMF) of 43.7% (7.6-167.8). Eight (11.1%), 8 (11.1%), 11 (15.3%), 43 (59.7%), and 2 (2.8%) patients received GINA-recommended treatment steps 1, 2, 3, 4, and 5,

TABLE 1 Patient characteristics

	Asthma			Reference population (n = 29)
	All patients (n = 72)	Patients without airflow limitation ^a (n = 39)	Patients with airflow limitation ^b (n = 33)	
Age, y	67 (16-88)	63 (16-80)***	70 (37-88)	60 (31-80)
Sex: female	48 (66.7)	28 (71.8)	20 (60.6)	14 (48.2)
Height (m)	1.56 (1.44-1.73)	1.57 (1.44-1.73)	1.56 (1.48-1.69)	1.57 (1.44-1.73)
Weight (kg)	56.1 (36.6-86.0)	56.3 (36.6-86.0)	55.4 (42.9-72.6)	55.5 (33.3-79.5)
BMI (kg/m ²)	22.7 (16.0-35.6)	23.0 (16.0-35.6)	22.5 (16.7-30.4)	22.7 (14.4-35.3)
Spirometry				
FEV ₁ , percentage predicted	83.6 (21.1-128.4)*	92.4 (62.5-128.4)****	71.4 (21.1-103.3)*	96.1 (80.1-163.8)
FEV ₁ /FVC (%)	72.1 (40.9-98.0)*	79.0 (70.4-98.0)****	60.8 (40.9-69.6)*	82.1 (71.9-98.8)
MMF, percentage predicted	43.7 (7.6-167.8)*	72.9 (37.6-167.8)****	28.0 (7.6-44.0)*	83.3 (55.7-153.3)
Inhaled corticosteroid	64 (88.9)	35 (89.7)	29 (87.9)	
Inhaled corticosteroid dose (μg) ^c	320 (80-1000)	320 (80-1000)	400 (100-800)	
Long-acting beta-2 agonist	49 (68.1)	27 (69.2)	22 (66.7)	
Long-acting muscarinic antagonist	8 (11.1)	4 (10.3)	4 (12.1)	
GINA treatment steps				
1	8 (11.1)	4 (10.3)	4 (12.1)	
2	8 (11.1)	5 (12.8)	3 (9.1)	
3	11 (15.3)	7 (18.0)	4 (12.1)	
4	43 (59.7)	21 (53.9)	22 (66.7)	
5	2 (2.8)	2 (5.1)	0	

BMI, body mass index; FVC, forced vital capacity; FEV₁, forced expiratory volume in 1 s; MMF, maximum mid-expiratory flow rate; GINA, Global Initiative for Asthma. Ai, airway intraluminal area; WT, wall thickness; BSA, body surface area.

Data are expressed as number (%) or median (range).

*P < .01 compared with reference population.

**P < .05 compared with reference population.

***P < .05 compared with asthmatic patients with airflow limitation.

****P < .01 compared with asthmatic patients with airflow limitation.

^aAsthmatic patients with FEV₁/FVC ≥ 70%.

^bAsthmatic patients with FEV₁/FVC < 70%.

^cData are expressed as fluticasone equivalent among 64 asthmatic patients, 35 asthmatic patients without airflow limitation, and 29 asthmatic patients with airflow limitation, who received inhaled corticosteroid.

respectively. Sixty-four (88.9%) patients received an inhaled corticosteroid with a median (range) dose (fluticasone equivalents) of 320 μg (80-1000 μg). Forty-nine (68.1%) patients received a long-acting beta-2 agonist, and 8 (11.1%) received a long-acting muscarinic antagonist. Compared with asthma patients, the 29 reference subjects demonstrated comparable characteristics except for a significantly higher FEV₁/FVC ratio, percentage predicted FEV₁, and percentage predicted MMF.

3.2 | Respiratory impedance and airway 3D CT in patients with asthma

Asthma patients had significantly higher R5, R20, R5-R20, Fres, and ALX values, and significantly lower X5 values, compared with

reference subjects (Figure 2, Table S1). Asthma patients had significantly decreased Ai/BSA from third- to sixth-generation bronchi, and significantly increased WT/√BSA from third- to sixth-generation bronchi compared with the reference population (Figure 3, Table S2).

3.3 | Correlations between airway 3D CT and spirometry

FEV₁/FVC ratio, percentage predicted FEV₁, and percentage predicted MMF significantly correlated with Ai/BSA from third- to sixth-generation bronchi in partial rank correlation analysis, adjusting for the potential confounding factors WT/√BSA, age, duration of asthma, and dose of inhaled corticosteroids (Table 2). In

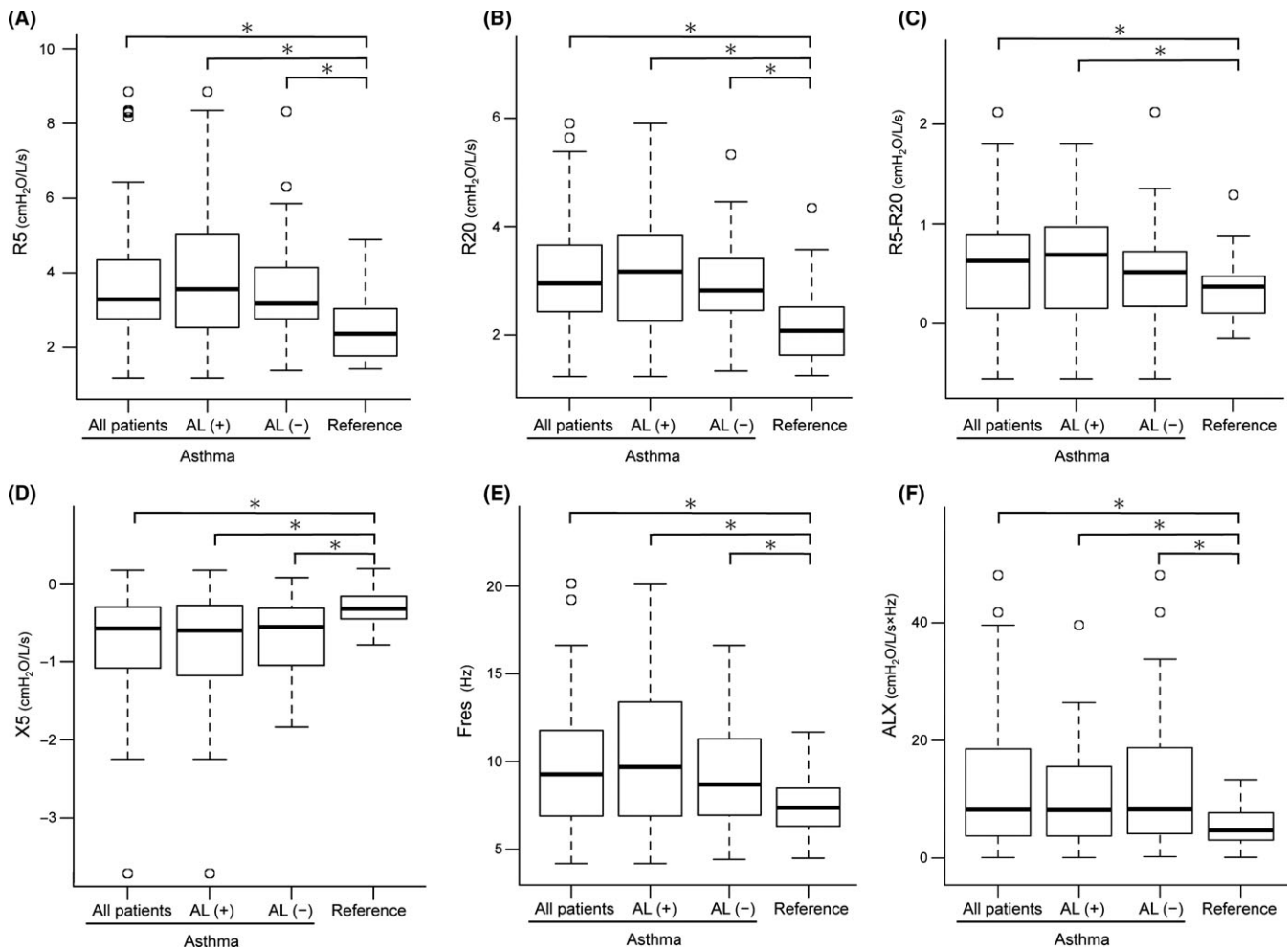


FIGURE 2 Comparison of respiratory impedance results between asthma patients and the reference population. A, R5, respiratory resistance at 5 Hz. B, R20, respiratory resistance at 20 Hz. C, R5-R20, the difference between R5 and R20. D, X5, respiratory reactance at 5 Hz. E, Fres, resonant frequency. F, ALX, low-frequency reactive area. The bottom and top of the boxes, and the horizontal lines in the boxes are the first and third quartile, and the second quartile (median), respectively. Whiskers represent the lowest datum still within $1.5 \times$ the interquartile range (IQR) of the lower quartile, and the highest datum still within $1.5 \times$ the IQR of the upper quartile; circles represent data not included in the whiskers. AL(+), asthmatic patients with airflow limitation, having an forced expiratory volume in 1 s (FEV₁)/forced vital capacity(FVC) ratio < 70%; AL(-), asthmatic patients without airflow limitation, having an FEV₁/FVC ratio $\geq$ 70%. * $P < .01$

contrast, there was no significant correlation between spirometry and WT/ $\sqrt{\text{BSA}}$, except that percentage predicted FEV₁ had very weak correlation with WT/ $\sqrt{\text{BSA}}$ in fourth-generation bronchi (Table 3).

3.4 | Correlations between airway 3D CT and respiratory impedance

R5 and R20 values demonstrated significant inverse correlations with Ai/BSA from third- to sixth-generation bronchi, whereas R5-R20, X5, Fres, and ALX did not (Table 2). Compared with R5, R20 demonstrated a stronger correlation with Ai/BSA, significantly in third-, fifth-, and sixth-generation bronchi ($P = .038$, $.005$, and $.031$, respectively), and a non-significant trend towards a stronger correlation in fourth-generation bronchi ($P = .151$). We found no significant correlation between respiratory impedance and WT/ $\sqrt{\text{BSA}}$, except that X5

had a very weak correlation with WT/ $\sqrt{\text{BSA}}$ in sixth-generation bronchi (Table 3).

3.5 | Subgroup analyses for asthma patients with and without airflow limitation

For subgroup analyses, asthma patients were divided into 2 groups: patients having an FEV₁/FVC ratio < 70% (patients with airflow limitation, $n = 33$) and patients having an FEV₁/FVC ratio $\geq$ 70% (patients without airflow limitation, $n = 39$). Patients without airflow limitation demonstrated comparable pulmonary function with reference subjects, except for having a lower FEV₁/FVC ratio. In contrast, patients with airflow limitation demonstrated a significantly lower percentage predicted FEV₁, FEV₁/FVC ratio, and percentage predicted MMF (Table 1). Both asthma patients with and without airflow limitation demonstrated significantly higher R5, R20, Fres, and

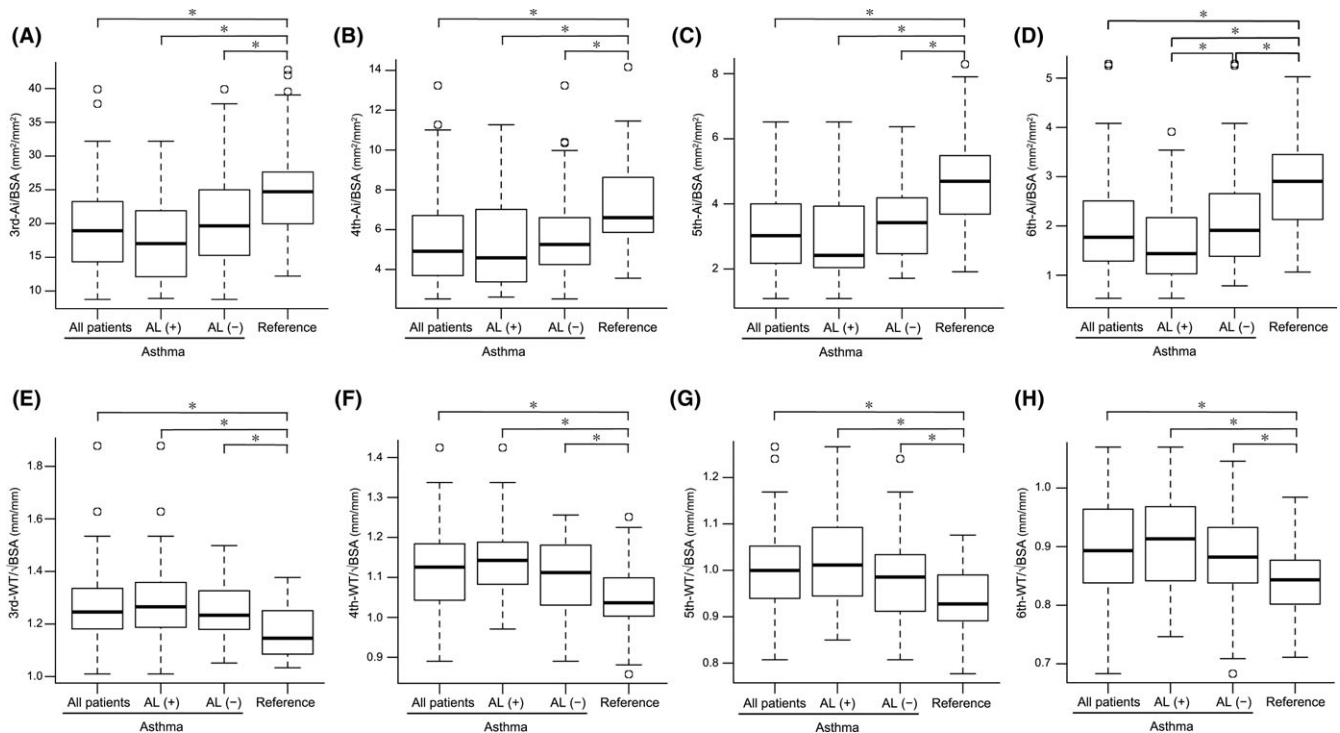


FIGURE 3 Comparison of airway three-dimensional CT images between asthma patients and the reference population. A-D, Airway intraluminal areas of third-, fourth-, fifth-, and sixth-generation bronchi, respectively. E-H, Airway wall thicknesses of third-, fourth-, fifth-, and sixth-generation bronchi, respectively. The bottom and top of the boxes, and horizontal lines in the boxes are the first and third quartile, and the second quartile (median), respectively. Whiskers represent the lowest datum still within $1.5 \times$ the interquartile range (IQR) of the lower quartile, and the highest datum still within $1.5 \times$ the IQR of the upper quartile; circles indicate data not included in the whiskers. Ai/BSA, airway luminal areas adjusted by body surface area; WT/√BSA, airway wall thicknesses adjusted by the square root of body surface area; 3rd-, third-generation bronchi; 4th-, fourth-generation bronchi; 5th-, fifth-generation bronchi; 6th-, sixth-generation bronchi; AL(+), asthmatic patients with airflow limitation, having an forced expiratory volume in 1 s (FEV₁)/forced vital capacity(FVC) ratio < 70%; AL(-), asthmatic patients without airflow limitation, having an FEV₁/FVC ratio $\geq$ 70%. * $P < .01$

ALX values, and significantly lower X5 values, compared with reference subjects (Figure 2, Table S1). Among asthma patients, those with airflow limitation demonstrated non-significantly higher R5, R20, Fres, and ALX values, and non-significantly lower X5 values, compared with patients without airflow limitation. Both asthma patients with and without airflow limitation demonstrated significantly decreased Ai/BSA and increased WT/√BSA from third- to sixth-generation bronchi compared with the reference population (Figure 3, Table S2). Among asthma patients, those with airflow limitation demonstrated non-significantly decreased Ai/BSA in third- to fifth-generation bronchi and significantly decreased Ai/BSA in sixth-generation bronchi, compared with patients without airflow limitation.

4 | DISCUSSION

In this study, we showed that respiratory resistance was correlated with reduced airway calibre in asthma patients, whereas respiratory reactance was not. The correlation with airway narrowing was stronger for R20 than R5, and even asthma patients without airflow

limitation, based on spirometry, had altered airway structure and impaired respiratory impedance. Respiratory impedance combined with spirometry may be useful for evaluating asthma.

In this study, we validated the theoretical model that respiratory resistance reflects airway narrowing in asthmatic patients. Respiratory resistance (forward airway pressure) increased inversely based on bronchial airway calibre in chronic obstructive pulmonary disease (COPD) in our previous study,²⁸ which was also seen in asthmatic patients. Respiratory resistance may be useful to assess airway narrowing in pulmonary diseases with airflow obstruction.

The magnitude of the correlations between Ai and resistance differed according to the wave frequency, R5, and R20. This differed from previous studies in patients with COPD that showed no difference between R5 and R20 regarding a correlation with Ai.²⁸ High-frequency waves reflect back from large airways, and low-frequency waves travel deep into the lungs.¹⁴ Therefore, it has been hypothesized that R5 denotes total airway resistance and R20 denotes central airway resistance.^{14,16} Both asthma and COPD have similarities and overlap as respiratory diseases with airflow limitation; however, these diseases also have different characteristics regarding the underlying mechanism and the process of airway

	Third generation		Fourth generation		Fifth generation		Sixth generation	
	ρ	P-value	ρ	P-value	ρ	P-value	ρ	P-value
Spirometry								
FEV ₁ , percentage predicted	.321	.001	.240	.018	.245	.016	.285	.005
FEV ₁ /FVC ratio	.502	<.001	.481	<.001	.507	<.001	.540	<.001
MMF, percentage predicted	.518	<.001	.484	<.001	.479	<.001	.502	<.001
Respiratory impedance								
R5	-.207	.041	-.251	.013	-.266	.008	-.329	.001
R20	-.274	.007	-.297	.003	-.355	<.001	-.396	<.001
R5-R20	-.021	.838	-.100	.329	-.050	.628	-.094	.359
X5	.092	.369	.123	.230	.129	.207	.138	.176
Fres	-.088	.392	-.152	.138	-.133	.195	-.138	.178
ALX	-.052	.613	-.107	.298	-.105	.308	-.117	.252

FVC, forced vital capacity; FEV₁, forced expiratory volume in 1 s; MMF, maximum mid-expiratory flow rate; R5, respiratory resistance at 5 Hz; R20, respiratory resistance at 20 Hz; X5, respiratory reactance at 5 Hz; Fres, resonant frequency; ALX, low-frequency reactive area.

Data are presented as partial rank correlation coefficients (ρ) and P-values. Airway luminal areas were adjusted by body surface area (Ai/BSA). Partial rank correlations between Ai/BSA and physiological function tests were adjusted for age, duration of asthma, dose of inhaled corticosteroid, and wall thickness adjusted by the square root of body surface area (WT/ $\sqrt{BSA}$).

TABLE 2 Correlations between airway luminal areas and physiological function testing in asthma patients

	Third generation		Fourth generation		Fifth generation		Sixth generation	
	ρ	P-value	ρ	P-value	ρ	P-value	ρ	P-value
Spirometry								
FEV ₁ , percentage predicted	-.030	.765	-.208	.041	-.089	.387	-.122	.233
FEV ₁ /FVC ratio	-.034	.741	-.094	.361	-.147	.152	-.197	.053
MMF, percentage predicted	.011	.917	-.083	.416	-.076	.462	-.132	.197
Respiratory impedance								
R5	.124	.225	.085	.407	.124	.225	.147	.151
R20	.150	.144	.093	.365	.117	.255	.134	.191
R5-R20	.075	.463	.044	.671	.077	.454	.111	.281
X5	-.101	.323	-.126	.218	-.083	.421	-.210	.039
Fres	.087	.399	.076	.457	.033	.751	.169	.098
ALX	.112	.273	.097	.345	.070	.497	.177	.083

FVC, forced vital capacity; FEV₁, forced expiratory volume in 1 s; MMF, maximum mid-expiratory flow rate; R5, respiratory resistance at 5 Hz; R20, respiratory resistance at 20 Hz; X5, respiratory reactance at 5 Hz; Fres, resonant frequency; ALX, low-frequency reactive area.

Data are presented as partial rank correlation coefficients (ρ) and P-values. Airway wall thicknesses were adjusted by the square root of body surface area (WT/ $\sqrt{BSA}$). Partial rank correlations between WT/ $\sqrt{BSA}$ and physiological function test results were adjusted for age, duration of asthma, dose of inhaled corticosteroid, and airway intraluminal areas adjusted by body surface area (Ai/BSA).

TABLE 3 Correlations between wall thicknesses and physiological function testing in asthma patients

remodelling.^{40,41} Although remodelling occurs in the small airways in both COPD and asthma, large airways play a major role in remodelling in asthma.^{40,42,43} The difference between R5 and R20

regarding the magnitude of correlation with airway narrowing may be attributed to the pathophysiological characteristics of the airway in asthma.

In contrast to resistance, respiratory reactance showed no correlation with airway narrowing. Respiratory reactance reflects small airway dysfunction.^{8,9,44} The third- to sixth-generation bronchi assessed in the current study were relatively large and central airways and therefore might have no correlation with reactance. Although the current study could not clarify how airway morphological changes affect respiratory reactance, respiratory resistance and reactance had different characteristics in their relationships with airway structure.

Structural changes in airways can be observed in mild-to-moderate asthma, as well as in severe asthma.^{17,20,42,45-47} In our study, even asthma patients without airflow limitation assessed by spirometry had altered airway structure in third- to sixth-generation bronchi. It is notable that these patients also demonstrated impaired respiratory impedance. Respiratory impedance is sufficiently sensitive to detect subtle changes in airway function and/or asthma symptoms that cannot be detected using conventional spirometry.²⁻⁷ Our data indicated that respiratory impedance has the potential to reflect airway structural changes that cannot be detected by spirometry. Respiratory impedance measures airway pressure, which is not measured in spirometry,^{15,48} and the specific features of respiratory impedance could provide additional information that is not available from spirometry.

In contrast to Ai, WT had only a very weak correlation with both spirometry and respiratory impedance, and the clinical importance remains unclear. Several studies reported a correlation between WT and spirometry^{17,20,21,23,25-27}; however, these studies did not consider potential confounding factors in the correlation analyses. Similarly, in the current study, WT significantly correlated with spirometric parameters in Spearman's rank correlation analyses without adjustments for confounding factors (Table S3), as was the case for Ai (Table S4). However, WT positively correlated with age and inversely correlated with Ai in the same Spearman's rank correlation analyses (Table S5), which could have affected the correlation analyses for airway morphology. Therefore, we used partial rank correlation analyses to eliminate potential confounding factors and demonstrated independent correlations between physiological function tests and Ai, but not WT. These results suggest that spirometry and respiratory impedance may be unsuitable for assessing morphological airway wall changes in asthmatic patients.

The present study had 3 main limitations. First, structural airway information cannot account completely for respiratory impedance. The latter reflects several factors in the respiratory system: airway tone, chest-wall resistance, and mucus secretion within airway lumina.^{4,16} In addition, age is an important factor. Although respiratory reactance is increased in childhood and decreases with ageing,^{49,50} age did not correlate with respiratory impedance, and potential confounding by age was excluded by partial correlation analysis in the current study. This study had a mostly middle-aged and elderly population and therefore might not be representative of juvenile patients with asthma. The potential influence of these factors should be considered when interpreting respiratory impedance data. Second, the resolution of the CT-based analysis had a technical

limitation. Although the current algorithm for extracting airways has been assessed previously using a standardized evaluation framework,⁵¹ the accuracy for assessing distal small bronchi was not validated completely. Sixth-generation bronchi might be the distal measurable limit in 3D CT.^{22,24,26,29} In the present study, the correlation of respiratory function and airway structure in sixth-generation bronchi showed almost identical trends to those in third- to fifth-generation bronchi. In addition, small airway disease, which has an important pathogenic role in asthma,⁵² could not be evaluated in this study because of limited resolution of chest CT images. How and whether more distal bronchi than sixth-generation bronchi correlate with respiratory resistance remains unknown. Third, static CT images at full inspiration provide limited information on within-breath differences in respiratory impedance in asthma patients.^{9,53} Further studies are needed to evaluate dynamic changes in the respiratory system during tidal breathing.

5 | CONCLUSIONS

Respiratory resistance correlated with bronchial airway narrowing. Asthma patients demonstrated abnormal changes in airways and impaired respiratory impedance even if they had normal spirometry. Respiratory impedance may be useful for evaluating asthma.

ACKNOWLEDGEMENT

We thank Jane Charbonneau, DVM, from Edanz Group (<http://www.edanzediting.com/ac>) for editing a draft of this manuscript.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

ORCID

N. Inui  <http://orcid.org/0000-0002-4624-7119>

Y. Suzuki  <http://orcid.org/0000-0001-6154-9791>

REFERENCES

1. Global Initiative for Asthma. Guide for asthma management and prevention. Published Online First; 2016. <http://ginasthma.org>. Accessed November 11, 2016.
2. Mansur AH, Manney S, Ayres JG. Methacholine-induced asthma symptoms correlate with impulse oscillometry but not spirometry. *Respir Med*. 2008;102:42-49.
3. Takeda T, Oga T, Niimi A, et al. Relationship between small airway function and health status, dyspnea and disease control in asthma. *Respiration*. 2010;80:120-126.
4. Shi Y, Aledia AS, Tatavosian AV, Vijayalakshmi S, Galant SP, George SC. Relating small airways to asthma control by using impulse oscillometry in children. *J Allergy Clin Immunol*. 2012;129:671-678.

5. Kelly VJ, Sands SA, Harris RS, et al. Respiratory system reactance is an independent determinant of asthma control. *J Appl Physiol*. 2013;115:1360-1369.
6. Juniper EF, Price DB, Stampone PA, Creemers JPHM, Mol SJM, Fireman P. Clinically important improvements in asthma-specific quality of life, but no difference in conventional clinical indexes in patients changed from conventional beclomethasone dipropionate to approximately half the dose of extrafine beclomethasone dipropion. *Chest*. 2002;121:1824-1832.
7. Komarow HD, Skinner J, Young M, et al. A study of the use of impulse oscillometry in the evaluation of children with asthma: analysis of lung parameters, order effect, and utility compared with spirometry. *Pediatr Pulmonol*. 2012;47:18-26.
8. Mikamo M, Shirai T, Mori K, et al. Predictors of expiratory flow limitation measured by forced oscillation technique in COPD. *BMC Pulm Med*. 2014;14:23.
9. Mori K, Shirai T, Mikamo M, et al. Colored 3-dimensional analyses of respiratory resistance and reactance in COPD and asthma. *COPD*. 2011;8:456-463.
10. Pisi R, Aiello M, Zanini A, et al. Small airway dysfunction and flow and volume bronchodilator responsiveness in patients with chronic obstructive pulmonary disease. *Int J Chron Obstruct Pulmon Dis*. 2015;10:1191-1197.
11. Kolsum U, Borrill Z, Roy K, et al. Impulse oscillometry in COPD: identification of measurements related to airway obstruction, airway conductance and lung volumes. *Respir Med*. 2009;103:136-143.
12. Jetmalani K, Timmins S, Brown NJ, et al. Expiratory flow limitation relates to symptoms during COPD exacerbations requiring hospital admission. *Int J Chron Obstruct Pulmon Dis*. 2015;10:939-945.
13. Shi Y, Aledia AS, Galant SP, George SC. Peripheral airway impairment measured by oscillometry predicts loss of asthma control in children. *J Allergy Clin Immunol*. 2013;131:718-723.
14. Bickel S, Popler J, Lesnick B, Eid N. Impulse oscillometry: interpretation and practical applications. *Chest*. 2014;146:841-847.
15. Oostveen E, MacLeod D, Lorino H, et al. The forced oscillation technique in clinical practice: methodology, recommendations and future developments. *Eur Respir J*. 2003;22:1026-1041.
16. Shirai T, Kurosawa H. Clinical application of the forced oscillation technique. *Intern Med*. 2016;55:559-566.
17. Niimi A, Matsumoto H, Amitani R, et al. Airway wall thickness in asthma assessed by computed tomography: relation to clinical indices. *Am J Respir Crit Care Med*. 2000;162:1518-1523.
18. Ueda T, Niimi A, Matsumoto H, et al. Role of small airways in asthma: investigation using high-resolution computed tomography. *J Allergy Clin Immunol*. 2006;118:1019-1025.
19. de Blic J, Tillie-Leblond I, Emond S, Mahut B, Duy TLD, Scheinmann P. High-resolution computed tomography scan and airway remodeling in children with severe asthma. *J Allergy Clin Immunol*. 2005;116:750-754.
20. Gupta S, Siddiqui S, Haldar P, et al. Quantitative analysis of high-resolution computed tomography scans in severe asthma subphenotypes. *Thorax*. 2010;65:775-781.
21. Hartley RA, Barker BL, Newby C, et al. Relationship between lung function and quantitative computed tomographic parameters of airway remodeling, air trapping, and emphysema in patients with asthma and chronic obstructive pulmonary disease: a single-center study. *J Allergy Clin Immunol*. 2016;137:1413-1422.e12.
22. Hasegawa M, Nasuhara Y, Onodera Y, et al. Airflow limitation and airway dimensions in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2006;173:1309-1315.
23. Coxson HO, Quiney B, Sin DD, et al. Airway wall thickness assessed using computed tomography and optical coherence tomography. *Am J Respir Crit Care Med*. 2008;177:1201-1206.
24. Hasegawa M, Makita H, Nasuhara Y, et al. Relationship between improved airflow limitation and changes in airway calibre induced by inhaled anticholinergic agents in COPD. *Thorax*. 2009;64:332-338.
25. Aysola RS, Hoffman EA, Gierada D, et al. Airway remodeling measured by multidetector CT is increased in severe asthma and correlates with pathology. *Chest*. 2008;134:1183-1191.
26. Shimizu K, Hasegawa M, Makita H, Nasuhara Y, Konno S, Nishimura M. Airflow limitation and airway dimensions assessed per bronchial generation in older asthmatics. *Respir Med*. 2010;104:1809-1816.
27. Shimizu K, Makita H, Hasegawa M, et al. Regional bronchodilator response assessed by computed tomography in chronic obstructive pulmonary disease. *Eur J Radiol*. 2015;84:1196-1201.
28. Karayama M, Inui N, Mori K, et al. Respiratory impedance is correlated with morphological changes in the lungs on three-dimensional CT in patients with COPD. *Sci Rep*. 2017;7:41709.
29. Yasui H, Inui N, Furuhashi K, et al. Multidetector-row computed tomography assessment of adding budesonide/formoterol to tiotropium in patients with chronic obstructive pulmonary disease. *Pulm Pharmacol Ther*. 2013;26:336-341.
30. Nakano Y, Muro S, Sakai H, et al. Computed tomographic measurements of airway dimensions and emphysema in smokers correlation with lung function. *Am J Respir Crit Care Med*. 2000;162:1102-1108.
31. Niimi A, Matsumoto H, Takemura M, Ueda T, Chin K, Mishima M. Relationship of airway wall thickness to airway sensitivity and airway reactivity in asthma. *Am J Respir Crit Care Med*. 2003;168:983-988.
32. Siddiqui S, Gupta S, Cruse G, et al. Airway wall geometry in asthma and nonasthmatic eosinophilic bronchitis. *Allergy*. 2009;64:951-958.
33. Japanese Respiratory Society. Guidelines of respiratory function tests: spirometry, flow-volume curve, and diffusion capacity of the lung. *Tokyo Japanese Respir Soc*; 2004.
34. Yamaguchi M, Niimi A, Ueda T, et al. Effect of inhaled corticosteroids on small airways in asthma: investigation using impulse oscillometry. *Pulm Pharmacol Ther*. 2009;22:326-332.
35. Niimi A, Matsumoto H, Amitani R, et al. Effect of short-term treatment with inhaled corticosteroid on airway wall thickening in asthma. *Am J Med*. 2004;116:725-731.
36. Capraz F, Kunter E, Cermik H, Ilvan A, Pocan S. The effect of inhaled budesonide and formoterol on bronchial remodeling and HRCT features in young asthmatics. *Lung*. 2007;185:89-96.
37. Kurashima K, Kanauchi T, Hoshi T, et al. Effect of early versus late intervention with inhaled corticosteroids on airway wall thickness in patients with asthma. *Respirology*. 2008;13:1008-1013.
38. Abe Y, Shibata Y, Igarashi A, et al. Reference values of MostGraph measures for middle-aged and elderly Japanese individuals who participated in annual health checkups. *Respir Investig*. 2015;54:148-155.
39. Berger P, Perot V, Desbarats P, Tunon-de-Lara JM, Marthan R, Laurent F. Airway wall thickness in cigarette smokers: quantitative thin-section CT assessment. *Radiology*. 2005;235:1055-1064.
40. Sköld CM. Remodeling in asthma and COPD – differences and similarities. *Clin Respir J*. 2010;4:20-27.
41. Postma DS, Reddel HK, ten Hacken NHT, van den Berge M. Asthma and chronic obstructive pulmonary disease: similarities and differences. *Clin Chest Med*. 2014;35:143-156.
42. Carroll N, Elliot J, Morton A, James A. The structure of large and small airways in nonfatal and fatal asthma. *Am Rev Respir Dis*. 1993;147:405-410.
43. Shimizu K, Hasegawa M, Makita H, Nasuhara Y, Konno S, Nishimura M. Comparison of airway remodelling assessed by computed tomography in asthma and COPD. *Respir Med*. 2011;105:1275-1283.
44. Bhatawadekar SA, Leary D, Maksym GN. Modelling resistance and reactance with heterogeneous airway narrowing in mild to severe asthma. *Can J Physiol Pharmacol*. 2015;93:207-214.
45. Awadh N, Müller NL, Park CS, Abboud RT, Fitzgerald JM. Airway wall thickness in patients with near fatal asthma and control groups:

- assessment with high resolution computed tomographic scanning. *Thorax*. 1998;53:248-253.
46. Lee Y, Park J, Hwang J, et al. High-resolution CT findings in patients with near-fatal asthma: comparison of patients with mild-to-severe asthma and normal control subjects and changes. *Chest*. 2004;126:1840-1848.
47. Marchac V, Emond S, Mamou-Mani T, et al. Thoracic CT in pediatric patients with difficult-to-treat asthma. *Am J Roentgenol*. 2002;179:1245-1252.
48. Berger KI, Goldring RM, Oppenheimer BW. Should oscillometry be used to screen for airway disease? yes. *Chest*. 2015;148:1131-1135.
49. Gochicoa-Rangel L, Torre-Bouscoulet L, Martinez-Briseno D, Rodriguez-Moreno L, Cantu-Gonzalez G, Vargas MH. Values of impulse oscillometry in healthy Mexican children and adolescents. *Respir Care*. 2015;60:119-127.
50. de Assumpcao MS, Goncalves RM, Martins R, Bobbio TG, Schivinski CI. Reference equations for impulse oscillometry system parameters in healthy Brazilian children and adolescents. *Respir Care*. 2016;61:1090-1099.
51. Inoue T, Kitamura Y, Li Y, Ito W. Robust airway extraction based on machine learning and minimum spanning tree. *Proc SPIE*. 2013;8670:86700L.
52. Kraft M, Djukanovic R, Wilson S, Holgate ST, Martin RJ. Alveolar tissue inflammation in asthma. *Am J Respir Crit Care Med*. 1996;154:1505-1510.
53. Shirai T, Mori K, Mikamo M, et al. Usefulness of colored 3D imaging of respiratory impedance in asthma. *Allergy Asthma Immunol Res*. 2013;5:322-328.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Karayama M, Inui N, Mori K, et al. Respiratory impedance is correlated with airway narrowing in asthma using three-dimensional computed tomography. *Clin Exp Allergy*. 2018;48:278-287. <https://doi.org/10.1111/cea.13083>

Supplementary data

Supplementary table S1: Respiratory impedance

	Asthma			Reference population
	All patients (n=72)	Patients without airflow limitation [†] (n=39)	Patients with airflow limitation [‡] (n=33)	(n=29)
R5 (cmH ₂ O/L/s)	3.29 (2.72 – 4.51)*	3.18 (2.72 – 4.17) [¶]	3.56 (2.39 – 5.04) [¶]	2.37 (1.77 – 3.04)
R20 (cmH ₂ O/L/s)	2.95 (2.42 – 3.66)*	2.82 (2.44 – 3.52)*	3.17 (2.24 – 3.85) [¶]	2.08 (1.63 – 2.55)
R5-R20 (cmH ₂ O/L/s)	0.63 (0.15 – 0.92) [¶]	0.52 (0.14 – 0.73)	0.69 (0.15 – 0.98) [¶]	0.37 (0.10 – 0.49)
X5 (cmH ₂ O/L/s)	-0.58 (-1.09 – -0.30) [¶]	-0.56 (-1.08 – -0.31) [¶]	-0.60 (-1.21 – -0.27) [¶]	-0.33 (-0.46 – -0.14)
Fres (Hz)	9.28 (6.73 – 11.78) [¶]	8.69 (6.73 – 11.33) [¶]	9.69 (6.82 – 13.45)*	7.37 (6.19 – 8.56)
ALX (cmH ₂ O/L/s×Hz)	2.23 (0.95 – 4.83) [¶]	2.07 (1.03 – 4.73) [¶]	2.76 (0.94 – 6.44) [¶]	1.18 (0.70 – 2.04)

Data are expressed as median (inter quartile range). R5, respiratory resistance at 5 Hz; R 20, respiratory resistance at 20 Hz; X5, respiratory reactance at 5 Hz; Fres, resonant frequency; ALX, low-frequency reactance area. [†]Asthmatic patients with FEV₁/FVC ≥ 70%. [‡]Asthmatic patients with FEV₁/FVC < 70%. **p*<0.01 compared with reference population. [¶]*p*<0.05 compared with reference population.

Supplementary data

Supplementary table S2: Three-dimensional CT of the airway

	Asthma			Reference population
	All patients (n=72)	Patients without airflow limitation [†] (n=39)	Patients with airflow limitation [‡] (n=33)	(n=29)
Ai/BSA (mm ² /m ²)				
Third-generation	18.9 (14.2 – 23.4)*	19.7 (15.0 – 26.0) [¶]	17.0 (11.7 – 21.9)*	24.7 (19.8 – 28.0)
Fourth-generation	4.9 (3.7 – 6.8)*	5.3 (4.2 – 6.6)*	4.6 (3.3 – 7.3)*	6.6 (5.7 – 8.7)
Fifth-generation	3.0 (2.2 – 4.1)*	3.4 (2.4 – 4.2)*	2.4 (2.0 – 3.9)*	4.7 (3.7 – 5.5)
Sixth-generation	1.8 (1.3 – 2.6)*	1.9 (1.4 – 2.7)* [‡]	1.4 (1.0 – 2.2)*	2.9 (2.1 – 3.5)
WT/ $\sqrt$ BSA (mm/m)				
Third-generation	1.25 (1.18 – 1.34)*	1.23 (1.18 – 1.33)*	1.27 (1.19 – 1.36)*	1.15 (1.08 – 1.25)
Fourth-generation	1.13 (1.04 – 1.19) [¶]	1.11 (1.03 – 1.18) [¶]	1.14 (1.07 – 1.19)*	1.04 (0.99 – 1.10)
Fifth-generation	1.00 (0.94 – 1.06)*	0.99 (0.91 – 1.04) [¶]	1.01 (0.94 – 1.10)*	0.93 (0.88 – 0.99)
Sixth-generation	0.89 (0.84 – 0.97)*	0.88 (0.84 – 0.93) [¶]	0.91 (0.84 – 0.93)*	0.84 (0.80 – 0.88)

Supplementary data

Data are expressed as median (inter quartile range). Ai, airway intraluminal area; WT, wall thickness; BSA, body surface area.

[†]Asthmatic patients with $FEV_1/FVC \geq 70\%$. [‡]Asthmatic patients with $FEV_1/FVC < 70\%$. * $p < 0.01$ compared with reference population. [¶] $p < 0.05$ compared with reference population. [§] $p < 0.05$ compared with asthmatic patients with airflow limitation.

Supplementary data

Supplementary table S3: Spearman rank correlation between wall thicknesses and physiologic function tests in asthma patients

	Third-generation		Fourth-generation		Fifth-generation		Sixth-generation	
	ρ	<i>p</i> -value	ρ	<i>p</i> -value	ρ	<i>p</i> -value	ρ	<i>p</i> -value
Spirometry								
FEV ₁ , %predicted	-0.125	0.213	-0.200	0.047	-0.069	0.505	-0.072	0.485
FEV ₁ /FVC ratio	-0.307	0.002	-0.332	0.001	-0.301	0.003	-0.275	0.007
MMF, %predicted	-0.233	0.020	-0.261	0.009	-0.190	0.064	-0.173	0.092
Respiratory impedance								
R5	0.172	0.090	0.142	0.167	0.159	0.126	0.138	0.184
R20	0.199	0.049	0.152	0.136	0.166	0.109	0.127	0.224
R5-R20	0.103	0.312	0.088	0.387	0.089	0.393	0.112	0.281
X5	-0.178	0.079	-0.212	0.037	-0.158	0.129	-0.244	0.018
Fres	0.185	0.068	0.190	0.062	0.116	0.265	0.210	0.042
ALX	0.181	0.074	0.185	0.069	0.142	0.171	0.217	0.036

Supplementary data

Data are presented as partial rank correlation coefficient (ρ) and p -value. Airway wall thicknesses were adjusted by the square root of body surface area ($WT/\sqrt{BSA}$). FVC, forced vital capacity; FEV_1 , forced expiratory volume in 1 second; MMF, maximum mid-expiratory flow rate; R5, respiratory resistance at 5 Hz; R 20, respiratory resistance at 20 Hz; X5, respiratory reactance at 5 Hz; Fres, resonant frequency; ALX, low-frequency reactance area.

Supplementary data

Supplementary table S4: Spearman rank correlation between airway luminal areas and physiologic function tests in asthma patients

	Third-generation		Fourth-generation		Fifth-generation		Sixth-generation	
	ρ	<i>p</i> -value	ρ	<i>p</i> -value	ρ	<i>p</i> -value	ρ	<i>p</i> -value
Spirometry								
FEV ₁ , %predicted	0.314	0.001	0.258	0.010	0.270	0.008	0.299	0.003
FEV ₁ /FVC ratio	0.408	<0.001	0.380	<0.001	0.423	<0.001	0.444	<0.001
MMF, %predicted	0.443	<0.001	0.408	<0.001	0.429	<0.001	0.449	<0.001
Respiratory impedance								
R5	-0.261	0.010	-0.295	0.003	-0.289	0.005	-0.323	0.001
R20	-0.335	0.001	-0.347	<0.001	-0.371	<0.001	-0.380	<0.001
R5-R20	-0.040	0.695	-0.111	0.278	-0.058	0.580	-0.095	0.360
X5	0.114	0.264	0.152	0.137	0.126	0.227	0.127	0.224
Fres	-0.092	0.368	-0.153	0.135	-0.105	0.313	-0.121	0.245
ALX	-0.074	0.470	-0.124	0.228	-0.097	0.354	-0.105	0.313

Supplementary data

Data are presented as partial rank correlation coefficient (ρ) and p -value. Airway luminal areas were adjusted by body surface area (A_i/BSA). FVC, forced vital capacity; FEV₁, forced expiratory volume in 1 second; MMF, maximum mid-expiratory flow rate; R5, respiratory resistance at 5 Hz; R 20, respiratory resistance at 20 Hz; X5, respiratory reactance at 5 Hz; Fres, resonant frequency; ALX, low-frequency reactance area.

Supplementary data

Supplementary table S5: Spearman rank correlation between clinical characteristics and 3D-CT

	Age		Duration of asthma		Dose of inhaled corticosteroids		Ai/BSA	
	ρ	<i>p</i> -value	ρ	<i>p</i> -value	ρ	<i>p</i> -value	ρ	<i>p</i> -value
Ai/BSA								
Third-generation bronchi	0.129	0.202	0.065	0.590	-0.003	0.979		
Fourth-generation bronchi	0.124	0.221	0.112	0.361	-0.057	0.640		
Fifth-generation bronchi	0.156	0.129	0.002	0.988	0.059	0.638		
Sixth-generation bronchi	0.151	0.143	-0.032	0.798	-0.012	0.922		
WT/ $\sqrt$ BSA								
Third-generation bronchi	0.393	<0.001	0.142	0.240	-0.024	0.844	-0.254	0.011
Fourth-generation bronchi	0.519	<0.001	0.089	0.465	-0.115	0.341	-0.215	0.033
Fifth-generation bronchi	0.468	<0.001	0.024	0.847	-0.197	0.109	-0.120	0.243
Sixth-generation bronchi	0.470	<0.001	0.044	0.727	-0.203	0.099	0.020	0.846

Data are presented as Spearman rank correlation coefficient (ρ) and *p*-value. Ai/BSA, airway luminal areas were adjusted by body surface area; WT/ $\sqrt$ BSA, airway wall thicknesses adjusted by the square root of body surface area. Correlations between Ai/BSA and WT/ $\sqrt$ BSA were evaluated in the same generation of bronchi