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Original article

Changes in lung sounds during asthma progression in a guinea pig model

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Abbreviations:
f, respiratory frequency; TV, tidal volume; MV, minute volume; sRaw, specific airway resistance; sGaw, specific airway conductance; Raw, airway resistance; FRC, functional residual capacity; PIF, peak inspiratory flow; PEF, peak expiratory flow; Ti, inspiratory time; Te, expiratory time; dT, delta time; EF50, flow at the point at which 50% of TV is expired; Aair, total airway area; ASM, airway smooth muscle; Aw, area of airway wall; Ai, area of internal airway area; rAw, ratio of the airway wall thickness to Aair; rAi, ratio of the internal area to Aair; 21wsC, challenged for 12 weeks; 12wsC, challenged for 12 weeks; HE, Hematoxylin-Eosin

ABSTRACT

Background: Lung sound analysis is useful for objectively evaluating airways even in children with asymptomatic asthma. However, the relationship between lung sounds and morphological changes in the airways has not been elucidated. We examined the relationship between lung sounds and chronic morphological changes in the airways during the progression of asthma from onset in guinea pigs.
Methods: Eleven male guinea pigs were examined; of these, seven were used as asthma models and four as controls. The asthma models were sensitized and repeatedly challenged by inhaling albumin chicken egg. We measured lung sounds and lung function twice a week for 21 weeks. After the final antigen challenge, the lungs were excised for histological examination. We measured the ratio of airway wall thickness to the total airway area and the ratio of the internal area to the total airway area in the trachea, third bronchi, and terminal bronchioles.
Results: Among the lungs sounds, the difference between the two groups was greatest with respect to inspiratory sound intensity. The ratio of airway wall thickness to the total airway area of the terminal bronchioles was greater in the asthma models than in the controls, and it correlated best with the changes in inspiratory sound intensity in the 501–1000-Hz range ($r = 0.76$, $p < 0.003$).
Conclusions: Lung sound intensity in the middle frequency range from 501 to 1000 Hz correlated with peripheral airway wall thickness. Inspiratory sound intensity appeared to be an indicator of morphological changes in small airways in asthma.
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Introduction

Chronic airway inflammation in bronchial asthma causes airflow limitation and leads to changes in the airway structure.[1,2] These morphological and functional changes are due to chronic allergic inflammation of the airways with increased airway wall thickness caused by thickening of smooth muscles, subepithelial fibrosis, accumulation of inflammatory cells, and airway edema.[3] These structural changes in the airways have been found in biopsy

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specimens of airways from adult patients with asthma.[4–7] In children and infants, these structural changes in the airways are observed from the onset of asthma.[8,9] Lung function tests are important tools for the assessment of these functional and possible morphological changes in asthma, but they are difficult to perform in small children.

Lung sound analysis is a noninvasive method that does not require cooperation of small children. Airway inflammation and airflow limitation affect breath sounds even in the absence of adventitious sounds.[10–12] We previously reported that lung sound intensity is affected by functional changes in the airways in children with asymptomatic asthma.[13,14]

It has been reported that acoustic transfer characteristics are affected by airway wall compliance, airway diameter, and energy dissipation caused by respiratory motion.[15–17] In this study, we investigated the changes in lung sounds during progression of asthma from onset and determined if lung sound analysis could detect histological changes.

Methods

Experimental animals

Eleven male Hartley strain guinea pigs (18 weeks old; weight, 812–922 g) were prepared for the examination (Japan SLC Inc., Shizuoka, Japan). All animal procedures were approved by our local animal care committee. The study protocol was approved by the ethics committee of our institution (approval number: 22-1-0). We divided the animals into two groups: seven asthma models and four controls. There was no significant difference in the weight of the asthma models and the controls.

Chronic asthma model: sensitization and exposure protocol

We sensitized the animals according to the sensitization methods previously described.[18,19] In brief, asthma models were sensitized by intraperitoneal injections of average 7 mg of ovalbumin (OA: albumin chicken egg, Grade V; Sigma Chemical, St. Louis, MO, USA) in 2 ml of Freund's complete adjuvant (Daitron, Tokyo, Japan) twice on days 0 and 14.

Chronic allergen challenge was started 1 week after the second intraperitoneal injection of the antigen. The allergen challenge was repeated once or twice a week for 18 weeks or 9 weeks.[18,19] We chose one of the sensitization methods according to the condition of the animals.

For the allergen challenge, the animals were placed in a nebulization unit box (8.5 × 9.3 × 22 cm) without being anesthetized. The animals were challenged with an aerosolized inhalational solution comprising 3 mg of OA in 3 ml of 0.9% saline delivered by an ultrasonic nebulizer (NE-U12; Omron, Tokyo, Japan) for 10 min.

The animals inhaled 3–30 mg of OA to avoid OA tolerance at a given concentration.[20] The nebulization box allowed up to four guinea pigs to simultaneously breathe OA under identical conditions. Diphenhydramine (20 mg/kg; Sigma Chemical) was intraperitoneally administered 1 h prior to each challenge to prevent severe anaphylaxis.

When an animal showed a severe change in breathing pattern or cyanosis within 10 min of exposure, the inhalation challenge was immediately discontinued for ethical reasons.

Measurement of respiratory resistance and lung function

Respiratory resistance was measured using a double-chamber whole-body plethysmograph (Buxco, Sharon, CT, USA) according to the method of Pennock *et al.*[21] We measured respiratory frequency (f), tidal volume (TV), minute volume (MV), specific airway resistance (sRaw), specific airway conductance (sGaw), airway resistance (Raw), functional residual capacity (FRC), peak inspiratory flow (PIF), peak expiratory flow (PEF), inspiratory time (Ti), expiratory time (Te), and delta time (dT). Delta time is the delay in time observed between nasal and thoracoabdominal flows, the flow at the point at which 50% of TV is expired (EF50).

Sound recording and signal processing

Before recording lung sounds, we shaved the hairs of the bilateral chest. Lung sounds were recorded on the right and left side of the anterior chest for ≥120 s while the animals breathed freely before measurement of airway resistance and the following antigen challenge.

Lung sounds were recorded using an air-coupled microphone. We inserted a Primo EM147 electret condenser microphone in plastic couplers with a cylindrical air chamber and recorded the lung sounds of the guinea pigs.

Audio signals of the lung sounds were amplified and high-pass filtered at 50 Hz (AT-MA2, AUDIO-TECHNICA). The optimal amplifier gain was adjusted empirically before the study and fixed throughout the experiments. The audio signal was digitized at 48 kHz and 16 bits per sample using an audio interface (Fireface UC; RME, Germany) and analyzed using audio editor software (Adobe Auditoin CS6; Adobe, USA). Fast Fourier transformation was performed on 4096 points of signal data using a Hanning window with 50% overlap into adjacent segments.

The upper section of Figure 1 shows an example of lung sound analysis in a guinea pig. The audio signal of the inspiratory breath sounds had greater amplitude than that of the expiratory breath sounds.

Noise handling

Prior to sound analysis, we carefully listened to all the recordings and reviewed the spectrogram to exclude noise, such as friction and environmental sound. It was apparent that the intensity of the inspiratory breath sounds was larger than that of the background noise; the average intensity of the inspiratory breath sounds was −74.4 ± 12.0 dB, whereas the average intensity of the background noise was −90.6 ± 13.1 dB.

Lung sound index

The spectral shape of the lung sounds shows that the intensity of the inspiratory breath sounds was larger than that of the expiratory breath sounds before the antigen challenge (The upper section of Fig. 1).

We calculated the lung sound index to compare lung sound intensities before and after the antigen challenge. The lung sound index is the difference between the averaged power of 3 inspiratory breath sounds before and after the antigen challenge.

We calculated the lung sound index in 4 frequency bands: 0–500 Hz, 501–1000 Hz, 1001–1500 Hz, and 1501–2000 Hz.

Histological examination and airway dimensions

We examined the lung tissues of 5 asthma models and 3 controls. The animals were sacrificed by administering an overdose of pentobarbital (500 mg/kg i.h). A cannula was inserted into the proximal portion of the trachea, and the lungs were inflated with ethanol at a constant pressure of 25 cmH_2O.

Tissue preparation was performed as previously described.[22,23] The lung tissue specimens were obtained from the trachea, third bronchi, and terminal bronchioles of the right and left lungs and then embedded in paraffin. Then, 4-μm-thick sections were cut and

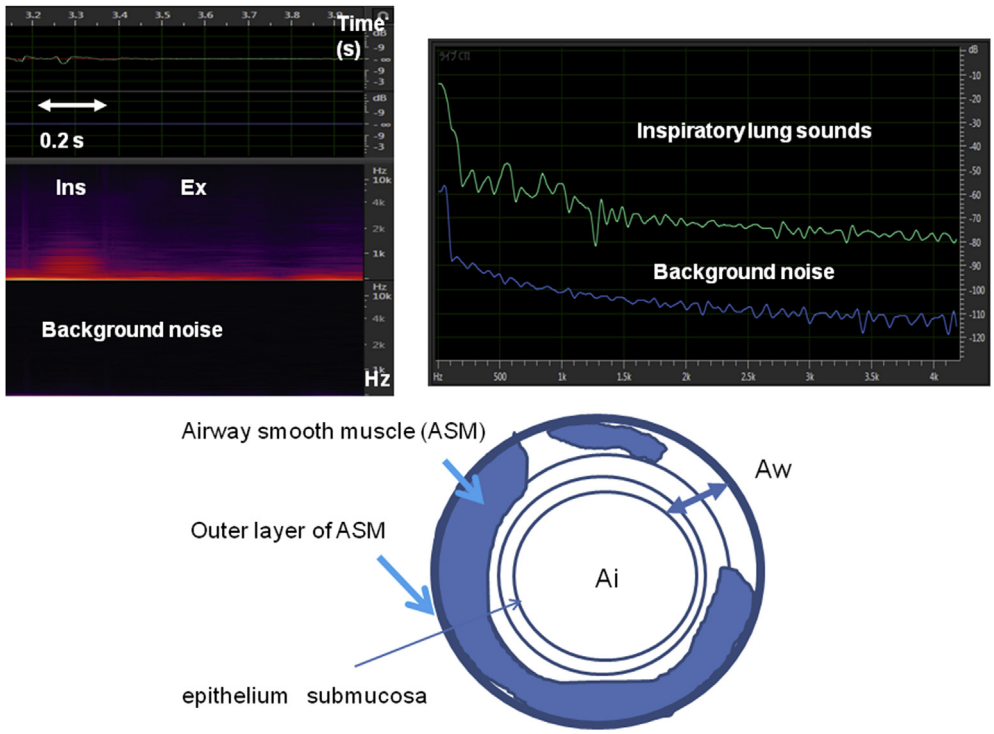

Fig. 1. Lung sound analysis and airway area dimensions in a guinea pig. The upper section shows the lung sound analysis in a guinea pig. The left side shows the sound spectrogram. The right side graph shows the shape of the power spectrum of the lung sounds. The intensity of inspiratory breath sounds was greater than that of the expiratory breath sounds and of the background noise. The lower section shows the airway area dimensions. Aair, the total airway area enclosed by the outer layer of ASM; Aw, Area within the airway wall enclosed between the internal layer of epithelium and the outer layer of ASM; Ai, Area within the internal airway enclosed by a luminal layer of epithelium. Ratio of the total area of airway wall thickness (rAw), rAw = Aw/Aair; Internal area ratio (rAi), rAi = Ai/Aair.

stained with hematoxylin and eosin (HE) and Masson's trichrome. The tissues were processed while images were magnified and traced to measure the dimensions of the airway area using a curvimeter. Membranous and cartilaginous airway areas were measured using a digitizer (Pixs2000pro; Innotech, Japan).

The lower section of Figure 1 shows the airway area dimensions. We measured the total airway area (Aair) enclosed by an outer layer of airway smooth muscle (ASM).[24,25] We also measured the area of the airway wall (Aw), which is surrounded by an internal layer of airway epithelium and an outer layer of ASM, and the area of the internal airway (Ai), which is surrounded by a luminal layer of epithelium. These indices were measured in the airways of the trachea, third bronchi, and terminal bronchioles of the right and left lungs.

We measured the ratio of the airway wall thickness to Aair (rAw) and the ratio of the internal area to Aair (rAi) in the trachea, third bronchi, and terminal bronchioles.

Statistical analyses

The data are presented as means ± SEMs. Comparisons of lung function parameters, lung sound intensity, rAw, and rAi between the asthma models and controls were performed using the unpaired t-test. The correlation coefficients were determined using Pearson's product moment correlation coefficient. Values of $p < 0.05$ were considered statistically significant.

Results

Comparison of lung function parameters between the asthma models and controls before experiments

Table 1 shows the results of lung function parameters of all guinea pigs before the experiments. There were no significant differences in the lung function parameters between the asthma models and controls.

Changes in lung function parameters between the asthma models and controls before and after the antigen challenge

There were significant differences in the changes of sRaw, Te, and dT between the asthma models and controls before and after the challenge ($p < 0.05$, 0.02, and 0.04, respectively; Table 2).

Frequency power spectrum of lung sounds in the asthma models and controls before the antigen challenge

Figure 2 shows the lung sounds of the asthma models and controls before the challenge. In the asthma models, the changes in

Table 1
Comparison of lung function parameters between the asthma models and controls before experiments.

		Asthma		Control	
		Average	SD	Average	SD
f		114.2	19	110.1	9.9
TV	ml	5.7	1.4	6.6	0.1
MV	ml/s	656.7	217.1	729.2	78.1
sRaw	cmH_20*s	2.3	0.4	2.6	0.1
sGaw	cmH_20*s	0.5	0.1	0.4	0
Raw	cmH_20*s	0.2	0.1	0.2	0
FRC	ml	5.9	2.7	6	1.2
PIF	ml/s	35.6	9.5	39.5	3.3
PEF	ml/s	25.9	9.5	27.4	4.2
Ti	ml/s	0.2	0	0.2	0
Te	s	0.3	0.1	0.4	0
dT	s	2.4	0.4	2.7	0.1
EF50	s	24.5	9	25.5	3.6

the averaged power values were −43.9 ± 10.8 dB (0–500 Hz), −53.3 ± 3.2 dB (501–1000 Hz), −64.6 ± 2.9 dB (1001–1500 Hz), and −75.1 ± 3.3 dB (1501–2000 Hz). In the controls, the changes in the averaged power values were −45.8 ± 10.3 dB (0–500 Hz), −58.5 ± 4.6 dB (501–1000 Hz), −69.9 ± 4.0 dB (1001–1500 Hz), and −80.3 ± 3.1 dB (1501–2000 Hz). There were no significant differences in the changes in the averaged sound power values between the asthma models and controls.

Changes in the lung sounds in typical cases of the asthma model and control

Figure 3 shows the changes in the lung sounds in a typical case of the asthma model and control. The left frame shows an asthma model that had been sensitized for 21 weeks. The right frame shows a control that was not sensitized. The lung sound intensity in the asthma model was greater than that in the control.

Comparison of lung sound changes between the asthma models and controls

Figure 4 shows the changes in lung sound intensity in the asthma models and controls. In the asthma models, the changes in the averaged power values were 13.3 ± 5.2 dB (0–500 Hz), 18.8 ± 7.2 dB (501–1000 Hz), 16.7 ± 4.9 dB (1001–1500 Hz), and 15.9 ± 5.6 dB (1501–2000 Hz).

In the controls, the changes in averaged power values were −6.2 ± 3.0 dB (0–500 Hz), −2.8 ± 6.1 dB (501–1000 Hz), −2.2 ± 3.4 dB (1001–1500 Hz), and −0.7 ± 4.6 dB (1501–2000 Hz). The change in lung sound intensity in the asthma models was significantly greater than that in the controls ($p < 0.001$, 0.003, 0.001, and 0.003, respectively).

Morphological changes in the airways in typical cases of the asthma models and controls

We obtained the histological tissue of 6 guinea pigs: 4 asthma models and 2 controls. Figure 5 shows the airway rings of the guinea pigs. The upper section of Figure 5 shows a trachea tissue sample stained with HE.

The middle section of Figure 5 shows the third bronchus tissue stained with Masson's trichrome. The bronchial wall of the asthma models that were challenged for 21 weeks (21wsC) was thicker than that of the controls and asthma models that were challenged for 12 weeks (12wsC).

Table 2
Changes in lung function parameters of the asthma models and controls before and after antigen challenge.

		Asthma		Control	
		Average	SD	Average	SD
f		−1.2	26.7	8.0	19.1
TV	ml	0.5	2.0	1.3	3.6
MV	ml/s	61.7	243.0	202.1	417.1
* sRaw	cmH_20*s	0.6	0.5	−0.3	0.5
sGaw	cmH_20*s	−0.1	0.1	0.3	0.1
Raw	cmH_20*s	0	0.1	0	0.1
FRC	ml	0.2	2.7	0.7	1.2
PIF	ml/s	8.6	12.6	4.8	9.6
PEF	ml/s	5.0	10.1	4.8	10.0
Ti	ml/s	0	0	0	0.1
* Te	s	0.1	0.1	−0.1	0.1
* dT	s	0.6	0.5	−0.4	0.6
EF50	s	3.7	11.5	10.2	13.3

* $p < 0.05$.

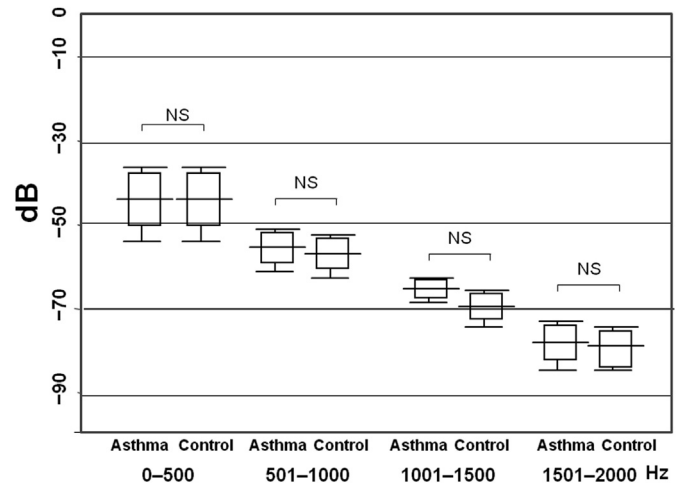

Fig. 2. Frequency power spectrum of lung sounds in the asthma models and controls before experiment. There were no significant differences between the asthma models and controls.

The lower section of Figure 5 shows the terminal bronchiole tissue stained with Masson's trichrome. The bronchial wall of the asthma models was thicker than that of the controls.

The averages of the rAw and rAi for each guinea pig were calculated from the measured data for the third bronchi and terminal bronchioles of the right and left lungs.

In the trachea, the rAw and rAi of the asthma models were 46.3% ± 4.2% and 54.4% ± 4.1%, respectively, whereas those of the controls were 46.8% ± 7.4% and 53.1% ± 7.4%, respectively. There were no significant differences in rAw and rAi between the asthma models and controls.

In the third bronchi, the rAw and rAi of the asthma models were 52.4% ± 16.1% and 47.6% ± 16.1%, respectively, whereas those of the controls were 24.2% ± 16.6% and 73.5% ± 19.9%, respectively. There were no significant differences in rAw and rAi between the asthma models and controls. The rAw and rAi of the 21wsC asthma models were 60.0% ± 5.8% and 39.9% ± 5.8%, respectively. The rAw and rAi of the 12wsC asthma models were 29.3% and 70.0%. Compared with 12wsC asthma models, 21wsC asthma models had larger rAw and smaller rAi.

In the terminal bronchioles, the rAw and rAi of the asthma models were 78.9% ± 15.8% and 21.0% ± 15.8%, respectively, whereas the rAw and rAi of the controls were 36.9% ± 0.1% and 63.0% ± 0.1%, respectively. Compared with the controls, rAw was significantly larger and rAi was significantly smaller in the asthma models ($p < 0.02$).

Relationships between lung sound changes and lung function parameters

The f correlated with the changes in lung sound intensity from 0 Hz to 500 Hz but did not correlate with the lung sound intensity of other frequency ranges ($r = 0.62$; $p < 0.05$). The TV, MV, sGaW, RaW, FRC, PIF, PEF, Ti, Te, and EF50 did not correlate with the changes in lung sound intensity of all the frequency ranges. The dT and sRaW correlated with the changes in lung sound intensity from 1001 Hz to 1500 Hz but did not correlate with the lung sound intensity of other frequency ranges ($r = 0.62$, $r = 0.63$; $p < 0.05$ and 0.05 respectively).

Relationships between lung sound changes and rAw and rAi of the terminal bronchioles

Figure 6 shows the correlations of lung sound changes with the rAw and rAi of the terminal bronchiole from the left and right lungs.

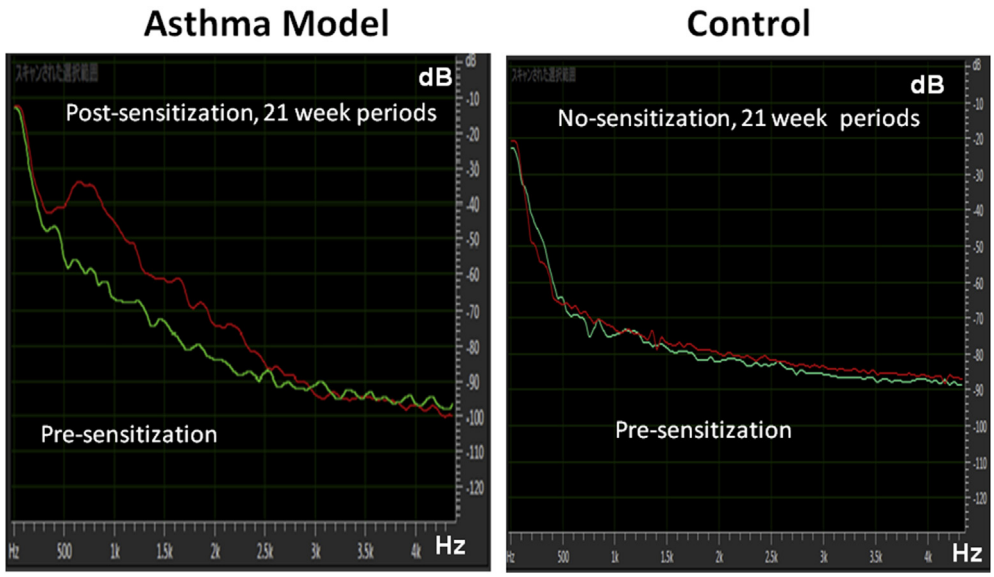

Fig. 3. Changes in lung sounds in typical cases of the asthma models and controls. In the sound spectrogram, the horizontal axis is intensity (dB) and the vertical axis is frequency (Hz). The intensity of lung sounds in the asthma models was greater than that in the controls.

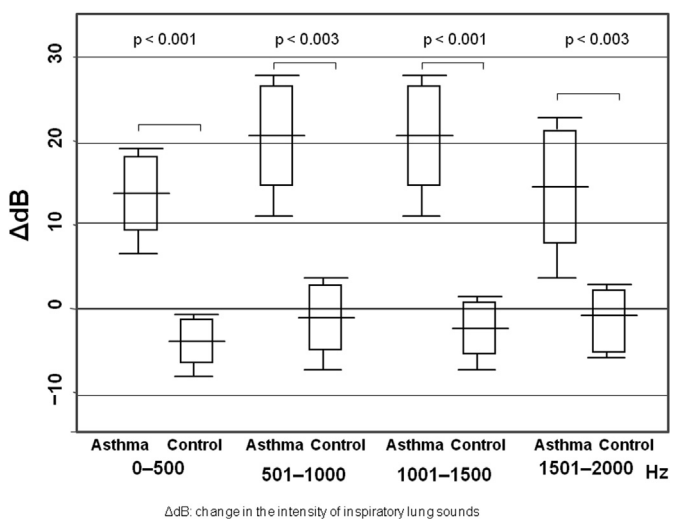

Fig. 4. Comparison of lung sound changes between the asthma models and controls. The change in lung sound intensity in the asthma models was greater than that in the controls. ΔdB, change in lung sound intensity.

The rAw of the third bronchi did not correlate with the changes in lung sound intensity [r = 0.46 (0–500 Hz), r = 0.07 (501–1000 Hz), r = 0.03 (1001–1500 Hz), and r = 0.16 (1501–2000 Hz)]. The rAi of the third bronchi did not correlate with the changes in lung sound intensity [r = −0.46 (0–500 Hz), r = −0.07 (501–1000 Hz), r = −0.03 (1001–1500 Hz), and r = −0.16 (1501–2000 Hz)].

The rAw of the terminal bronchioles correlated with the changes in lung sound intensity from 501 to 2000 Hz but did not correlate with the lung sound intensity from 0 to 500 Hz [r = 0.51 (0–500 Hz), r = 0.76 (500–1000 Hz), r = 0.69 (1000–1500 Hz), and r = 0.67 (1500–2000 Hz); NS, $p < 0.003$, $p < 0.05$ and 0.05 respectively].

The rAi of the terminal bronchioles correlated with the changes in lung sound intensity from 501 to 2000 Hz but did not correlate with the lung sound intensity from 0 to 500 Hz [r = −0.51 (0–500 Hz), r = −0.76 (500–1000 Hz), r = −0.69 (1000–1500 Hz), and r = −0.67 (1500–2000 Hz); NS, $p < 0.003$, $p < 0.05$ and 0.05 respectively].

The rAw of the terminal bronchioles showed the highest correlation with the changes in inspiratory sounds from 501 to 1000 Hz.

Discussion

In the present study, the lung sound intensity in the middle frequency ranged from 501 to 1500 Hz, particularly from 501 to 1000 Hz, in the inspiratory phase, indicating a morphological change in the small airways in a chronic asthma model. The change in the lung sounds during the disease process from the onset of asthma appeared to reflect thickening of the bronchial wall and narrowing of the lumen of the peripheral airways.

We reported that our index, named ic700, which is an index of lung sound intensity, could detect airway dysfunction in children with asymptomatic asthma while avoiding the influences of airflow and body size.[14] The ic700 is the sound intensity at 700 Hz in the inspiratory phase that can be an indicator of chronic structural changes in asthmatic airways in asymptomatic subjects without wheezes.

Many studies on the changes in breath sounds in asthmatic conditions have focused on the changes in breath sound generation and/or transmission.[13] However, there have been no reports on the change in lung sounds and its correlation with bronchial structural change directly. In the present study, we attempted to clarify the relationship between the changes in inspiratory sounds and morphological changes in the airways in asthma.

Normal inspiratory sounds are generated primarily within the lobar and segmental airways, whereas expiratory sound are generated from more proximal locations.[26,27] Our results are consistent with these previous findings.

In the present study, the sRaw of lung function parameters in the asthma models was increased after repeated antigen challenges. The sRaw of lung function parameters correlated well with the changes in lung sound intensity from 1001 Hz to 1500 Hz. The changes in lung sound intensity from 501 Hz to 1000 Hz were well correlated with the changes (hyperplasia) in the bronchial wall, even only in the peripheral airways. The changes in lung sound

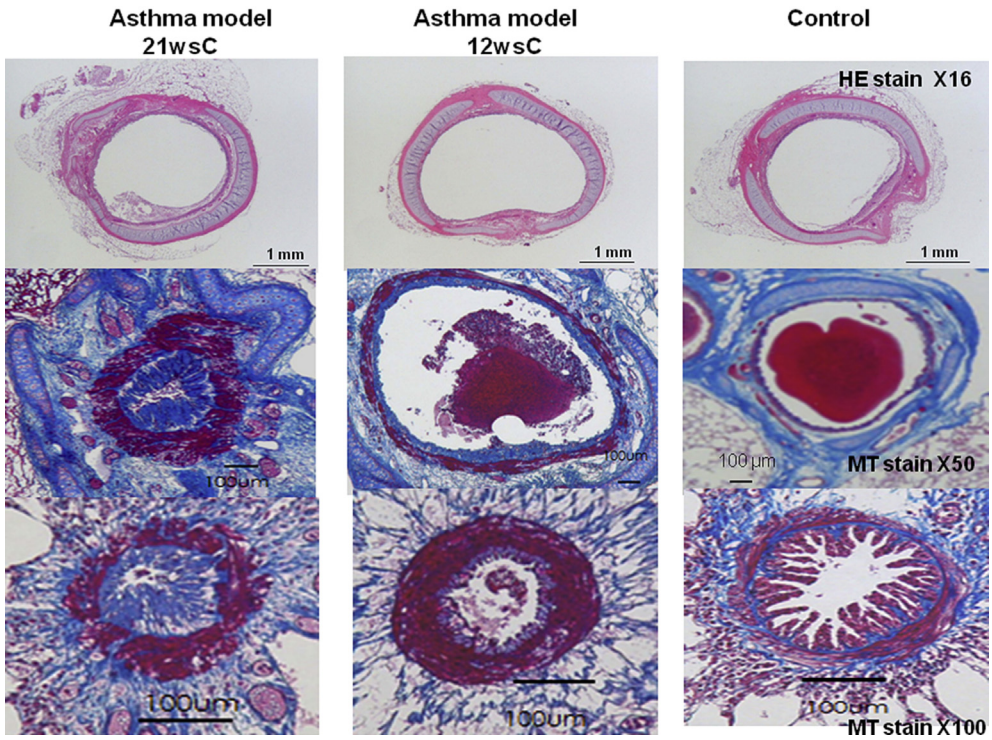

Fig. 5. Morphological changes in the trachea, third bronchi, and terminal bronchiole in typical cases of the asthma models and controls.

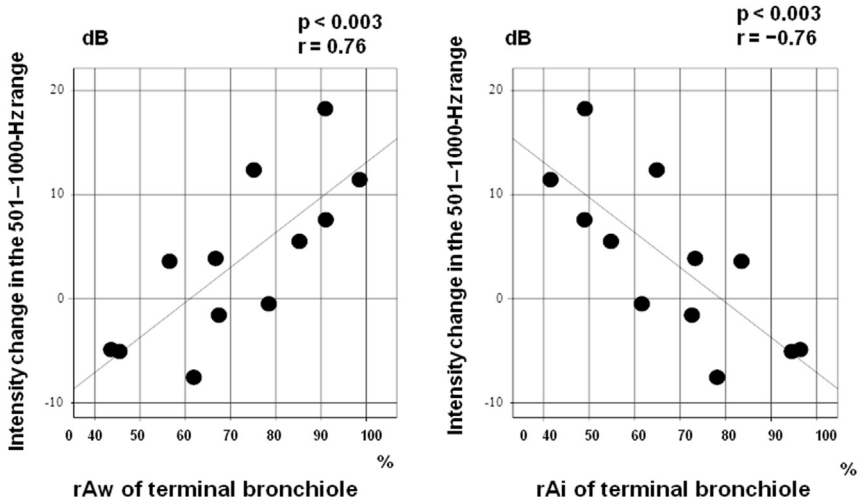

Fig. 6. Relationship between lung sound changes and rAw and rAi of terminal bronchioles. The rAw of the terminal bronchioles showed the highest correlation with the changes in lung sounds from 500 to 1000 Hz [$r = 0.76$ (500–1000 Hz)]. rAw, Ratio of airway wall thickness to total airway area; rAi, Ratio of internal area to total airway area.

intensity from 1001 Hz to 1500 Hz reflected the airway resistance rather than the hyperplasia of the bronchial wall.

The lung sound intensity from 501 Hz to 1000 Hz was louder than that from 1001 Hz to 1500 Hz. The lung sound intensity from 501 to 1000 Hz was considered to be suitable for the accurate analysis of the airway wall.

Malmberg *et al.*[28] reported a significant difference in the frequencies of baseline lung sounds between healthy subjects and patients with asthma or chronic obstructive pulmonary disease. Shreur *et al.*[29] reported that at similar levels of airway obstruction, changes in both the frequency and intensity of sounds with airflow were higher in patients with asthma than in normal subjects. These studies showed the lung sound characteristics while airflow and volume were standardized during acutely induced airway obstruction. Thus, the lung sounds in patients with asthma reflect not only the degree of airway obstruction but also reflect the changes in their breath sound transmissions.

Matsumoto *et al.* assessed airway wall thickening quantitatively by computed tomography and reported its clinical implication in asthma.[30] Airway wall thickening should include histological airway structural changes, such as subbasement membrane thickening, increase in mucous gland and goblet cells, airway wall hyperplasia, and increased vascularity, i.e., chronic inflammation. Morphological changes in asthma, such as increased airway wall thickness, may enhance the changes in lung sounds even at a similar change in forced expiratory volume in 1 s.[31]

These inflammatory changes are assumed to increase the stiffness of the airway; thus, the intensity of sound transmission can be increased. The rAw of the terminal bronchioles showed the highest correlation with the changes in inspiratory sounds between 501 and 1000 Hz. In this study, we showed that the changes in lung sound intensity reflected the hyperplasia of the wall area of the small airways. The sound intensity of the middle frequency range appears to be a useful biomarker for the detection of structural changes in small airways in asthma models. We need to investigate usefulness of the sound intensity of the middle frequency range for bronchial asthma.

In summary, we analyzed the changes in lung sounds during the progression of asthma in guinea pigs used as an experimental chronic asthma model and observed hyperplasia of the bronchial wall and narrowing of the lumen in the airways. We found that the changes in inspiratory sound intensity in the middle frequency range from 501 to 1000 Hz in the inspiratory phase correlated well with the morphological changes in small airways. Lung sound change during disease progression from onset correlated with the morphological changes in the small airways in a chronic asthma guinea pig model.

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Conflict of interest

The authors have no conflict of interest to declare.

Authors' contributions

CH, KM and SW designed and conceived the study. CH, KS, TO, GPS, KK and SW collected data. CH, KM, and YN interpreted the data. CH prepared the manuscript with input from KM, KS, TO, GPS, KK, YN and SW.

References

1. Laitinen LA, Laitinen A, Haahtela T. Airway mucosal inflammation even in patients with newly diagnosed asthma. *Am Rev Respir Dis* 1993;**147**:697–704.
2. Djukanovic R, Roche WR, Wilson JW, Beasley CR, Twentyman OP, Howarth RH, et al. Mucosal inflammation in asthma. *Am Rev Respir Dis* 1990;**142**:434–57.
3. Boulet LP, Belanger M, Carrier G. Airway responsiveness and bronchial-wall thickness in asthma with or without fixed airflow obstruction. *Am J Respir Crit Care Med* 1995;**152**:865–71.
4. Stewart AG, Tomlinson PR, Wilson J. Airway wall remodeling in asthma: a novel target for the development of anti-asthma drugs. *Trends Pharmacol Sci* 1993;**14**:275–9.
5. Kuwano K, Bosken CH, Pare PD, Bai TR, Wiggs BR, Hogg JC. Small airway dimensions in asthma and in chronic obstructive pulmonary disease. *Am Rev Respir Dis* 1993;**148**:1220–5.
6. Pear JK, Woolcock AJ, Cullen K. Rate of decline of lung function in subjects with asthma. *Eur J Respir Dis* 1987;**70**:171–9.
7. Lange P, Parner J, Vestbo J, Schnohr P, Jensen G. A 15-year follow-up study of ventilatory function in adults with asthma. *N Engl J Med* 1998;**339**:1194–200.
8. Pohunek P, Warner JO, Turzíková J, Kudrmann J, Roche WR. Markers of eosinophilic inflammation and tissue re-modelling in children before clinically diagnosed bronchial asthma. *Pediatr Allergy Immunol* 2005;**16**:43–51.
9. Berankova K, Uhlik J, Honkova L, Pohunek P. Structural changes in the bronchial mucosa of young children at risk of developing asthma. *Pediatr Allergy Immunol* 2014;**25**:136–42.
10. Malmberg LP, Sorva R, Sovijärvi AR. Frequency distribution of breath sounds as an indicator of bronchoconstriction during histamine challenge test in asthmatic children. *Pediatr Pulmonol* 1994;**18**:170–7.
11. Malmberg LP, Sovijärvi AR, Paajanen E, Piirilä P, Haahtela T, Katila T. Changes in frequency spectra of breath sounds during histamine challenge test in adult asthmatics and healthy control subjects. *Chest* 1994;**105**:122–31.
12. Habukawa C, Nagasaka Y, Murakami K, Takemura T. High-pitched breath sounds indicate airflow limitation in asymptomatic asthmatic children. *Respirology* 2009;**14**:399–403.
13. Habukawa C, Murakami K, Horii N, Yamada M, Nagasaka Y. A new modality using breath sound analysis to evaluate the control level of asthma. *Allergol Int* 2013;**62**:29–35.
14. Habukawa C, Murakami K, Endo M, Yamada M, Horii N, Nagasaka Y. Evaluation of bronchial dysfunction using a new modality of breath sound analysis in asthmatic children. *Allergol Int* 2015;**64**:84–9.
15. Mahagnah M, Gavriely N. Gas density does not affect pulmonary acoustic transmission in normal men. *J Appl Physiol* 1995;**78**:928–37.
16. Rasanen J, Gavriely N. Detection of porcine oleic acid-induced acute lung injury using pulmonary acoustics. *J Appl Physiol* 2002;**93**:51–7.
17. Rasanen J, Gavriely N. Response of acoustic transmission to positive airway pressure therapy in experimental lung injury. *Intensive Care Med* 2005;**31**:1434–41.
18. Katsunuma T, Fujita K, Mak JC, Barnes PJ, Ueno K, Iikura Y. Beta-adrenergic agonists and bronchial hyperreactivity: role of beta2-adrenergic and tachykinin neurokinin-2 receptors. *J Allergy Clin Immunol* 2000;**106**:104–8.
19. Ohki Y, Tokuyama K, Sato A, Nishimura H, Tabata M, Tomiyoshi K, et al. Maturational changes in airway remodeling after chronic exposure to ovalbumin in sensitized guinea pigs: role of cell renewal of airway resident cells. *Pediatr Res* 2002;**52**:525–32.
20. Olivo CR, Vieira RP, Arantes-Costa FM, Perini A, Martins MA, Carvalho CR. Effects of aerobic exercise on chronic allergic airway inflammation and remodeling in guinea pigs. *Respir Physiol Neurobiol* 2012;**182**:81–7.
21. Pennock BE, Cox CP, Rogers RM, Cain WA, Wells JH. A noninvasive technique for measurement of changes in specific airway resistance. *J Appl Physiol Respir Environ Exerc Physiol* 1979;**46**:399–406.
22. Mochizuki H, Ohki Y, Arakawa H, Kato M, Tokuyama K, Morikawa A. Effect of inhaled indomethacin on distilled water-induced airway epithelial cell swelling. *J Appl Physiol* 2002;**92**:155–61.
23. Mochizuki H, Ohki Y, Arakawa H, Tokuyama K, Morikawa A. Effect of ultrasonically nebulized distilled water on airway epithelial cell swelling in guinea pigs. *J Appl Physiol* 1999;**86**:1505–12.
24. Moreno RH, Hogg JC, Paré PD. Mechanics of airway narrowing. *Am Rev Respir Dis* 1986;**133**:1171–80.
25. Bai A, Eidelman DH, Hogg JC, James AL, Lambert RK, Ludwig MS, et al. Proposed nomenclature for quantifying subdivisions of the bronchial wall. *J Appl Physiol (1985)* 1994;**77**:1011–4.
26. Forgacs P. The functional basis of pulmonary sounds. *Chest* 1978;**73**:399–405.
27. Bohadana AB, Kanga JF, Kraman SS. Does airway closure affect lung sound generation. *Clin Physiol* 1988;**8**:341–9.
28. Malmberg LP, Pesu L, Sovijarvi AR. Significant differences in flow standardised breath sound spectra in patients with chronic obstructive pulmonary disease, stable asthma, and healthy lungs. *Thorax* 1995;**50**:1285–91.
29. Shreur HJW, Vanderschoot J, Zwinderman AH, Dijkman JH, Sterk PJ. The effect of methacholine-induced acute airway narrowing on lung sounds in normal and asthmatic subjects. *Eur Respir J* 1995;**8**:257–65.
30. Matsumoto H, Niimi A, Tabuena RP, Takemura M, Ueda T, Yamaguchi M, et al. Airway wall thickening in patients with cough variant asthma and non-asthmatic chronic cough. *Chest* 2007;**131**:1042–9.
31. Olson DE, Hammersley JR. Mechanism of lung sound generation. *Semin Respir Med* 1985;**6**:171–9.