

Original Article

Prediction of Airway Inflammation in Patients with Asymptomatic Asthma by Using Lung Sound Analysis

Terufumi Shimoda, MD, PhD^a, Yukio Nagasaka, MD, PhD^b, Yasushi Obase, MD, PhD^c, Reiko Kishikawa, MD, PhD^a, and Tomoaki Iwanaga, MD, PhD^a *Fukuoka, Kyoto, and Okayama, Japan*

What is already known about this topic? The intensity and frequency of sounds in a lung sound analysis may be related to airway constriction. The possibility of airway inflammation prediction by lung sound analysis was suggested.

What does this article add to our knowledge? A lung sound analysis can evaluate the eosinophilic airway inflammation in persons with asthma even without symptoms.

How does this study impact current management guidelines? Asthma control may be able to be evaluated by this totally noninvasive new method to evaluate lung sounds, but further studies will be necessary to confirm this.

BACKGROUND: The intensity and frequency of sounds in a lung sound analysis (LSA) may be related to airway constriction; however, whether any factors of an LSA can predict airway eosinophilic inflammation in patients with asthma is unknown. **OBJECTIVE:** To determine whether an LSA can predict airway eosinophilic inflammation in patients with asymptomatic asthma.

METHODS: The expiratory-inspiratory ratios of sound power in the low-frequency range (E-I LF) from 36 patients with asymptomatic asthma were compared with those of 14 healthy controls. The relations of E-I LF with airway eosinophilic inflammation were analyzed. The E-I LF cutoff value for predicting airway eosinophilic inflammation also was analyzed. **RESULTS:** The mean $\pm$ SD E-I LF was higher in the patients with asthma and with increased sputum eosinophils than in those patients without increased sputum eosinophils (0.45 ± 0.24 vs 0.20 ± 0.12 ; $P < .001$) or in the healthy controls (0.25 ± 0.10 ; $P = .003$). A multiple regression analysis showed that the sputum eosinophil ratio and exhaled nitric oxide were independently correlated with E-I LF, $P = .0003$ and $P = .032$, respectively. For the prediction of increased sputum eosinophils and increased fractional exhaled nitric oxide levels, the E-I LF thresholds of 0.29 and 0.30 showed sensitivities of 0.80 and 0.74 and specificities of 0.83 and 0.77, respectively.

CONCLUSIONS: We showed that LSAs can safely predict airway inflammation of patients with asymptomatic asthma. © 2014 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2014;■:■-■)

Key words: Lung sound analysis; Airway inflammation; Bronchial asthma

The persistent airway inflammation in bronchial asthma enhances airway hyperresponsiveness and causes airway narrowing. Wheezes and rhonchi are recognized as accessory sounds in bronchial asthma and may be used to estimate the levels of airway inflammation and/or airway constriction in a clinical setting.^{1,2} Although the auscultation is fairly subjective, the objectivity of a computerized lung sound analysis (LSA) of the intensity and frequency of lung sounds has been reported.^{3,4} The intensity or frequency of lung sounds increases with increasing airway constriction⁵ and correlates with pulmonary function and airway hyperresponsiveness.⁶⁻⁸ An LSA can be performed even for patients with asthma and without audible wheezing during auscultation. Each of the highest frequencies at inspiration or expiration is inversely correlated with airway obstruction.^{9,10}

Notably, airway inflammation is an important factor in the diagnosis and/or evaluation of the effects of treatment of patients with asthma. The degree of airway inflammation is now assessed by performing a bronchial biopsy and a bronchial lavage with a fiberoptic bronchoscope, which are invasive; by inducing sputum by using a hypertonic saline solution inhalation, which is cumbersome or unstable to be obtained; and by analyzing exhaled breath condensate or measuring exhaled nitric oxide (FeNO), which are expensive.^{11,12} If airway inflammation in bronchial asthma can be estimated by an LSA, which can be performed repeatedly and noninvasively, then this tool could be very useful for clinical use. However, this method has not yet been thoroughly evaluated.¹³ If any of the factors measured in an LSA can detect the level of airway inflammation consistently, automatically, and easily, then such a tool will be useful for controlling asthma in the clinic. In this study, we investigated whether any of the elements of an LSA could predict airway

^aClinical Research Center, Fukuoka National Hospital, Fukuoka, Japan

^bKyoto Respiratory Center, Otowa Hospital, Kyoto, Japan

^cDepartment of Respiratory Medicine, Kawasaki Medical School, Kurashiki, Okayama, Japan

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Corresponding author: Terufumi Shimoda, MD, PhD, Clinical Research Center, Fukuoka National Hospital, 4-39-1 Yakatabaru, Minami-ku, Fukuoka 811-1394, Japan. E-mail: t-shimoda@mfukuoka2.hosp.go.jp.

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Abbreviations used*Ach*-Acetylcholine*E-I LF*-Expiratory-inspiratory ratio of sound power in the low-frequency range*Exp LF*-Sound power during expiration*FeNO*-Exhaled nitric oxide*FVC*-Forced vital capacity*Ins LF*-Sound power during inspiration*LABA*-Long-acting β -agonist*LAS*-Lung sound analysis*NO*-Nitric oxide*PC₂₀*-Concentration of acetylcholine when the *FEV₁* had decreased by >20%*SABA*-Short-acting β -agonist*V₂₅*-Maximal expiratory flow at 25%*V₅₀*-Maximal expiratory flow at 50%

eosinophilic inflammation in patients with asymptomatic asthma who do not have audible wheezing on auscultation.

METHODS**Subjects and study design**

From October 2004 to June 2011, we examined 36 patients, new to our hospital, diagnosed with mild persistent asthma and 14 healthy controls (Table I). An LSA, a blood examination, pulmonary function tests, an FeNO measurement, an acetylcholine (Ach) bronchial provocation test, and an induced sputum analysis were performed.^{12,14} The following inclusion criteria were used: fulfillment of the Global Initiative for Asthma criteria¹¹; a history of asthma symptoms, including recurrent cough, wheezing, or dyspnea, and had positive airway hyperresponsiveness (PC₂₀) (ie, PC₂₀ for Ach <8 mg/mL) or positive bronchial reversibility (ie, 12% and 200 mL or more of FEV₁ increase); and no current treatment with inhaled or systemic corticosteroids. The use of antiasthma drugs, including bronchodilators, was discontinued for at least 24 hours before the examination. Patients were included if whole-lung auscultation failed to detect any evidence of wheezing. The subjects with a history of cigarette smoking, chronic obstructive pulmonary disease, any cardiovascular disease, or current viral or bacterial infection were excluded from this study.¹⁵ The healthy controls had no respiratory symptoms and no overt illnesses, and exhibited no abnormalities in their lung function tests and chest radiographies. All of the healthy controls and the patients with asthma were younger than 60 years old, and their body mass index values ranged between 18.2 and 26.8 kg/m². The ethics committee of the Fukuoka National Hospital approved the study protocol (protocol 20-12); all of the participants received verbal and written study information before providing their informed consent.

LSA

Lung sounds were recorded for ≥ 30 seconds over the base of the left lung by using a hand-held microphone. The sound recording was performed in a quiet room, but not in a soundproof booth, in the outpatient department. The patients breathed freely through a disposable mouthpiece to synchronize their breath cycles while the breath sounds were recorded. The recording system consisted of an electrostethoscope that contained a wide-range audio sensor adhered inside a diaphragm (Bio-Sound Sensor BSS-01; Kenz Medico, Saitama, Japan), a signal processing system, and

TABLE I. Subject characteristics

	Healthy controls, mean $\pm$ SD (n = 14)	Patients with asthma, mean $\pm$ SD (n = 36)	P value
Age (y)	36.2 $\pm$ 13.3	41.1 $\pm$ 13.2	.25
Body mass index (kg/m ²)	22.1 $\pm$ 4.3	22.5 $\pm$ 2.1	.77
Men/women	1/13*	12/24*	.06
Atopic/nonatopic	—	22/14*	
Disease duration (y)	—	7.2 $\pm$ 10.4	—
FEV ₁ -FVC (%)	88.0 $\pm$ 6.7	74.5 $\pm$ 9.9	<.001
FEV ₁ %predicted	102.1 $\pm$ 14.0	88.0 $\pm$ 16.9	.005
V ₅₀ %predicted	98.5 $\pm$ 16.2	62.7 $\pm$ 24.8	<.001
V ₂₅ %predicted	87.4 $\pm$ 24.9	44.7 $\pm$ 18.4	<.001
Log PC ₂₀ (mcg)	—	2.7 $\pm$ 0.6†	—
IgE (IU/mL)	74.8 $\pm$ 102.6	613.7 $\pm$ 1272.0	.020
FeNO (ppb)	16.9 $\pm$ 9.6	105.9 $\pm$ 100.8	<.001
Sputum macrophages (%)	65.4 $\pm$ 19.6	44.0 $\pm$ 20.1‡	.002
Sputum neutrophils (%)	27.0 $\pm$ 13.5	32.7 $\pm$ 20.0‡	.27
Sputum eosinophils (%)	0.2 $\pm$ 0.4	17.4 $\pm$ 22.3‡	<.001
E-I LF	0.25 $\pm$ 0.10	0.37 $\pm$ 0.25	.02

*No. subjects.

†n = 33.

‡n = 32.

a personal computer (see Figure E1A in this article's Online Repository at www.jaci-inpractice.org). The sensor had a band-pass filter range of 40 to 2500 Hz and sound-collecting ability in the 40 to 2000 Hz range. The recorded sounds were analyzed by using a sound spectrometer (LSA-2008; Kenz Medico, Saitama, Japan). The recorded sounds were resampled to 10,000 Hz and analyzed by 1024-point fast Fourier transformation with 60% overlap into adjacent segments by using a Hanning data window. The results are presented as a sound spectrograph with frequencies in Hz on the vertical axis and time on the horizontal axis (see Figure E1B and E1C in this article's Online Repository at www.jaci-inpractice.org). The sound intensity (dBm) was shown with color and brightness; dBm, which was used to express the power of the sound in this study, is an abbreviation for the ratio of the measured power in decibels (dB) in reference to 0.001 W. Thus, dBm was an absolute unit that was used when measuring the absolute power. We developed a new indicator because expiratory sound power correlated with sputum eosinophils positively, and inspiratory sound power correlated with sputum eosinophils inversely. The expiratory-inspiratory ratio of sound power (dB) in the low frequency range (E-I LF) is defined by the following formula:

E-I LF = $10^A([e - i]/20)$, in which "e" is the sound power during expiration and "i" is the sound power during inspiration.

Measurement of flow-volume curves

The forced vital capacity (FVC), the FEV₁, and the maximal expiratory flow at 50% (V₅₀) and maximal expiratory flow at 25% (V₂₅) were measured with a spirometer (Chest Graph HI-701; Chest M.I., Tokyo, Japan). The results are expressed as a percentage of the predicted values (FEV₁-FVC, FEV₁ %predicted, V₅₀ %predicted, and V₂₅ %predicted), based on the relevant reference standards.¹⁵

Measurement of FeNO concentration

By following the guidelines published by the American Thoracic Society, FeNO was measured by using the single-breath

online method and a fast response (0.02 seconds) chemiluminescence analyzer (Sievers Nitric Oxide Analyzer NOA 280i; GE Analytical Instruments, Boulder, Colo).¹⁴ While seated, the subjects breathed through a mouthpiece attached to a 1-way valve. The valve had 2 sampling ports near the mouthpiece. The nitric oxide (NO) was sampled directly into the analyzer through a Teflon side-arm tube attached to one of the sampling ports (NO Breath kit; GE Analytical Instruments, Boulder, Colo). The exhalation pressure was measured by a pressure transducer in the analyzer via the second sampling port. All of the measurements were made by using a mouth pressure of 16 cm H₂O, which corresponds to an expiratory flow of 50 mL/s. After inhaling to total lung capacity, the subjects immediately exhaled into the mouthpiece. The mouth pressure then was displayed on a computer screen as a prompt for the subjects to maintain a steady flow. The NO values were recorded as a plateau during the last part of the exhalation. Repeated exhalations were performed to achieve 3 NO values that agreed at the 5% level. The NO concentrations were recorded as the average of these 3 values.

Measurement of airway hyperresponsiveness to Ach

The challenge test was performed by using standardized methodology. Antiasthma medications that patients received were withheld for at least 24 hours before the measurement of airway hyperresponsiveness. The subjects inhaled aerosolized Ach from a hand-held nebulizer (PARI BOY 038; PARI GmbH, Starnberg, Germany) while performing tidal breathing for 2 minutes. The operating airflow rate was 5 L/min. Isotonic saline solution was inhaled first as a control, followed by progressive doublings of the Ach concentration, from 39 to 20,000 mcg/mL. The FEV₁ was measured after each inhalation by using a spirometer (Chest Graph HI-70). The percentage decrease in FEV₁ was calculated from the post-saline solution FEV₁. The test was continued until the FEV₁ had decreased by >20% or until the maximum concentration of Ach had been administered. Airway hyperresponsiveness was expressed as the PC₂₀. At the end of the test, any decrease in the FEV₁ was reversed with an inhalation of salbutamol (0.3 mL Venetlin for inhalation; GSK, Tokyo, Japan). The subjects with a PC₂₀ < 8000 mcg/mL were considered to have airway hyperresponsiveness.¹¹

Sputum induction and processing

Sputum induction. The participants inhaled 5 mL 3% sodium chloride solution through a small ultrasonic nebulizer (Nescosonic nebulizer, UN-511; Azwell, Tokyo, Japan) to induce sputum.¹² According to the manufacturer, the output was approximately 5 L/min, and the mass median aerodynamic diameter of the nebulized saline solution ranged from 1 to 5 μ m. Before coughing up sputum, the subjects were asked to rinse their mouths, to swallow saliva and water, and to blow their noses to minimize contamination with saliva and postnasal drip. The subjects were asked to cough during and after the inhalation, and to expectorate into empty containers. Sputum was induced over a 20-minute period. An inhaled β_2 -stimulant (2 mL saline solution and 0.3 mL salbutamol solution with a pressurized nebulizer) was administered as a premedication. To examine the participants' lung function, flow-volume curve measurements were taken before and after the sputum induction. If the FEV₁ decreased by more than 10%, then the subject was treated with

an additional inhalation of a β_2 -stimulant. None of the subjects experienced marked adverse effects.

Sputum processing. The sputum sample was processed within 30 minutes. The sputum samples were transferred to a Petri dish, and the more-viscous parts were collected by using forceps. The sputum plugs were first mixed with forceps. The samples were then processed by a method described by Metso et al¹² to collect cells for the cytospin procedure. The samples were diluted with 6.5 mmol/L dithiothreitol (diluted 10-fold in distilled water) (Sputolysin; Calbiochem, La Jolla, Calif) and diluted 4-fold with distilled water, and the mixture was incubated for 15 minutes on a roller mixer at room temperature. An equal volume of PBS solution (pH 7.2) was added, and the incubation continued for an additional 5 minutes. The mixture was filtered through a 53- μ m mesh nylon filter (SEFAR Mesh+Technology NYTAL 21T-53; Sefar, Heiden, Switzerland). The sputum cells were separated by centrifugation at 2000 g for 10 minutes. The cells obtained after centrifugation were resuspended in 1 mL PBS solution, and the total number of cells was determined by using a hemocytometer. The cell viability was measured by using the trypan blue exclusion test (trypan blue stain 0.4%; Gibco BRL Life Technologies, Paisley, UK). The cell suspension was cytocentrifuged (Cytospin 3; Shandon, Astmoor, UK) onto microscope slides (Super Frost S2441, Matsunami, Tokyo, Japan) at 450 rpm for 6 minutes. The cytospin products from the sputum were air-dried for 30 minutes and then stained by using the Giemsa staining method (Muto Pure Chemicals Co Ltd, Tokyo, Japan). At least 400 nonsquamous cells, including eosinophils, neutrophils, lymphocytes, macrophages, and ciliated epithelial cells, were counted differentially. The results are expressed as the percentages of the total nonsquamous cell counts. When the slide examination revealed macrophages and ciliated epithelial cells, the sample was considered to be of bronchial origin and was included in the study.

Statistical analysis

The data analysis was performed with StatMate IV (ATMS Co Ltd, Tokyo, Japan) and JMP (SAS Institute, INC, Tokyo, Japan). The variables of the patients with asthma were compared with those of the healthy controls by using a Mann-Whitney *U* test. Next, a Spearman rank correlation and a multiple regression analysis were used to examine the relationship between the airway inflammation and the other factors for the patients with asthma. The results were considered positive for airway inflammation if the patients with asthma had $\geq 3\%$ eosinophils in their induced sputum¹² or had ≥ 40 ppb FeNO values.¹¹ The receiver operating characteristic curves were generated for E-I LF to evaluate the sensitivity and specificity for predicting airway eosinophilic inflammation (FeNO and sputum eosinophils). The level of significance (*P* value) was set at .05. The data are presented as the means $\pm$ SDs.

RESULTS

Patient characteristics

Acceptable sputum samples were obtained from 32 patients. An Ach bronchial provocation test was completed for 33 patients with asthma. There were no significant differences in age and body mass index between the patients with asthma and the healthy controls (Table 1). The patients with asthma had significantly lower FEV₁-FVC, FEV₁ %predicted, V₅₀ %predicted, and V₂₅ %predicted values than the healthy controls. The

TABLE II. E-I LF of patients with asthma divided by the presence of airway eosinophilic inflammation (sputum eosinophil percentage and FeNO level) and healthy controls: comparison of the E-I LF of the patients with asthma divided by sputum eosinophil percentage

	Healthy controls, mean $\pm$ SD (n = 14)	Patients with asthma and with less sputum eosinophils ($<3\%$), mean $\pm$ SD	P value, versus healthy controls	Patients with asthma and with more sputum eosinophils ($>3\%$), mean $\pm$ SD	P value, versus healthy controls	P value, versus patients with asthma and with less sputum eosinophils
E-I LF	0.25 $\pm$ 0.10	0.20 $\pm$ 0.12	.50	0.45 $\pm$ 0.24	.003	$<.001$

TABLE III. E-I LF of patients with asthma divided by the presence of airway eosinophilic inflammation (sputum eosinophil percentage and FeNO level) and healthy controls: comparison of the E-I LF of the patients with asthma divided by FeNO level

	Healthy controls, mean $\pm$ SD (n = 14)	Patients with asthma and with low FeNO (<40 ppb), mean $\pm$ SD	P value, versus healthy controls	Patients with asthma and with high FeNO (>40 ppb), mean $\pm$ SD	P value, versus healthy controls	P value, versus patients with asthma and with low FeNO (<40 ppb)
E-I LF	0.25 $\pm$ 0.10	0.23 $\pm$ 0.20	.87	0.44 $\pm$ 0.24	.006	.005

patients with asthma had higher IgE, FeNO, and sputum eosinophils, and less sputum macrophage than the healthy controls. Previous to visiting our hospital, no patients had received any inhaled or oral corticosteroids. Eleven patients were on some kind of asthma medication: short-acting β -agonist (SABA), 4; theophylline and SABA, 3; and long-acting β -agonist (LABA) only, LABA plus leukotriene receptor antagonist plus SABA, LABA plus theophyllin plus SABA, LABA plus theophyllin, 1 each. None of the others received any anti-asthma medication.

E-I LFs of patients with asthma and healthy controls

The patients with asthma had significantly higher mean $\pm$ SD E-I LF values versus the healthy controls (0.37 ± 0.25 vs 0.25 ± 0.10 ; $P = .02$) (Table I). Some other factors of LSA (eg, sound intensity or frequencies in inspiratory or expiratory) had no differences between the groups (data not shown). The mean $\pm$ SD E-I LF was higher with the patients with asthma and with increased sputum eosinophils ($>3\%$) than in the healthy controls (0.45 ± 0.24 vs 0.25 ± 0.10 ; $P = .003$) or those without increased sputum eosinophils (0.20 ± 0.12 ; $P < .001$) (Table II). The mean $\pm$ SD E-I LF was higher in the patients with asthma and with a high FeNO (more than 40 ppb) than in the healthy controls (0.44 ± 0.24 vs 0.25 ± 0.10 , respectively; $P = .006$) and those without a high FeNO level (0.23 ± 0.20 ; $P = .005$) (Tables III).

Single correlations and multiple regression analyses between eosinophilic airway inflammation (increased sputum eosinophils or FeNO) and other factors of patients with asthma

The eosinophil number in induced sputum had negative correlations with the FEV₁-FVC, FEV₁ %predicted, V₅₀ %predicted, V₂₅ %predicted, and log PC₂₀ ($r = -0.51, -0.61, -0.60, -0.56$, and -0.69 , respectively), and had a positive correlation with E-I LF ($r = 0.59$) (Tables IV and V). FeNO had negative correlations with the FEV₁-FVC, FEV₁ %predicted, V₅₀ %predicted, V₂₅ %predicted, and log PC₂₀, ($r = -0.42, -0.58, -0.53, -0.43$, and -0.69 , respectively), and had a positive correlation with E-I LF ($r = 0.62$).

The multiple regression analyses for patients with asthma showed that the sputum eosinophils ratio was independently correlated with E-I LF ($t = 4.08$; $P = .0008$) and that FeNO was independently correlated with the E-I LF ($t = 2.16$; $P = .043$).

TABLE IV. Single correlations and multiple regression analysis between eosinophilic airway inflammation (eosinophils in induced sputum and FeNO) and other factors (E-I LF, pulmonary function tests factors, airway hyperresponsiveness) in the patients with asthma: single correlations

	Eosinophils in induced sputum		FeNO	
	<i>r</i>	<i>P</i> value	<i>r</i>	<i>P</i> value
Age	−0.27	.14	−0.33	.05
Body mass index	0.04	.82	−0.06	.71
Disease duration	0.31	.0677	0.32	.0577
Exp LF	0.77	.015	0.19	.63
Ins LF	−0.67	.049	−0.47	.20
E-I LF	0.59	.0001	0.62	$<.0001$
FEV ₁ -FVC	−0.51	.0016	−0.42	.0099
FEV ₁ %predicted	−0.61	$<.0001$	−0.58	.0002
V ₅₀ %predicted	−0.60	.0001	−0.53	.0009
V ₂₅ %predicted	−0.56	.0004	−0.43	.0084
Log PC ₂₀	−0.69	$<.0001$	−0.69	$<.0001$
Log IgE	0.23	.216	−0.69	$<.0001$

Exp LF- Sound power during expiration; Ins LF- Sound power during inspiration.

The Spearman correlation analysis revealed the multicollinearity among FEV₁-FVC, FEV₁ %predicted, V₅₀ %predicted, and V₂₅ %predicted; FEV₁ %predicted was used as the representative value of these factors (lung functional factors) was analyzed. E-I LF was calculated from sound power during inspiration and sound power during expiration, and E-I LF was used as the representative value of these factors (LSA factors).

The receiver operating characteristic curve for E-I LF to evaluate the sensitivity and specificity for predicting airway eosinophilic inflammation (FeNO and sputum eosinophils) was analyzed. With regard to the prediction of increased eosinophils in the induced sputum ($>3\%$), the maximum area under the curve was 0.81 of the receiver operating characteristic curve. When the E-I LF threshold was set at 0.29, the sensitivity was 0.80 and the specificity was 0.83 with maximum χ^2 value of 12.2, and the odds ratio 20.0 (95% CI, 3.7-10.7). With regard to the prediction of increased FeNO level, the maximum area under the curve was 0.77. When the E-I LF threshold was set at 0.30, the sensitivity was 0.74, and the specificity was 0.77 with maximum χ^2 value of 12.4 and odds ratio 15.8 (95% CI, 3.4-73.6).

TABLE V. Single correlations and multiple regression analysis between eosinophilic airway inflammation (eosinophils in induced sputum and FeNO) and other factors (E-I LF, pulmonary function tests factors, airway hyperresponsiveness) in the patients with asthma: multiple regression analysis

	Eosinophils in induced sputum, $R^2 = 0.81$		FeNO, $R^2 = 0.65$	
	<i>t</i>	<i>P</i> value	<i>t</i>	<i>P</i> value
Age	−1.05	.31	−0.63	.54
Women	−0.78	.45	−0.91	.37
Body mass index	0.53	.60	−0.59	.56
Disease duration	−0.59	.56	−0.02	.98
FEV ₁ %predicted	0.25	.80	−0.25	.81
Log PC ₂₀	0.17	.86	−0.82	.42
Log IgE	0.39	.70	−0.21	.84
E-I LF	4.08	.0008	2.16	.043

DISCUSSION

There are some problems to be solved in the use of LSAs. Which strategy is adequate for evaluating airway inflammation: bronchial or vesicular breath sounds? Can an LSA be analyzed with patients without audible wheezing or rhonchi? Which indicators are adequate for predicting airway inflammation: the frequencies or the intensities or the power at low or high frequencies in the expiratory or inspiratory phase? There are 2 main conclusions that can be drawn from the present study. First, the E-I LF of the LSA in patients with mild-to-moderate asthma and without audible wheezing can be an independent predictor of eosinophilic airway inflammation. Second, the E-I LF cutoff values of 0.30 and 0.29 can predict eosinophilic airway inflammation with sensitivity levels of 0.74 and 0.80, respectively, and specificity levels of 0.77 and 0.83, respectively (FeNO > 40 ppb, or sputum eosinophils ratio >3%).

First, the E-I LF was significantly higher for the patients with asthma and with airway eosinophilia than for those without airway eosinophilia and for the healthy controls. The E-I LF was the only independent indicator of airway eosinophilic inflammation according to the multiple regression analysis. Therefore, the E-I LF can be an index not only of obstructive pulmonary impairment but also of airway eosinophilic inflammation in patients with asthma. There currently are no published reports that suggest that any LSA index can predict airway eosinophilic inflammation. In most reports on the utility of an LSA, the primary focus has been on the correlations between airway narrowing and the frequency or the power of the bronchial breath sounds in the low- or high-frequency range during expiration or inspiration.^{5–10} In 1 study, both vesicular and bronchial breath sounds at inhalation and exhalation were analyzed in the context of antigen-induced provocation; however, the majority of the discussion focused on the bronchial breath sounds. Despite this focus, these investigators indicate that an LSA can distinguish the airway inflammation of late asthmatic responses from that of early asthmatic responses despite similar levels of airway constriction; they also noted that an LSA was more sensitive than other measurements of lung function.⁷ Therefore, their results also may support our hypothesis that an LSA can identify airway inflammation.

With regard to the differences in the LSA between expiratory and inspiratory flow, Habukawa et al⁹ reported on 2 interesting studies. One study emphasized the importance of inspiratory

frequency, which had a stronger relationship with FEV₁ %predicted and %FEV₇₅, and FEV₁-FVC than expiratory frequency in children with asymptomatic asthma, and the other study emphasized the usefulness of analyzing the changes in expiratory frequency, which were significantly associated with the changes in spirometric parameters and bronchial hyperresponsiveness during methacholine inhalation provocation.¹⁰ The inspiratory and expiratory phases may have their own characteristics that have not yet been confirmed. As a possible solution, we calculated the E-I LF as an index of the ratio of the sound power at inhalation and exhalation, which may be more informative. In this study, we chose the sound power of the low-frequency range (100–195 Hz) as a suitable lung sound parameter. This frequency range encompasses more than 80% of the total power of inspiratory and expiratory lung sounds, and it is not affected by ambient noise.

We found that the E-I LF was higher for the patients with asthma and with airway inflammation than for the patients with asthma and without airway inflammation, or than in the normal controls. Because bronchial breath sounds have greater power than vesicular breath sounds, the presence of an airway obstruction or airway inflammation is likely in the patients whose vesicular expiratory sound is stronger than their vesicular inspiratory sound, which is consistent with our observations.¹³ Second, we found that using an E-I LF cutoff value of 0.29 or 0.30 predicted eosinophilic airway inflammation with sensitivity and specificity of approximately 80%. Results of previous reports suggested that an LSA can be useful for predicting airway constriction or airway hyperresponsiveness.^{5–10} There are 2 possible reasons for the increase in the E-I LF: one is that the airflow is increased by the narrowing of the airway, and the other is that turbulence is caused by the airway mucosal edema associated with asthmatic inflammation.¹³ It was interesting that the E-I LF was the only significant independent factor related to the airway eosinophil count in the multiple regression analysis. The low E-I LF of healthy controls and patients with asthma and without elevated airway inflammation may indicate the lower turbulence. To our knowledge, this study is the first to recommend that an LSA may be useful for predicting eosinophilic airway inflammation in patients with asthma and with inaudible wheezing.

There were some limitations with this study. The first limitation was that some of the data could not be analyzed because of noise interference. Friction noise has a relatively higher frequency, which reaches more than 2000 Hz, and the most powerful of environmental noises are in the frequency range of less than 100 Hz. Because this study was carried out in an outpatient clinic and not in a sound research laboratory, it was difficult to completely avoid environmental and friction noise. In this study, we compared the highest frequency lung sounds with parameters of airway inflammation and pulmonary function; for example, we compared the sound power of a higher frequency range as a ratio of the sound powers of 200 to 395 Hz and 400 to 795 Hz. The absolute power of recorded lung sounds is another major problem when studying lung sounds in a clinical setting.

Many factors affect lung sounds, including body size, breathing pattern, and the pressure applied to a hand-held microphone. To avoid friction noise, patients with dry skin need to exert more pressure on a hand-held microphone compared with patients with normal skin. The sound power of subjects who are obese is usually less than that of subjects who are not obese. To avoid individual

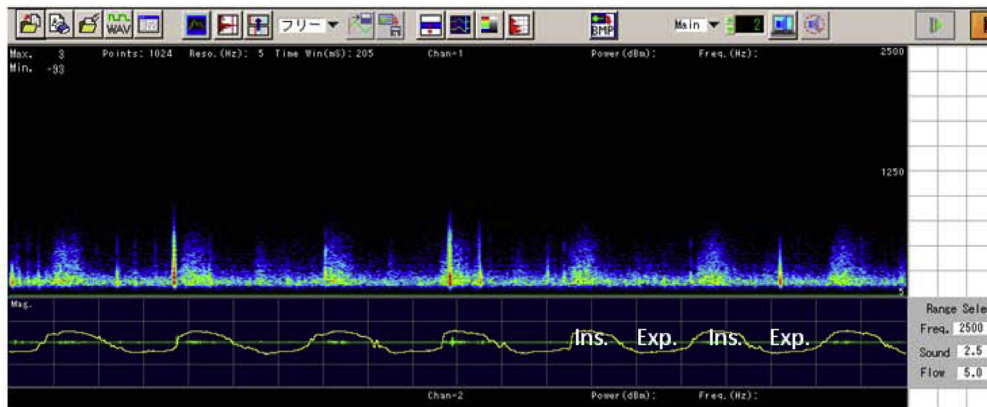
variations in the absolute power of the recorded lung sounds, we calculated the E-I ratio of the sound power. By using the E-I LF of the recorded lung sounds, we were able to minimize the problems related to noise and sound power. Reducing the friction with the contact surface of the skin and improving the analysis software to remove such noise would be desirable. In this study, we were able to analyze all of the patient data thanks to their cooperation and our careful recordings. The second limitation of this study was that it was difficult to maintain a constant flow, which may have affected the frequency of the breath sounds. We believe that using a ratio of the power of inspiration and expiration helped to stabilize the analysis and to obtain meaningful results. For example, improved software that shows the flow level on the screen would be helpful. Finally, airway narrowing can occur in the absence of airway eosinophilic inflammation. Age, smoking, obesity, and complications of other respiratory diseases such as bronchiectasis or chronic obstructive pulmonary disease also may cause airway narrowing in patients with asthma; such subjects were excluded from the study. Similarly, the patients with severe asthma and a long history of disease may have airway constriction due to irreversible airway remodeling.¹¹ Eosinophilic inflammation is not the only cause of airway obstruction; it also can be attributable to neutrophilic inflammation caused by complications associated with chronic obstructive pulmonary disease, bacterial or viral infections, or smoking.¹⁶⁻²¹ It should be noted that the E-I LF varies when these factors cause airway narrowing. In the present study, those subjects also were excluded. We are planning another study that evaluates this E-I LF correlation with bronchial hyper-responsiveness, neutrophilic inflammation, obesity, or other background factors (eg, obesity, atopic factor, smoking status).

In conclusion, we have demonstrated that an LSA can be used to detect the degree of persistent airway inflammation in asymptomatic patients with mild-to-moderate asthma by analyzing the components of the recorded lung sounds. Until now, airway inflammation has been evaluated with FeNO measurements, bronchoscopy, and sputum induction. In contrast, this study is the first to report that LSA can be used to evaluate airway inflammation externally.

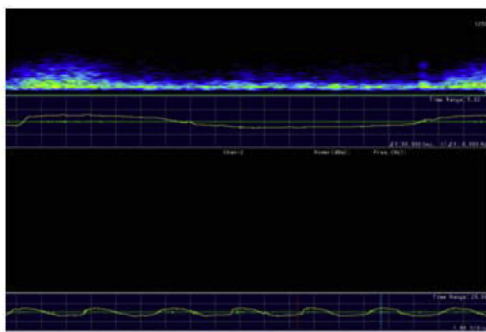
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A



B



C

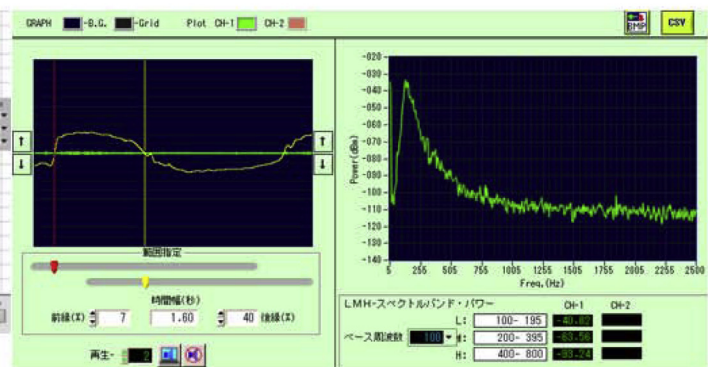


FIGURE E1 . Sound spectrogram display of lung sounds in a patient. A, The recorded sounds were analyzed by using fast Fourier analysis, and the results are displayed as a spectrograph with the frequencies in Hz on the vertical axis and time on the horizontal axis; the sound intensity (dBm) is illustrated with color and brightness. B, A selected portion of the analysis. C, A selected portion of the range of an inspiratory or expiratory position; we calculated the average power (dB) of low frequencies from 100 to 195 Hz.

The screenshot shows the LMH-Analyzer software interface. The top bar includes a 'GRAPH' tab, a 'DSO' tab, and a 'DSO' button. The left panel displays a time-domain waveform with a yellow trace on a black background, with a horizontal scale bar below it. The right panel displays a frequency spectrum plot with a yellow trace on a black background, showing a peak at approximately 250 Hz. The bottom panel contains various settings, including 'LIMH-スペクトルバンド・パワー' and 'ベース周波数'.