

Lung Sound Analysis and Airway Inflammation in Bronchial Asthma



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What is already known about this topic? Lung sound analysis (LSA) was suggested as a new marker for airway inflammation in bronchial asthma.

What does this article add to our knowledge? LSA can be used to assess airway inflammation and obstruction in bronchial asthma. Furthermore, LSA can detect both central and peripheral airway inflammation.

How does this study impact current management guidelines? LSA is a noninvasive method for evaluating airway inflammation that could be useful for identifying and treating airway inflammation in asthmatic patients.

BACKGROUND: Our previous study on lung sound analysis (LSA) revealed that the expiration-to-inspiration sound power ratio in a low-frequency range (E/I LF) was increased in patients with bronchial asthma, even when they have no wheezes.

OBJECTIVE: We also monitored the expiration-to-inspiration sound power ratio in a mid-frequency range (E/I MF) and the mid- to low-frequency sound power ratio for inspiration and expiration (ie, I MF/LF and E MF/LF, respectively) using a new software program to examine which parameter is most suitable as an index of airway inflammation in patients with asthma.

METHODS: A study was conducted in 31 patients with mild-to-moderate bronchial asthma to examine potential correlations of LSA parameters (E/I LF, E/I MF, I MF/LF, and E MF/LF) with spirogram parameters, airway hyperresponsiveness (PC₂₀), fractional exhaled nitric oxide (NO), and sputum eosinophils.

RESULTS: E/I LF was significantly correlated with airway narrowing (forced expiratory volume in 1 second [FEV_{1.0}]/forced vital capacity [FVC]%; $r = -0.50$, maximal expiratory flow at 50% [V₅₀], %pred: $r = -0.50$) and peripheral airway inflammation (alveolar NO: $r = 0.36$, eosinophils in peripheral sputum: $r = 0.41$). E/I MF was significantly correlated with airway narrowing (FEV_{1.0}/FVC%: $r = -0.46$, V₅₀, %pred: $r = -0.49$), airway inflammation (bronchial NO: $r = 0.43$,

alveolar NO: $r = 0.47$, eosinophils in peripheral sputum: $r = 0.50$), and airway hyperresponsiveness (logPC₂₀: $r = -0.49$). E MF/LF was significantly correlated with airway inflammation (NO: $r = 0.36$, eosinophils in sputum: $r = 0.40$) and airway hyperresponsiveness (logPC₂₀: $r = -0.40$). I MF/LF was not significantly correlated with any parameters.

CONCLUSIONS: Among the 4 LSA parameters investigated, E/I MF demonstrated the highest correlation with airway inflammation, and also with bronchial

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

Bronchial asthma is a chronic inflammatory disease characterized by airway infiltration of inflammatory cells, including eosinophils, mast cells, macrophages, neutrophils, and lymphocytes, as well as airway narrowing and increased airway hyperresponsiveness. The examination of airway inflammation is important for the diagnosis and treatment of bronchial asthma. Airway inflammation is assessed by various methods, including bronchofiberscopy, differential cell counts, and inflammatory cytokine assays in bronchoalveolar lavage and induced sputum, inflammatory cytokine assay in exhaled breath condensate, and fractional exhaled nitric oxide (FeNO). FeNO measurement with a varying expiratory flow rate has enabled bronchial and alveolar NO levels to be distinguished.¹⁻³ In addition, induced sputum can be distinguished as having a central or peripheral origin.⁴⁻⁶

We previously conducted a study on lung sound analysis (LSA) in patients with bronchial asthma with no rumbles or wheezes that showed that the expiration-to-inspiration sound power ratio in a low-frequency range (E/I LF), between 100 and 195 Hz, was increased along with airway inflammation.⁷ LSA has advantages of being simple, noninvasive, and repeatable.⁷ Our previous study used LSA analysis software, and it was incapable of analyzing data in cases when friction noise was introduced and

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Abbreviations used

E/I LF- The expiration-to-inspiration sound power ratio in a low-frequency range
E/I MF- The expiration-to-inspiration sound power ratio in a mid-frequency range
E LF- The expiration sound power in a low-frequency range
E LF/MF- The expiration sound power ratio in a low-frequency range to that in a mid-frequency range
E MF- The expiration sound power in a mid-frequency range
FVC- Forced vital capacity
FEV_{1.0}- Forced expiratory volume in 1 second
FeNO- Fractional exhaled nitric oxide
I LF- The inspiration sound power in a low-frequency range
I LF/MF- The inspiration sound power ratio in a low-frequency range to that in a mid-frequency range
I MF- The inspiration sound power in a mid-frequency range
LSA- Lung sound analysis
V₅₀ and *V₂₅*- Maximal expiratory flow at 50% and 25%

overlapped with breath sounds at auscultation. In this study, we analyzed lung sounds using the new analysis software. Easy LSA software (Easy-LSA, Fukuoka National Hospital, Fukuoka, Japan) allows for the correction of background noise and exclusion of a small portion of data that are affected by friction noise from the analysis. We examined the expiration and inspiration sound powers (dB) and the ratios of individual measurements (ie, E/I LF, E/I MF, I MF/LF, and E MF/LF), not only in the low-frequency range, between 100 and 200 Hz, but also in the mid-frequency range, between 200 and 400 Hz, which had previously been difficult to analyze because of the greater influence of noise. We hypothesized that the lung sound power analysis in the mid-frequency can improve the prediction of the airway inflammation over that in the low frequency. We assessed whether the new LSA index is a better predictor of peripheral airway eosinophilic inflammation based on peripheral sputum eosinophil number.

METHODS**Subjects and study design**

We examined 31 patients who newly visited our hospital from November 2011 to November 2014 and were diagnosed with mild-to-moderate persistent asthma and 22 healthy control subjects. LSA, blood examination, pulmonary function tests, FeNO measurement, acetylcholine (Ach) bronchial provocation test, and induced sputum analysis were performed.^{8,9} To evaluate the usefulness of the LSA, we carefully selected patients without auscultatory wheeze and also without decreased lung function. All patients included in this study fulfilled the Global Initiative for Asthma criteria.¹⁰ All included patients had a history of asthmatic symptoms, including recurrent cough, wheezing, or dyspnea, and had positive airway hyperresponsiveness, that is, PC₂₀ for Ach < 8 mg/mL. Airway reversibility was confirmed in 80% of the patients in whom bronchial asthma was diagnosed based on medical history and positive PC₂₀ results. All subjects had no smoking history and had a forced expiratory volume in 1 second/forced vital capacity (FEV_{1.0}/FVC) ratio after bronchodilator inhalation of greater than 70%. Chest X ray and high-resolution computed tomography revealed no findings indicative of emphysema, chronic obstructive

pulmonary disease, or other organic lesions in any patients. All patients demonstrated normal diffusion capacity. No patients had previously used inhaled or oral corticosteroids. Antiasthma drugs, including bronchodilators, were discontinued for at least 24 hours before this examination. Wheezing was not heard on auscultation in any patient. After the examination had been performed, appropriate treatment, including inhaled corticosteroids, was provided to all patients.

The ethics committee of Fukuoka National Hospital approved the study protocol (protocol no.: 20-12), and all participants received verbal and written study information before providing their informed consent.

Lung sound analysis

Lung sounds were recorded for 30 seconds or more using a totally noninvasive hand-held microphone over the left lung base, where we could most clearly recognize the alveolar respiratory sound. The recording system consisted of an electro-stethoscope containing a wide-range audio sensor adhered to the inside of a diaphragm (Bio-Sound Sensor BSS-01; Kenz Medico, Saitama, Japan), a signal processing system, and a personal computer. The sensor had a band-pass filter range of 40-2500 Hz and good sound-collecting ability in the 40-2000 Hz range. The recorded sound was analyzed using a sound spectrometer (Easy-LSA).

The recorded sound was analyzed by fast Fourier analysis and displayed as a spectrograph, with the frequency in Hz on the vertical axis and time on the horizontal axis. Thus, dBm is an absolute unit that is used when measuring absolute power. On the display, the vertical axis indicates the cycles in kilohertz, and the horizontal axis indicates the time in seconds. The intensity (dBm) of the sound is shown as color and brightness. The recording system was calibrated with a reference sound pressure (1 kHz; 94 dB [0 dB = 20 μ Pa]).

We defined low frequencies (LF) as frequencies of 100-200 Hz and middle frequencies (MF) as frequencies of 200-400 Hz and determined inspiration sound power, expiration sound power, inspiration-to-expiration sound power ratio in the low-frequency and mid-frequency ranges (E/I LF and E/I MF, respectively), and the MF/LF sound power ratio for inspiration and expiration (I MF/LF and E MF/LF, respectively). E/I LF, E/I MF, I MF/LF, and E MF/LF data were converted into logarithmic values (dBm).⁷

The sound recording was performed in a quiet room, but not in a soundproof booth, in the outpatient department. The patients breathed freely during the breath sound recording.

Measurement of flow-volume curves

The FVC, FEV₁, and maximal expiratory flow at 50% and 25% 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 based on relevant reference standards.¹¹

Measurement of FeNO concentration

Following the guidelines published by the American Thoracic Society, FeNO was measured 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).⁹ All of the measurements were performed using a mouth pressure of 16 cm H₂O, which corresponds to expiratory flows of 50, 100, 150, and 200 mL/s. Alveolar and central airway contributions to FeNO were calculated using the slope intercept model and the NO measurements at 50-200 mL/s.^{1,2} By referring to these reports, we measured FeNO parameters, that is,

FeNO (50 mL/s), bronchial NO, and alveolar NO, and performed differential cell counts in induced sputum, including “central sputum” collected after the first 10 minutes of hypertonic saline inhalation and “peripheral sputum” collected after an additional 10 minutes of saline inhalation.

Measurement of airway hyperresponsiveness to Ach

Ach provocation was performed by the inhalation of increasing concentrations of dilute Ach solution from 0.039 to 20 mg in 2-fold increments. The Ach concentration at which FEV_{1.0} was decreased from baseline by 20% was recorded as PC₂₀, which was used as an index of airway hyperresponsiveness.¹²

Sputum induction and processing

Sputum was induced by the inhalation of 5 mL of 3% NaCl solution using an ultrasonic nebulizer (UN-511, Nescio). The output was approximately 5 L/min. Before coughing up sputum, the subjects were asked to rinse their mouths and blow their nasal drip. The subjects were asked to cough during and after inhalation and to expectorate into empty containers. We modified the methods described by Gershman et al⁴ to collect both central and peripheral sputum. Sputum was induced over 20 minutes. The portion induced for the first 10 minutes was defined as “central sputum,” and the portion induced for the second 10 minutes was defined as “peripheral sputum.” The sputum was processed within 30 minutes. The samples were processed using the method described by Metso et al⁸ for the collection of cells for cytospin and the collection of cell-free supernatant. Cytospins from sputum were allowed to air-dry for 30 minutes and were then stained using the Giemsa staining method. At least 400 nonsquamous cells, including eosinophils, neutrophils, lymphocytes, macrophages, and ciliated epithelial cells, were differentially counted. 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

Data for individual parameters were compared between patients with bronchial asthma and healthy subjects using the *t*-test. Then, correlations between individual LSA parameters and other measurements were determined using Pearson's correlation coefficient in the patients with bronchial asthma. Finally, the data of the patients with asthma were corrected for factors that potentially influence LSA, that is, gender, age, and past smoking exposure (pack-years), and variables with *P* < .25 were added in a stepwise manner to determine which of the LSA parameters (E/I LF, E/I MF, I MF/LF, or E MF/LF) had the strongest correlation with FEV_{1.0}/%pred, V₅₀/%pred, logPC₂₀, bronchial flux NO, alveolar NO, and eosinophils (%) (central sputum, peripheral sputum, and blood). E/I LF, E/I MF, I MF/LF, and E MF/LF were individually introduced into analyses with other parameters because of their multicollinearity. Values of *P* < .05 were considered statistically significant.

RESULTS

Patient characteristics

The bronchial asthma patient group consisted of 14 males and 17 females, whereas the healthy volunteer group consisted of 3 males and 19 females. All sounds were recorded easily without adverse events.

Age and body mass index were not significantly different between the 2 groups. FEV_{1.0}/FVC% and V₅₀/%pred were significantly lower in patients with the bronchial asthma than in the healthy subjects (*P* < .01 and *P* < .05, respectively). In addition, FeNO, blood eosinophils (%), central sputum eosinophils (%), and peripheral sputum eosinophils (%) were also higher in patients with bronchial asthma than in healthy subjects (*P* < .01, *P* < .001, *P* < .01, and *P* < .01) (Table I).

Comparison of individual LSA parameter data between patients with bronchial asthma and healthy subjects

E MF(dB), E/I LF, E/I MF, I MF/LF, and E MF/LF were significantly higher in the patients with bronchial asthma than in the healthy subjects (*P* < .05, *P* < .01, *P* < .05, *P* < .001, and *P* < .05, respectively) (Table II).

Correlation of individual LSA parameters with spirogram, PC₂₀, and airway inflammation (FeNO, sputum differential cell counts) in the patients with bronchial asthma

Univariate analysis (Table III)

Correlation with spirogram. FEV_{1.0}/FVC% and V₅₀/%pred were significantly negatively correlated with E/I LF and E/I MF (FEV_{1.0}/FVC%: *r* = −0.50 and −0.46, respectively, *P* < .01, both; V₅₀/%pred: *r* = −0.50 and −0.49, respectively, *P* < .01, both) and positively correlated with I LF and I MF (FEV_{1.0}/FVC%: *r* = 0.40 and 0.42, respectively, *P* < .05, both; V₅₀/%pred: *r* = 0.48 and 0.51, respectively, *P* < .01, both). FEV_{1.0}/%pred was significantly negatively correlated with E/I LF (*r* = −0.38, *P* < .05) and positively correlated with I LF (*r* = 0.38, *P* < .05).

Correlation with logPC₂₀. E/I MF and E MF/LF were significantly correlated with logPC₂₀ (*r* = −0.49, *P* < .01 and *r* = −0.40, *P* < .05).

Correlation with airway inflammation. E/I MF and E MF/LF were significantly correlated with FeNO and bronchial NO (FeNO: *r* = 0.46, *P* < .01 and *r* = 0.36, *P* < .05, respectively; bronchial NO: *r* = 0.43, *P* < .05 and *r* = 0.36, *P* < .05, respectively). E/I LF, E/I MF, and E MF/LF were significantly positively correlated with alveolar NO and peripheral sputum eosinophils (%) (alveolar NO: *r* = 0.36, *P* < .05; *r* = 0.47, *P* < .01; *r* = 0.36, *P* < .05, respectively; peripheral sputum eosinophils (%): *r* = 0.41, *P* < .05; *r* = 0.50, *P* < .01; *r* = 0.36, *P* < .05, respectively). E MF/LF was significantly correlated with central sputum eosinophils (%) (*r* = 0.40, *P* < .05).

Multivariate analysis in patients with bronchial asthma

E/I LF and E/I MF were significantly correlated with FEV_{1.0}/%pred and V₅₀/%pred (*P* < .01 and *P* < .01, respectively). E/I MF was significantly correlated with logPC₂₀ (*P* < .01). E/I MF and E MF/LF were significantly correlated with bronchial flux NO (*P* < .05, both). E/I LF, E/I MF, and E MF/LF were significantly correlated with alveolar NO (*P* < .05, *P* < .01, and *P* < .05, respectively). E MF/LF was significantly correlated with central sputum eosinophils (%) (*P* < .05). E/I MF was significantly correlated with peripheral sputum

TABLE I. Patient characteristics

	Bronchial asthma (n = 31), mean (95% CI)	Healthy volunteer (n = 22), mean (95% CI)	P value
Age (y)	46.7 (40.6-52.9)	40.3 (33.1-47.4)	.16
BMI	23.2 (22.1-24.2)	20.7 (18.1-23.2)	.071
Male/female	14/17	3/19	.015
Duration (y)	7.1 (3.23-10.96)	—	—
Atopic/nonatopic (type)	17/14	—	—
Nonsmoker/ex-smoker	19/12	18/4	.11
Mild persistent/moderate persistent	7/24	—	—
FEV _{1,0} /FVC (%)	77.0 (73.7-80.2)	83.3 (79.8-86.8)	.008
FEV _{1,0} %pred (%)	97.7 (91.6-103.8)	99.7 (93.4-106.0)	.64
V ₅₀ %pred (%)	69.0 (59.9-78.0)	84.0 (75.5-92.5)	.016
V ₂₅ %pred (%)	57.0 (47.7-66.4)	68.1 (56.4-79.8)	.13
logPC ₂₀	3.04 (2.84-3.24)	—	—
FeNO (50 mL/s) (ppb)	55.6 (34.0-77.2)	17.0 (12.8-21.2)	.001
Bronchial flux NO (nL/s)	2.48 (1.47-3.50)	0.89 (0.49-1.29)	.005
Alveolar NO (ppb)	7.97 (5.41-10.54)	3.80 (2.08-5.53)	.008
Eosinophils (%) in blood	5.97 (4.48-7.47)	2.83 (2.06-3.59)	.0004
Eosinophils (%) in central sputum	14.39 (5.87-22.91)	0.32 (0.09-0.55)	.002
Eosinophils (%) in peripheral sputum	13.61 (4.65-22.57)	0.26 (0.01-0.52)	.005

BMI, Body mass index; CI, confidence interval; FeNO, fractional exhaled nitric oxide; FEV_{1,0}/FVC, forced expiratory volume in one second/forced vital capacity; FEV_{1,0}%pred, forced expiratory volume in one second, %predicted; logPC₂₀, logarithm of the acetylcholine concentration at which FEV_{1,0} was decreased from baseline by 20%; NO, nitric oxide; V₂₅%pred, maximal expiratory flow at 25%; V₅₀%pred, maximal expiratory flow at 50%.

Bold indicates $P < 0.05$.

eosinophils (%) and blood eosinophils (%) ($P < .05$ and $P < .05$, respectively) (Table IV).

DISCUSSION

Our previously proposed LSA analysis using E/I LF as an index of inflammation is simple, noninvasive, and replicable.⁷ The original procedure, which used LSA 2008 (Kenz Medico) to analyze expiratory and inspiratory sound data acquired in a low-frequency range, between 100 and 195 Hz, from LSA recorded data had the issue of noise interference. The system was incapable of analyzing data in cases where friction noise was introduced and overlapped with breath sounds at auscultation. In this study, we used the new analysis software Easy LSA, which can eliminate a portion of data containing friction noise from the analysis, making it possible to analyze samples that were otherwise unanalyzable. This recording system is also easy to handle and noninvasive.

Then, we compared 4 different LSA parameters, that is, E/I LF, E/I MF, I MF/LF, and E MF/LF, using Easy LSA and demonstrated that all of these parameters were elevated in patients with bronchial asthma compared with healthy subjects. In addition, a single power value, E MF, was found to be significantly higher in patients with bronchial asthma than in healthy subjects. These findings show that the LSA parameters can identify airway inflammation, but they do not distinguish the type of the inflammation as neutrophilic versus eosinophilic.

TABLE II. Comparison of individual LSA parameters in patients with bronchial asthma and healthy volunteers

	Patients with bronchial asthma (n = 31), mean (95% CI)	Healthy volunteer (n = 22), mean (95% CI)	P value
I LF (dB)	65.2 (63.5-66.9)	66.0 (64.2-67.8)	.49
I MF (dB)	58.2 (56.7-59.8)	57.3 (55.6-59.0)	.42
E LF (dB)	58.6 (56.9-60.2)	56.6 (55.1-58.1)	.080
E MF (dB)	48.2 (46.5-49.9)	45.3 (43.6-46.9)	.014
E/I LF	0.51 (0.43-0.60)	0.36 (0.30-0.42)	.004
E/I MF	0.36 (0.29-0.43)	0.27 (0.22-0.31)	.029
I MF/LF	0.46 (0.42-0.49)	0.37 (0.34-0.40)	.0003
E MF/LF	0.31 (0.28-0.34)	0.27 (0.25-0.30)	.037

CI, Confidence interval; E/I LF, the expiration-to-inspiration sound power ratio in a low-frequency range; E/I MF, the expiration-to-inspiration sound power ratio in a mid-frequency range; E LF, the expiration sound power in a low-frequency range; E MF, the expiration sound power in a mid-frequency range; E MF/LF, the expiration sound power in a mid-frequency range to the expiration sound power in a low-frequency range; I LF, the inspiration sound power in a low-frequency range; I MF, the inspiration sound power in a mid-frequency range; I MF/LF, the inspiration sound power in a mid-frequency range to the inspiration sound power in a low-frequency range ratio; LSA, lung sound analysis.

Bold indicates $P < 0.05$.

Further studies would be necessary to determine whether LSA can distinguish subtypes of airway inflammation.

Correlation analyses between respiratory function and individual LSA parameters were performed. The univariate analysis only identified E/I LF as being significantly correlated with FEV_{1,0}%pred; however, the multivariate analysis, with correction for age, gender, and smoking exposure, revealed that both E/I LF and E/I MF were significantly correlated with FEV_{1,0}%pred. E/I LF and E/I MF were also significantly correlated with V₅₀%pred. Because I LF and I MF were positively correlated with V₅₀%pred and FEV_{1,0}%pred, a lesser degree of airway narrowing seems to be associated with an increased expiratory flow volume and a greater sound volume. A relationship between air flow volume and sound power has been reported in several studies conducted in healthy subjects.^{13,14} Auscultation sound amplitude is proportional to flow rate squared, and tracheal sound power is proportional to flow rate to the power of 4. Changes in auscultation position and breathing flow volume alter sound volume.¹⁵ Lung sounds are produced by laminar and turbulent flow in the airway, but they differ with the position of auscultation because sound transmits differently depending on the distance from the origin of the sound to where it is heard, the direction of air flow, and features of the lung. In the normal state, bronchial sounds are stronger during expiration than inspiration, whereas alveolar sounds are stronger during inspiration than expiration.^{16,17} In this study, E/I MF and E MF/LF were found to be significantly correlated with airway hyperresponsiveness. The correlation analyses of airway inflammation and individual LSA parameters showed that E/I MF and E MF/LF were significantly correlated with FeNO and bronchial NO. In addition, E MF/LF was significantly correlated with central sputum eosinophils (%). In contrast, E/I MF, E/I LF, and E MF/LF were significantly correlated with alveolar NO and peripheral sputum eosinophils (%), which are indicators of peripheral airway inflammation. In summary, these findings may indicate that expiration and/or inspiration in the frequency range of 100-400 Hz reflects the

TABLE III. Correlation of individual LSA parameters with FeNO, respiratory function, and airway hyperresponsiveness in patients with bronchial asthma

n = 31		E/I LF	E/I MF	I MF/LF	E MF/LF	I LF (dB)	I MF (dB)	E LF (dB)	E MF (dB)
FEV _{1.0} /FVC%	r 95% CI	−0.50** (−0.73, −0.18)	−0.46** (−0.70, −0.13)	−0.04 (−0.39, 0.32)	−0.19 (−0.51, 0.18)	0.40* (0.06, 0.66)	0.42* (0.08, 0.68)	−0.01 (−0.36, 0.35)	−0.06 (−0.41, 0.30)
FEV _{1.0} %pred.	r 95% CI	−0.38* (−0.64, −0.02)	−0.32 (−0.60, −0.13)	−0.14 (−0.47, 0.23)	−0.18 (−0.50, 0.18)	0.38* (0.03, 0.64)	0.34 (−0.01, 0.62)	0.10 (−0.26, 0.44)	0.004 (−0.35, 0.36)
V ₅₀ %pred.	r 95% CI	−0.50** (−0.72, −0.17)	−0.49** (−0.72, −0.16)	−0.01 (−0.36, 0.34)	−0.28 (−0.58, 0.08)	0.48** (0.15, 0.71)	0.51** (0.19, 0.73)	0.10 (−0.26, 0.44)	−0.02 (−0.37, 0.34)
V ₂₅ %pred.	r 95% CI	−0.33 (−0.61, 0.03)	−0.35 (−0.63, 0.00)	0.06 (−0.30, 0.40)	−0.20 (−0.52, 0.16)	0.37* (0.01, 0.64)	0.42* (0.07, 0.67)	0.12 (−0.25, 0.45)	0.03 (−0.32, 0.38)
logPC ₂₀ [#]	r 95% CI	−0.35 (−0.64, 0.01)	−0.49** (−0.73, −0.15)	−0.03 (−0.39, 0.34)	−0.40* (−0.67, −0.04)	0.20 (−0.18, 0.52)	0.20 (−0.18, 0.53)	−0.07 (−0.42, 0.31)	−0.27 (−0.58, 0.11)
FeNO (50 mL/s)	r 95% CI	0.26 (−0.10, 0.56)	0.46** (0.13, 0.70)	−0.13 (−0.46, 0.24)	0.36* (0.01, 0.64)	−0.04 (−0.39, 0.32)	−0.10 (−0.44, 0.26)	0.19 (−0.18, 0.51)	0.33 (−0.02, 0.61)
Bronchial NO	r 95% CI	0.23 (−0.13, 0.54)	0.43* (0.09, 0.68)	−0.12 (−0.46, 0.24)	0.36* (0.01, 0.63)	−0.04 (−0.39, 0.32)	−0.11 (−0.44, 0.26)	0.15 (−0.21, 0.48)	0.30 (−0.06, 0.59)
Alveolar NO	r 95% CI	0.36* (0.01, 0.63)	0.47** (0.14, 0.71)	0.002 (−0.35, 0.36)	0.36* (0.00, 0.63)	−0.07 (−0.42, 0.29)	−0.08 (−0.42, 0.28)	0.23 (−0.13, 0.54)	0.36* (0.01, 0.64)
Eosinophils (%) in blood	r 95% CI	0.33 (−0.03, 0.61)	0.41* (0.07, 0.67)	−0.10 (−0.44, 0.26)	0.26 (−0.10, 0.57)	−0.20 (−0.51, 0.17)	−0.25 (−0.55, 0.12)	0.04 (−0.32, 0.38)	0.16 (−0.20, 0.49)
Eosinophils (%) in central sputum	r 95% CI	0.08 (−0.28, 0.42)	0.25 (−0.11, 0.56)	−0.03 (−0.38, 0.32)	0.40* (0.05, 0.66)	0.03 (−0.33, 0.38)	0.01 (−0.34, 0.37)	0.07 (−0.29, 0.42)	0.25 (−0.11, 0.56)
Eosinophils (%) in peripheral sputum	r 95% CI	0.41* (0.06, 0.66)	0.50** (0.17, 0.72)	−0.03 (−0.38, 0.33)	0.36* (0.01, 0.64)	−0.13 (−0.46, 0.24)	−0.15 (−0.48, 0.21)	0.16 (−0.20, 0.49)	0.32 (−0.04, 0.61)

[#]n = 29; **P < .01, *P < .05.

CI, Confidence interval; E/I LF, the expiration-to-inspiration sound power ratio in a low-frequency range; E/I MF, the expiration-to-inspiration sound power ratio in a mid-frequency range; E LF, the expiration sound power in a low-frequency range; E MF, the expiration sound power in a mid-frequency range; E MF/LF, the expiration sound power in a mid-frequency range to the expiration sound power in a low-frequency range; FeNO, fractional exhaled nitric oxide; FEV_{1.0}/FVC%, forced expiratory volume in one second/forced vital capacity; FEV_{1.0}%pred, forced expiratory volume in one second, %predicted; I LF, the inspiration sound power in a low-frequency range; I MF, the inspiration sound power in a mid-frequency range; I MF/LF, the inspiration sound power in a mid-frequency range to the inspiration sound power in a low-frequency range ratio; logPC₂₀, logarithm of the acetylcholine concentration at which FEV_{1.0} was decreased from baseline by 20%; LSA, lung sound analysis; NO, nitric oxide; V₂₅%pred, maximal expiratory flow at 25%; V₅₀%pred, maximal expiratory flow at 50%.

TABLE IV. Multivariate analysis in patients with bronchial asthma

	E/I LF		E/I MF		E MF/LF	
	<i>t</i> value	<i>P</i> value	<i>t</i> value	<i>P</i> value	<i>t</i> value	<i>P</i> value
FEV _{1,0} %pred	−3.25	.003	−2.94	.007		
Sex (female)	−2.72	.011	−2.81	.009		
Age (y)	3.36	.002	3.14	.004		
Pack-years	−2.23	.035	−2.6	.015		
	R ² = 0.45		R ² = 0.42			
V ₅₀ %pred	−3.09	.005	−3.42	.002		
Sex (female)	−2.23	.034	−2.64	.014		
Pack-years	−1.38	.180	−1.84	.077		
	R ² = 0.37		R ² = 0.41			
logPC ₂₀			−3.23	.003		
Sex (female)			−1.44	.16		
			R ² = 0.30			
Bronchial flux NO			2.59	.015	2.08	.046
			R ² = 0.19		R ² = 0.13	
Alveolar NO	2.09	.046	2.88	.007	2.06	.048
	R ² = 0.22		R ² = 0.13		R ² = 0.13	
Eosinophils (%) in central sputum					2.33	.027
					R ² = 0.16	
Eosinophils (%) in peripheral sputum			2.77	.010		
Pack-years			2.03	.052		
			R ² = 0.34			
Eosinophils in blood			2.45	.021		
			R ² = 0.17			

E/I LF, The expiration-to-inspiration sound power ratio in a low-frequency range; E/I MF, the expiration-to-inspiration sound power ratio in a mid-frequency range; E MF/LF, the expiration sound power ratio in a low-frequency range to that in a mid-frequency range; FEV_{1,0}%pred, forced expiratory volume in one second; logPC₂₀, logarithm of the acetylcholine concentration at which FEV_{1,0} was decreased from baseline by 20%; NO, nitric oxide; V₅₀%pred, maximal expiratory flow at 50%.

degree of airway narrowing and is influenced by changes in inspiratory flow volume and enhancement of expiratory sound because of airway narrowing. Because sound power ratios in the middle-frequency range, that is, E/I MF and E MF/LF, are significantly correlated with airway hyperresponsiveness and central airway inflammation, the expiratory sound power in the LSA frequency range of 200–400 Hz seems to have the most importance with regard to airway inflammation and airway hyperresponsiveness. Alveolar breath sounds are generally heard with a peak volume in a frequency range of 100–150 Hz, and the volume rapidly decreases outside this range. Expiratory sounds are difficult to detect at the lung bases where alveolar breath sounds are heard in their normal state, but it has been reported that airway inflammation makes sound transmission easier and bronchial breath sounds more audible.¹⁷ This result can also be explained by our finding that expiratory sound power in the frequency range of 200–400 Hz increased as the airway inflammation worsened. However, it is known that sound power ratios correlate better with airway hyperresponsiveness and inflammatory parameters, such as PC₂₀, FeNO, and sputum eosinophils, than single power parameters; thus, it is important to calculate ratios to correct for variations in the manner in which each patient takes a deep breath.^{7,15}

In conclusion, among the LSA parameters evaluated in this study, E/I LF and E/I MF seem to be indicators of airway narrowing. E/I MF showed the strongest correlation with airway hyperresponsiveness, and also had the strongest correlation with inflammatory parameters. E/I MF is considered to be a good

index for assessing inflammation in bronchial asthma. LSA may be clinically useful as an additional measure for the assessment of airway inflammation in bronchial asthma.

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