Lung sound analysis is useful for monitoring the therapeutic course in bronchial asthma patients

Running title: Lung sound analysis in asthma treatment

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ABSTRACT

Background: Lung sound analysis (LSA) has been reported to be useful for predicting airway obstruction and inflammation in patients with bronchial asthma.

Objectives: We examined whether the exhalation-to-inhalation sound pressure ratio in the middle frequency range of 200-400 Hz (E/I MF) is useful for monitoring the therapeutic course in patients with asthma.

Methods: This study included 84 patients with mild to moderate asthma whose LSA data were available before and after a 1 year daily 800 μg budesonide treatment. We analyzed whether the E/I MF before and after treatment was associated with the fractional exhaled nitric oxide (FeNO) level, sputum eosinophil percentage, respiratory function, and airway hyperresponsiveness.

Results: Prior to the budesonide treatment, the E/I MF was significantly correlated with respiratory function, airway hyperresponsiveness, FeNO, and sputum eosinophil percentage. The cutoff values for the E/I MF to detect the abnormalities of respiratory function, FeNO, and sputum eosinophil percentage were 0.367, 0.358, and 0.363 respectively. The E/I MF was significantly improved in groups with budesonide-induced improvements in respiratory function or FeNO with respect to the appropriate reference value compared with groups with no improvement (odds ratios of 6.39 and 4.78, respectively). According to the multivariate analysis, patients without improvements in E/I MF had a longer smoking history (p=0.038), a lower post-treatment respiratory function (p=0.028), and a higher post-treatment FeNO (p=0.0095).

Conclusion: Similarly to respiratory function and FeNO, E/I MF based on LSA is a useful indicator for monitoring therapeutic efficacy in asthmatic patients.

Key words: bronchial asthma, fractional exhaled nitric oxide, induced sputum, inhaled corticosteroid, lung sound analysis

RESUMEN

Introducción: El análisis de los sonidos pulmonares ha demostrado ser una prueba de utilidad para objetivar la presencia de obstrucción e inflamación en las vías respiratorias de pacientes con asma bronquial.

Objetivos: Hemos evaluado si el cociente sonido inspiración-espiración por presión en el rango de frecuencias medias, de 200 a400 Hz, (E/I MF) tenía utilidad en la evaluación de la respuesta al tratamiento en pacientes con asma bronquial.

Métodos: el estudio incluyó 84 pacientes con asma leve o moderada que tuvieran registros de LSA antes y tras un año de tratamiento con 800 μg de budesonida inhalada. Analizamos si los cambios en E/I MF tras el tratamiento se correlacionaban con los cambios en los niveles de óxido nítrico en aire exhalado (FeNO), el porcentaje de eosinófilos en muestras de esputo inducido, la función pulmonar y la hiperreactividad bronquial.

Resultados: Antes de iniciar el tratamiento con budesonida inhalada, el cociente E/I MF se correlacionaba significativamente con la función pulmonar, la hiperreactividad bronquial, los niveles de FeNO y el porcentaje de eosinófilos en las muestras de esputo. Los puntos de corte del cociente E/I MF para detectar valores anómalos en la función pulmonar, los niveles de FeNO, y el porcentaje de eosinófilos en esputo eran 0.367, 0.358, and 0.363 respectivamente. El cociente E/I MF mejoraba significativamente en el grupo de pacientes en los que la budesonida inhalada inducía cambios significativos en la función pulmonar o en los niveles, con respecto a los valores de referencia apropiados comparados con los de los grupos de pacientes que no presentaban mejoría en estos parámetros (odds ratios de 6.39 and 4.78, respectivamente). En un análisis multivariante los pacientes que no presentaban mejoras significativas en el cociente, E/I MF presentaban una historia de tabaquismo activo significativamente más larga (p=0.038), unos niveles de función pulmonar tras tratamiento significativamente más bajos (p=0.028), y paralelamente unos niveles de FeNO, tras tratamiento, más elevados (p=0.0095).

Conclusiones: Al igual que la función pulmonar y los niveles de FeNO, el cociente E/I MF obtenido mediante el LSA es un indicador útil para evaluar la eficacia del tratamiento en pacientes con asma bronquial.

Palabras clave: asma bronquial, fracción de óxido nítrico en aire exhalado, esputo inducido, corticoide inhalado, análisis de sonidos pulmonares

INTRODUCTION

Abnormal breath sounds are audible when a change in lung features alters sound transmission; sounds not normally heard may become audible, or sounds normally heard may become inaudible [1]. Lung sounds are generally considered to arise from thickened airways from the trachea up to the 9th division of bronchioles and are transmitted through the lung parenchyma, including the alveoli, and the chest wall before they are heard upon auscultation [2,3]. Detection of abnormal breath sounds on auscultation requires experienced skills and may occasionally be difficult. In contrast, lung sound analysis (LSA) has advantages including greater sensitivity than auscultation and the ability to conduct objective recording. We anticipate the application of LSA to studies of the pathology in patients with bronchial asthma, regardless of the sound type. LSA is performed by adjusting airflow. When lung sounds are monitored in the lung area as the inspiratory airflow is maintained at a constant level, patients with bronchial asthma exhibit a higher median frequency of inspiratory sound (F50) than healthy individuals, and the F50 increases as the airway obstruction becomes more severe [4]. Even in patients with bronchial asthma without audible adventitious sounds, F50 increases and the forced expiratory volume in one second (FEV1) decreases in response to histamine challenge [5,6]. However, it is difficult for different bronchial asthma patients breathe at the same constant airflow because the asthma severity varies from patient to patient.

We previously performed a study of nonsmoking steroid-naive patients in the interictal period of bronchial asthma and reported that the exhalation-to-inhalation sound pressure ratio in the low frequency (LF) range between 100 and 200 Hz (E/I LF), a parameter detected using LSA, was strongly correlated with airway inflammation [7]. Furthermore, we used new software capable of removing noise (EasyLSA) to perform analysis on pre-treatment bronchial asthma patients and confirmed that the exhalation-to-inhalation sound pressure ratio in the middle frequency (MF) range between 200 and 400 Hz (E/I MF) was more strongly correlated with airway inflammation, obstruction, and hyperresponsiveness than E/I LF [8]. No previous studies have indicated that the exhalation-to-inhalation sound pressure ratio in a specified frequency range, such as E/I MF, is useful as an indicator in the treatment of bronchial asthma.

In the present study, we examined how E/I MF was correlated with fractional exhaled nitric oxide (FeNO), sputum eosinophil percentage, respiratory function, and airway hyperresponsiveness to assess whether E/I MF is useful for monitoring the therapeutic course in bronchial asthma patients.

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METHODS

Subjects and study design

This study included 84 patients for whom LSA data were available before and after 1 year of inhaled corticosteroid (ICS) treatment (budesonide inhalation 800 μg/day) among all patients with mild to moderate bronchial asthma who were treated as new patients at our hospital between 2003 and 2013 and who were willing to participate in our study. An LSA, a blood examination, pulmonary function tests, a FeNO measurement, an acetylcholine (Ach) bronchial provocation test, and an induced sputum analysis were performed. All the patients included in this study fulfilled the criteria of GINA [9]. All included patients had a history of asthmatic symptoms, including recurrent cough, wheezing or dyspnea, and exhibited positive airway hyperresponsiveness (PC_{20} for acetylcholine (Ach) <8 mg/mL). Patients with conditions complicated with COPD (chronic pulmonary obstructive disease) and patients with cardiopulmonary disease that affected pulmonary function were excluded. All patients retained normal diffusion capacity. No patients had previously used an inhaled or oral corticosteroid. The use of anti-asthma drugs, including bronchodilators, was discontinued for at least 24 h prior to this examination. Wheezing was not detected on auscultation in any patient.

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

Lung sound analysis (LSA)

Lung sound recording was performed in a quiet room, although not in a soundproof booth, in the outpatient department. The patients breathed deeply during the breath sound recording. Lung sounds were recorded using a hand-held microphone for ≥30 s over the left lung base. The recording system consisted of an electro-stethoscope containing a wide-range audio sensor attached to a diaphragm (Bio-Sound Sensor BSS-01; Kenz Medico, Saitama, Japan), a signal processing system, and a personal computer. The sensor used a band-pass filter range of 40–2500 Hz and displayed good sound-collecting ability in the 40–2000 Hz range. The recorded sound was analyzed using a sound spectrometer (Easy-LSA; Nakano, Fukuoka, Japan). The recorded sounds were re-sampled to 5,012 Hz, analyzed via fast Fourier analysis and displayed as a spectrograph, with frequency in Hz on the vertical axis and time in seconds on the horizontal axis. The recording system was calibrated with a reference sound pressure (1 kHz; 94 dB [0 dB=20 μPa]). One breath with relatively little noise was chosen by visual observation, and average sound intensity during inhalation and expiration was analyzed in the

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200-400 Hz frequency range. We defined this frequency range to be the MF range and determined the inspiration sound pressure level in the MF range (I MF), expiration sound pressure level in the MF range (E MF), and E/I MF. The E/I MF data were converted from logarithmic values to real values.

Other examinations

Flow–volume curves were generated, FeNO and airway hyperresponsiveness to acetylcholine (Ach) were measured, and sputum induction and processing were performed in accordance with previously reported procedures [7,8,10,11].

Statistical analysis

The correlations between E/I MF and various test parameters related to bronchial asthma before and after ICS treatment were analyzed using Spearman rank correlation coefficients. The cutoff values for the E/I MF were compared between the airway obstruction-positive and -negative groups, which were stratified using an FEV_1/FVC% threshold of 70%, an FeNO threshold of 21 ppb or a sputum eosinophil percentage of 3%, based on the receiver operating characteristic (ROC) curve analysis [12]. The proportion of patients with a pre-treatment E/I MF below the cutoff value and the proportion of patients with a pre-treatment E/I MF at or above the cutoff were determined. Subsequently, patients with and without normalization of E/I MF to a value below the cutoff after treatment were further divided into the normalization and non-normalization groups. Post-treatment data separated by cutoff values, including the spirogram results, PC_{20}, FeNO, and eosinophil percentage, were compared between the normalized and non-normalized groups using χ^2 tests. Multiple regression analyses (using the least squares method) were performed to identify factors related to the differences between the normalized and non-normalized groups.

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RESULTS

Patient characteristics

The age of the patients ranged from 16 to 72 years, with a mean (95% CI) of 43.7 (40.6, 46.8) years; there were 47 females and 37 males. The patient cohort included 51 nonsmokers, 16 ex-smokers, and 17 current smokers with a mean smoking rate of 6.06 (1.31, 3.44) packs/year. The means (95% CI) of the tested parameters were as follows: 91.7% (88.4%, 94.9%) for $FEV_{1\%predicted}$; 2.74 (2.62, 2.85) for $logPC_{20}$; 90.6 (72.1, 109.1) ppb for FeNO; and 16.8% (12.1%, 21.5%) for the sputum eosinophil percentage **(Table 1)**.

Correlations of E/I MF with spirogram results, PC_{20}、FeNO, and sputum eosinophil percentage before and after ICS treatment

Before treatment, E/I MF exhibited significant negative correlations with FEV_1/FVC%, $FEV_{1\%predicted}$, $V50_{\%predicted}$, and $logPC_{20}$ (r_s=-0.34, -0.38, -0.36, and -0.29, respectively) and significant positive correlations with FeNO and the eosinophil percentage (r_s=0.31 and 0.38, respectively). In contrast, after treatment, E/I MF exhibited negative correlations with FEV_1/FVC% and $V50_{\%predicted}$ (r_s=-0.45 and -0.49, respectively) and positive correlations with FeNO and the eosinophil percentage (r_s=0.41 and 0.33, respectively), but not with $FEV_{1\%predicted}$ or $logPC_{20}$ **(Table 2)**. The decrease in E/I MF correlated with the decrease in FeNO (r_s=0.28).

E/I MF cutoff value for airway inflammation and obstruction

The E/I MF cutoff values were 0.367 using a FEV_1/FVC% threshold of 70%, 0.358 using a FeNO threshold of 21 ppb, or 0.363 using a sputum eosinophil percentage threshold of 3%; all three E/I MF cutoff values were similar **(Figure 1)**.

Comparison of E/I MF normalization ratios after ICS treatment

The E/I MF was normalized to less than 0.37 after the ICS treatment and was significantly correlated to the normalized FEV_1/FVC% (p=0.005), $V50_{\%predicted}$ (p=0.004), and FeNO (p=0.01). There were no significant correlations between rate of E/I MF normalization and either $FEV_{1\%predicted}$ or $logPC_{20}$ values after ICS treatment (**Table 3**).

Factors related to the differences between the E/I MF values in the normalized and non-normalized groups after ICS treatment based on the multiple regression analysis

According to the multivariate analysis, the non-normalized post-treatment E/I MF

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group had a longer smoking history (p=0.038), a lower post-treatment $V50_{\%predicted}$ (p=0.028), and a higher post-treatment FeNO (p=0.010) **(Table 4)**.

DISCUSSION

In this study, the decreases in E/I MF and FeNO were correlated each other during the ICS treatment. E/I MF may represent the relationship between the two mechanisms influenced by ICS treatment: the enhancement of expiratory and inspiratory sound pressure, which depends on an increase in respiratory flow, and a mechanism related to the alteration of respiratory sound transmission from the sound source due to changes in asthmatic airway conditions as a result of reduced inflammation and dilation of the airway after ICS treatment. Bronchodilator treatment improves respiratory function and reduces the tracheal sound spectral power [13].

With respect to the sounds heard on auscultation, the amplitude is proportional to the square of the flow rate, and the tracheal sound power is proportional to the fourth power of the flow rate [14,15]. We speculate that ICS treatment reduced airway inflammation and increased the airway lumen diameter, which in turn increased the respiratory flow volume and rate upon deep breathing. As a result, patients with normalized E/I MF exhibited an increase in I MF. The same mechanism appears to apply to the positive correlation between the inspiratory sound intensity and $FEV_{1\%predicted}$, consistent with the report by Bohadana et al [16]. The asthma-induced airway narrowing alters sound transmission from the source and enhances the expiration sound pressure and frequency; appropriate treatment reduces the expiratory sound pressure.

The results demonstrated a significant correlation of the pre-treatment E/I MF with airway obstruction, hyperresponsiveness, and inflammation as well as a significant correlation of post-treatment E/I MF with peripheral airway obstruction and airway inflammation; however, no correlation was observed between post-treatment E/I MF and central airway obstruction or airway hyperresponsiveness. Thus, E/I MF may be affected by peripheral airway obstruction and airway inflammation.

Because FEV_1/FVC%, FeNO, and sputum eosinophil percentage were found to significantly correlate with E/I MF both before and after ICS treatment, the cutoff value for E/I MF was examined after stratifying the study data using known cutoff values for these parameters [9,17]. Interestingly, each E/I MF cutoff value used to predict the FEV_1/FVC% threshold of 70%, the FeNO threshold of 21 ppb, or the sputum eosinophil percentage threshold of 3% was within a narrow range (0.358 to 0.367). Based on this result, an E/I MF

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cutoff value of 0.37 was used to divide the patients into the normalized group and the non-normalized group. E/I MF was significantly improved in the groups for which FEV_1/FVC(%), $V50_{\%predicted}$ or FeNO were improved after ICS treatment compared with the groups without an improvement (odds ratios of 6.39, 5.55, and 4.07, respectively). Therefore, E/I MF appears to be able to indicate airway obstruction and airway inflammation before and after treatment. Smoking history, residual peripheral airway constriction and residual airway inflammation were independent risk factors for the non-normalized E/I MF.

These characteristics may be attributed to residual neutrophilic airway inflammation related to smoking. As shown in previous reports, an increase in the sputum neutrophil percentage is correlated with airway construction [18], and neutrophilic asthma is associated with a poor response to inhaled steroid treatment [19]. As shown in our previous study, heavier smoking was associated with higher E/I MF levels [8]. Therefore, for the patients whose E/I MF does not decrease after ICS treatment, physicians should also monitor FeNO and/or sputum cell differentiation, and other parameters to assess whether the airway inflammation is neutrophilic, such as in smoking-related inflammation, or eosinophilic, and should reconsider the therapeutic strategy.

In conclusion, in bronchial asthma patients, the presence of airway obstruction and airway inflammation is associated with increased E/I MF, which is reduced by ICS treatment. Similarly to FeNO and respiratory function test results, E/I MF based on LSA is a useful parameter for monitoring therapeutic efficacy in bronchial asthma patients.

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Conflict of interest

The authors have no conflicts of interest to declare.

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Table 1. Patient characteristics

		Before treatment		After treatment		
		mean	(95% CI)	mean	(95% CI)	
Age (y)		43.7	(40.6, 46.8)			
Body mass index (kg/m^2)		22.7	(22.0, 23.4)			
Sex (F/M)	47/ 37					
Severity (mild persistent/moderate persistent)	74/ 10					
Allergic rhinitis (no/yes)	42/ 42					
Atopic dermatitis (no/yes)	70/ 14					
Atopic/non-atopic	53/ 29					
Nonsmoker/ex-smoker/current smoker	51/ 16/ 17					
Pack-years		6.06	(1.31, 3.44)			
Asthma duration(y)		6.5	(4.5, 8.4)			
FEV_1/FVC% (%)		74.8	(72.6, 77.0)	78	(76.5, 79.6)	*
$FEV_{1\%predicted}$ (%)		91.7	(88.4, 94.9)	98.8	(96.2, 101.4)	***
$V_{50\%predicted}$ (%)		63.1	(57.5, 68.7)	73	(67.8, 78.3)	*
$V_{25\%predicted}$ (%)		49.6	(44.0, 55.2)	56.1	(51.1, 61.2)	
$logPC_{20}$		2.74	(2.62, 2.85)	3.32	(3.20, 3.44)	***
FeNO (ppb)		90.6	(72.1, 109.1)	35.8	(28.6, 43.1)	***
Sputum eosinophils (%)		16.8	(12.1, 21.5)	3.6	(1.9, 5.3)	***
E/I MF		0.44	(0.40, 0.48)	0.34	(0.31, 0.37)	***

p<0.001: ***, p<0.01: **, p<0.05: *

Table 2. Correlations between the E/I MF and the spirogram findings, PC_{20}, FeNO, and sputum eosinophil percentage before and after treatment

	Before treatment		After treatment		ΔE/I MF and Δfactor	
	r_s	P-value	r_s	P-value	r_s	P-value
FEV_1/FVC% (%)	**-0.34**	**0.002**	**-0.45**	**<.0001**	0.03	0.82
$FEV_{1\%predicted}$ (%)	**-0.38**	**0.0003**	0	0.98	0.02	0.83
$V_{50\%predicted}$ (%)	**-0.36**	**0.0008**	**-0.49**	**<.0001**	0.02	0.88
logPC_{20}	**-0.29**	**0.007**	-0.06	0.59	-0.07	0.50
FeNO (ppb)	**0.31**	**0.005**	**0.41**	**0.0002**	**0.28**	**0.011**
Sputum eosinophils (%)	**0.38**	**0.0004**	**0.33**	**0.003**	0.23	0.09

Table 3. Comparisons of the normalized E/I MF rates after the ICS treatment

	Normalized percentage (Yes/No)	P-value	Odds ratios (95%CI)
FEV_1/FVC%			
≥70%	**68.1 (49/23)**		
<70%	**25.0 (3/9)**	**0.005**	**6.39 (1.58-25.85)**
$FEV_{1\%predicted}$			
≥80%	63.6 (49/28)		
<80%	42.9 (3/4)	0.28	2.33 (0.49-11.19)
$V_{50\%predicted}$			
≥80%	**85.2 (23/4)**		
<80%	**50.9 (29/28)**	**0.004**	**5.55 (1.70-18.10)**
PC_{20}			
≥8000 μg/ml	61.2 (41/26)		
<8000 μg/ml	64.7 (11/6)	0.79	0.86 (0.28-2.61)
FeNO			
<21 ppb	**81.5 (22/5)**		
≥21 ppb	**51.9 (27/25)**	**0.01**	**4.07 (1.34-12.40)**
Sputum eosinophils			
<3%	67.8 (40/19)		
≥3%	45.8 (11/13)	0.06	2.49 (0.94-6.57)

Table 4. Factors related to the differences between the E/I MF values of the normalized and non-normalized groups after the ICS treatment (based on the multiple regression analysis)

	Estimate	SE	$\chi 2$	P-value
Age (y) (**N)**	0.002	0.038	0	0.96
Pack-years (**N)**	-0.010	0.048	4.3	0.038
Post-treatment $V50_{\%predicted}$ (**N)**	0.075	0.034	4.85	0.028
Post-treatment FeNO (**N)**	-0.080	0.031	6.73	0.010

(N): normalized

Parameters that showed a significant difference based on univariate analysis were selected and tested using the least squares method.

Figure legends

Figure 1. E/I MF cutoff value for airway inflammation and obstruction (receiver operating characteristic (ROC)). (A) When the data are stratified as FEV_1/FVC% ≥70% or <70%, the E/I MF cutoff value is 0.367, with a sensitivity of 0.82 and a specificity of 0.63. (B) When the data are stratified using a FeNO ≥21 ppb or <21 ppb, the E/I MF cutoff value is 0.358, with a sensitivity of 0.60 and a specificity of 0.71. (C) When the data are stratified as sputum eosinophil percentage ≥3% or <3%, the E/I MF cutoff value is 0.363, with a sensitivity of 0.71 and a specificity of 0.70.

Figure 1