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Original article

Airway inflammation phenotype prediction in asthma patients using lung sound analysis with fractional exhaled nitric oxide

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Abbreviations:
LSA, Lung sound analysis; FeNO, fractional exhaled nitric oxide; $FEV_{1.0}$, forced expiratory volume in one second; FVC, forced vital capacity; V_{50} and V_{25}, maximal expiratory flow at 50% and 25%; 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; PC_{20}, provocative concentration of acetylcholine causing a 20% decrease in FEV1.0

ABSTRACT

Background: We previously reported the results of lung sound analysis in patients with bronchial asthma and demonstrated that the exhalation-to-inhalation sound pressure ratio in the low frequency range between 100 and 200 Hz (E/I LF) was correlated with the presence of airway inflammation and airway obstruction. We classified asthma patients by airway inflammation phenotype using the induced sputum eosinophil and neutrophil ratio and determined whether this phenotype could be predicted using E/I LF and fractional exhaled nitric oxide values.

Methods: Steroid-naive bronchial asthma patients were classified into four phenotypes, including "Low inflammation" (35 patients), "Eosinophilic type" (58 patients), "Neutrophilic type" (15 patients), and "Mixed type" (15 patients) based on the results of induced sputum examinations. The E/I LF data and FeNO levels were then evaluated for the four phenotype groups; the prediction powers of these two indices were then analyzed for each phenotype.

Results: The median E/I LF value was highest in the "Mixed type" and lowest in the "Low inflammation" group. FeNO differentiated between the "Low inflammation" and "Eosinophilic type" groups, "Low inflammation" and "Neutrophilic type" groups, and "Neutrophilic type" and "Mixed type" ($p < 0.0001$, $p = 0.007$, and $p = 0.04$, respectively). E/I LF differentiated between the "Low inflammation" and "Eosinophilic type" groups ($p = 0.006$). E/I LF could distinguish the "Mixed type" group from the "Low inflammation" and "Eosinophilic type" groups ($p = 0.002$).

Conclusions: A combination of the E/I LF value and FeNO may be useful for the classification of the airway inflammation phenotype in patients with bronchial asthma.

Introduction

Bronchial asthma is a chronic inflammatory disease that presents as a mixture of various phenotypes.[1–3] The classification of phenotypes using cluster analysis is expected to help elucidate asthma pathophysiology and impact personalized medicine according to individual responses to drugs.[4,5] Currently available methods to evaluate airway inflammation in bronchial asthma include exhaled nitric oxide (FeNO) measurement, bronchofiberscopic biopsy and bronchoalveolar lavage (BAL), differential cell counts and inflammatory cytokine measurement in induced sputum, and inflammatory cytokine measurement in exhaled breath condensate.[6–10] Differential cell counts of induced sputum can distinguish between eosinophilic and neutrophil inflammation; however, this method requires complex processing and may involve sample collection errors.

In contrast, FeNO measurement and lung sound analysis (LSA) have the advantages of being simple and noninvasive.[7,11] FeNO is

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currently used as an airway eosinophilic inflammation index. Regarding LSA, we previously reported that the exhalation-to-inhalation sound pressure ratio in the low frequency (LF) range between 100 and 200 Hz (E/I LF) was correlated with airway inflammation.[11] E/I LF may indicate the inflammation level by evaluating airway turbulence but does not indicate the inflammation phenotype (neutrophilic or eosinophilic). However, this index may shed light on the physiological characteristics of asthma by assessing the sound pressure ratio of inhalation versus exhalation.

Based on these findings and hypothesis, we classified asthma patients into four phenotype groups (low inflammation, eosinophilic, neutrophilic, and both eosinophilic and neutrophilic) based on the proportion of eosinophils vs. neutrophils of induced sputum. By comparing E/I LF and FeNO values across the four groups, we determined whether E/I LF is useful for distinguishing airway inflammation phenotypes in bronchial asthma.

Methods

Subjects and study design

We examined 190 patients diagnosed with persistent asthma who first visited our hospital from November 2009 to November 2012. An LSA, a blood examination, pulmonary function tests, an FeNO measurement, an acetylcholine (Ach) bronchial provocation test, and an induced sputum analysis were performed. All of the patients included in this study fulfilled the diagnostic criteria of the Global Initiative for Asthma (GINA) Guidelines.[1] All included patients reported a history of asthmatic symptoms, including recurrent cough, wheezing or dyspnea, and exhibited airway hyperresponsiveness, i.e., PC_{20} for Ach <8000 mcg/mL. Airway reversibility was confirmed in 80% of patients but not in the remaining 20% of patients, for whom bronchial asthma was diagnosed based on medical history and positive PC_{20} results. Chest X rays and high resolution computed tomography (CT, as needed) identified no indications of chronic obstructive pulmonary disease (COPD) in any patient. All patients demonstrated normal diffusion capacity. COPD was ruled out by an FEV/FVC over 70% after bronchodilator inhalation and smoking history. No patients had previously used inhaled or oral corticosteroids. The asthmatic subjects treated with bronchodilators were not excluded. Anti-asthma drugs, including bronchodilators, were discontinued for at least 24 h prior to examination. Of the 190 patients examined, 137 patients were nonsmokers or past smokers who were successful in collecting induced sputum. Among these patients, those with a large number of squamous cells in the sputum and those considered ineligible due to suspected infection were excluded from the analysis. The remaining 123 patients were classified into four phenotype groups as follows: eosinophils <3% with neutrophils <60% (Low inflammation); eosinophils ≥3% with neutrophils <60% (Eosinophilic type); eosinophils <3% with neutrophils ≥60% (Neutrophilic type); and eosinophils ≥3% with neutrophils ≥60% (Mixed type).[12,13] As a result, 35 patients (28%), 58 patients (47%), 15 patients (12%), and 15 patients (12%) were classified into the “Low inflammation”, “Eosinophilic type”, “Neutrophilic type”, and “Mixed type” groups, respectively.

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.

LSA

LSA was performed according to the procedure described in previous studies.[11,14] The sound recording was performed in quiet room, but 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 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 a reliable sound collecting ability in the 40–2000 Hz range. The recorded sound was analyzed by fast Fourier analysis using a sound spectrometer (Easy-LSA; Fukuoka, Japan) and was displayed as a spectrograph, with the frequency in Hz on the vertical axis and time on the horizontal axis. The recording system was calibrated with a reference sound pressure (1 kHz; 94 dB [0 dB = 20 μPa]).

We defined a frequency range of 100–200 Hz as LF and determined the inspiration sound power, expiration sound power, and the E/I in the LF range. E/I LF data were converted from logarithmic values (dBSPL) to actual values.

Measuring the fractional exhaled nitric oxide (FeNO) concentration

Following the guidelines published by the American Thoracic Society (ATS), FeNO was measured using the online single-breath method and a fast response (0.02 s) chemiluminescence analyzer (Sievers Nitric Oxide Analyzer NOA 280i, GE Analytical Instruments, Boulder, CO, USA).[11] All measurements were obtained using a mouth pressure of 16 cm H_2O, corresponding to an expiratory flow of 50 mL/s. The FeNO concentrations were recorded as the average of 3 FeNO values.

Sputum induction and processing

The participants inhaled 5 mL 3% NaCl solution through an ultrasonic nebulizer to induce sputum collection. The subjects were asked to cough during and after the inhalation and to expectorate into empty containers. Sputum was induced over a 20-min period. The sputum samples were processed within 30 min according to a method described by Metso *et al.*[10] The sputum cells were separated by centrifugation at 2000*g* for 10 min. The cell suspension was cytocentrifuged (Cytospin 3; Shandon, Astmoor, UK) onto microscope slides at 450 rpm for 6 min. The cytospin products from the sputum were air-dried for 30 min and then stained using the Giemsa staining method (Muto Pure Chemicals, Tokyo, Japan). At least 400 non-squamous cells were counted differentially, including eosinophils, neutrophils, lymphocytes, macrophages, and ciliated epithelial cells. The results are expressed as the percentages of the total non-squamous cell counts.

Other examinations

The measurements of flow–volume curves and bronchial hyperresponsiveness to Ach were performed in accordance with previous papers.[11,14]

Statistical analysis

We examined potential differences in the background or measured values between four groups (Low inflammation”, “Eosinophilic type”, “Neutrophilic type” and “Mixed type”) using a box plot. The Steel–Dwass test was used to test the significance of differences between the pairs of groups.

The ROC curve and chi square test were performed for each index to clarify the useful threshold to distinguish any two groups. Next, the chi square values were compared between using FeNO only and using FeNO and E/I LF together to determine E/I LF value addition.