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Characteristics of Breath Sounds During Methacholine-induced Bronchoconstriction in Children with Asthma

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Objective: The utility of an analysis of breath sounds as a non-invasive lung function test in children and adults has been studied. Analyzing specific breath sounds during methacholine inhalation challenge is useful for evaluating airway constriction in asthmatic patients.

Patients and methods: The study population included 57 children with atopic asthma (male: female = 38: 19; median age, 10 years [range, 5–16 years]). The breath sound spectrum was measured before a methacholine inhalation test, just after the methacholine inhalation challenge and after β_2 agonist inhalation. The values of breath sound parameters were analyzed and the direct changes of the sound spectrum during methacholine inhalation challenge were evaluated.

Results: The values of breath sound parameters, RPF_{75} and RPF_{50}, were significantly decreased after methacholine inhalation ($P < 0.001$, $p < 0.001$, respectively), indicationg bronchoconstriction, and increased after β_2 agonist inhalation ($P < 0.001$, $p < 0.001$, respectively), indicating bronchodilation. The high-pitch area of the sound spectrum curve around 1,500 Hz was significantly increased after methacholine inhalation ($P < 0.001$). The values returned to the baseline level after β_2 agonist inhalation.

Conclusions: Bronchoconstriction by methacholine inhalation induced a reversible high-pitch sound. The assessment of changes in the high-pitch area of the breath sound spectrum may be useful for the detection of airway narrowing in asthmatic patients.

Key words: asthma, breath sound analysis, bronchoconstriction, methacholine

INTRODUCTION

In recent years, the evolution of breath sound analysis techniques has been remarkable [1, 2]. With technical improvements, breath sound analysis techniques have come to be used in the clinical setting and practical studies have been reported [3, 4]. The participants of these studies include adults to newborns and infants [5, 6]. What is commonly reported is that the breath sound spectrum changes with bronchoconstriction and bronchodilation [4, 7].

If characteristic bronchoconstriction-induced sounds can be distinguished, it may be possible to confirm the bronchial condition based on the presence of such sounds at the time of an outpatient visit. This would be of great significance for the early diagnosis [8, 9] or long-term management of asthma in patients who cannot undergo standard lung function tests or perform forced oscillation techniques [7]. Thus, actual measurement values are required to identify the characteristic changes in breath sound spectrum associated with bronchoconstriction is, as well the power and frequency of such sounds.

Bronchial hyperresponsiveness (BHR) is considered to be the main physiological condition of asthma [10]. Asthma patients are usually measured BHR by inhalation test of bronchoconstrictors, such as methacholine or histamine. We can objectively quantify the hypersensitivity of bronchi against non-specific stimuli in asthmatics [11]. During the provocation test, a transient bronchial constriction is confirmed by changes in FEV_1 or respiratory resistance, and an individual threshold can be determined in many cases [12]. This means that a bronchoconstriction is commonly observed during the provocation tests of BHR.

Unlike the case of an acute exacerbation due to allergen inhalation or respiratory infections, this constriction is transient and recovery is seen with the inhalation of β_2 agonists [13] Obvious bronchoconstriction during the provocation test is important for evaluating the objective characteristics of changes in the breath sound spectrum associated with bronchoconstriction. In this study, we used a new breath sound analysis technique to investigate the changes in the breath sound spectrum associated with bronchoconstriction in asthmatic children who underwent a methacholine inhalation test.

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PATIENTS AND METHODS

Study subjects

The study participants who agreed to participate in this study included a total of 57 pediatric outpatients (male:female = 38:19; median age, 10 [range, 5–16 years]) who were treated at the Tokai University Hospital from April 1, 2012 to July 31, 2015. The inclusion criteria were as follows: one or more positive specific IgE value (> 0.7 UA/ml), recurrent wheezing and bronchial hyperresponsiveness following a methacholine inhalation challenge [14]. All of the participants had been diagnosed with atopic-type asthma by a physician [15]. The analysis of raw data of breath sounds using our previously reported technique [4, 5] has been reported for 49 of the 57 participants [16].

All drugs were withdrawn for 12 hours before the test. None of the subjects had respiratory symptoms on the day of testing. Written informed consent was obtained from all of the children or their legal guardians and the study protocol was approved by the institutional review board of Tokai University Hospital (No. 11R-158, approval date; December 21, 2011, No. 14R-133 approval date; December 15, 2015, No. 17R-161 approval date; October 10, 2017).

Study design

The assessments were performed before the methacholine challenge, just after the methacholine challenge, and at 15 minutes after the β_2 agonist inhalation [16]. As a general rule, each subject was requested to take tidal breaths when the breath sounds were recorded [4]. It was confirmed that the breath sound samples included no wheezes, crackles and outside noises based on the findings of the physician's auscultation findings and the image of the breath sound analyzer image. After the sound analysis, the participants' pulmonary function was tested using spirometry.

Lung function tests

The lung function of the participants was determined via spirometry using a computerized spirometer (Chestgraph HI-105, Chest Co., Tokyo) [17]. The resting baseline was selected using the best-of-three resting results based on the highest sum of the FVC and FEV_1. The results are shown as the percent predicted value, which was calculated using the prediction equations for Japanese children [18].

Methacholine inhalation challenge

The methacholine inhalation challenge was performed according to the method described by Takishima [14]. Briefly, methacholine was diluted two-fold with saline on the day of the test to provide a series of 10 strengths, ranging from 25 mg/ml to approximately 49 μg/ml. During the methacholine inhalation challenge, the respiratory resistance (Rrs) was continuously measured using an Astograph® (Chest Co., Tokyo, Japan) [17]. The administration of methacholine was stopped when the Rrs reached double the baseline value.

The minimum dose of methacholine to cause bronchial constriction (Dmin), represents the bronchial sensitivity. One Dmin unit was considered to be equal to 1 minute of inhalation of 1.0 mg/ml of aerosolized methacholine solution [14]. The speed of bronchoconstriction in response to methacholine (St), which represents the bronchial sensitivity, was also calculated.

Breath sound analysis

A breath sound analysis was performed for all participants, as described previously [4, 5]. Breath sounds were recorded using a handheld microphone for ≥ 10 seconds. The microphone was placed on the right upper anterior chest at the second intercostal space along the mid clavicular line. A sound analysis of the inspiration phase was performed using an LSA-2000 sound spectrometer (Kenz Medico Co., Saitama, Japan).

The sound-amplifying unit was found to be effective for analyzing sounds in the range of 100–3,000 Hz. The recorded sounds were analyzed according to fast Fourier transformation. The sampling frequency was 10,240 Hz and the spectra were obtained using a Hamming window. The sounds were displayed as a spectrograph. The point of the maximum frequency (Hz) in the shape during inspiration was used for the sound spectrum analysis.

To evaluate the dBm-based spectrum images, we used a new analyzing software (LSA2020/ANA, Kenz Medico Co., Saitama, Japan). By using the software, we decided to set the zero point of the Y-axis (dBm) based on the mean of the background noise of all of the participants using. In this report, the zero level (0 dB of breath sound spectrum) was visually corrected based on the breath sound spectrums in each sample before the zero point (the frequency at 0 dB) was decided [19]. The zero level and the zero point were used to calculate of the area under the curve (AUC) of the sound spectrum.

The sound spectrum parameters were determined by one point of the maximum frequency (Hz) in the shape. The data were automatically calculated using a custom software program [19]. The common parameters, the total area of the power spectrum (A_T) and the frequency at 99% (F_{99}) were measured according to the methods of a previous report [19, 21]. The spectrum curve indices, the A_3/A_T, B_4/A_T, RPF_{75} and RPF_{50} values, were also calculated [4]. RPF_{75} is the ratio of power at F_{75} (dBF_{75}) to the frequency value (F_{99}–F_{75}), and RPF_{50} is the ratio of power at F_{50} (dBF_{50}) to the frequency value (F_{99}–F_{50}). The total sound spectrum was divided into three or four sections from low to high frequencies, which allowed for the quantitation of the energy distribution within the spectrum [4]. A five-point moving average was used as a smoothing technique to determine the suitable dB value for identifying the 0 dB in the slope of each sound spectrum.

In this study, breath sound samples were obtained three times; before methacholine inhalation, just after methacholine inhalation (when the Rrs value increased to twice the baseline Rrs value), and 15 minutes after β_2 agonist inhalation [16]. Each personal breath sound parameter was analyzed conventionally, using a sample with a median value from three tidal breaths.

We also measured which part of the spectrum curve was increased by bronchial constriction by directly measuring the AUC of sound spectrum curves. Conventionally, we used the AUC index according to the dB and Hz (1 arbitrary unit of AUC [1 dB x10 Hz]

Table 1 Patient characteristics

	No.	Age (Years)	Sex (m:f)	FVC (%pred)	FEV_1 (%pred)	Rrs.cont (cmH_2O/L/sec)	Dmin (Unit)	St (cmH_2O/sec/min)
Atopic asthma	57	10* (8, 12)	38:19	82.0 (77.9, 93.3)	87.9 (80.7, 97.0)	7.30 (5.60, 8.95)	1.19 (0.44, 2.62)	1.75 (1.13, 3.45)

*: Median (first quartile, third quartile).

Table 2 The results of the data analysis

	① Before methacholine	② Just after methacholine	③15 min after β_2 agonist inhalation	P value ① vs ②	 ② vs ③	 ① vs ③
F_{99} (Hz)	1416* (1337, 1485)	1643 (1535, 1812)	1426 (1317, 1535)	**< 0.001**	**< 0.001**	0.912
Slope (-dBm/octave)	0.48 (0.35, 0.58)	0.39 (0.28, 0.54)	0.51 (0.35, 0.60)	0.115	0.229	0.967
A_3/A_T (%)	15.2 (13.4, 16.6)	14.7 (13.1, 15.9)	15.2 (14.3, 17.6)	0.040	**< 0.001**	0.069
B_4/A_T (%)	9.5 (8.6, 10.4)	8.9 (8.2, 9.9)	9.9 (8.8, 11.1)	0.027	**0.001**	0.094
RPF_{75} (dBm/Hz)	8.4 (7.1, 9.4)	6.2 (5.1, 7.2)	8.9 (7.1, 10.3)	**< 0.001**	**< 0.001**	0.234
RPF_{50} (dBm/Hz)	6.7 (6.2, 7.2)	5.3 (4.9, 6.1)	7.0 (6.0, 8.0)	**< 0.001**	**< 0.001**	0.085

Bold type indicates a significant difference. *: Median (first quartile, third quartile), p value; Mann-Whitney U test.

in each 10 Hz from 100 Hz to 3,000 Hz on a spectrum image) [4, 5].

Statistical analyses

The statistical analyses were conducted using the SPSS software program (IBM SPSS Statistics, Version 22 for Windows; IBM Corp., Armonk, N.Y., USA). The parameters were compared using the Wilcoxon's signed-rank test. Bonferroni's multiple comparison test was used for multiple comparisons, and *P* values of < 0.017 were considered to indicate statistical significance in Table 2. The Table data are expressed as the median and the first and the third quartile values.

RESULTS

The lung function and the breath sound analysis

All of the 57 subjects underwent spirometry. The data of spirometric parameters, the FVC and FEV_1, were within the normal range for children, and chest auscultation by pediatric respiration specialist found no abnormalities (Table 1). None of the breath sound images showed wheezes, crackles or distinct outside noises. The shape of the sound spectrum showed good similarity in the same patients.

Differences in the breath sound parameters before, just after methacholine inhalation, and after β_2 agonist inhalation

In 57 subjects, the Rrs. cont was 7.30 (5.60, 8.95) cmH_2O/L/sec [median (first quartile, third quartile)], the Dmin was 1.19 (044, 2.62) units, and the St was 1.75 (1.13, 3.45) cmH_2O/L/sec/min. Based on observations of real-time images of respiratory resistance, in all patients, methacholine inhalation induced an increase in respiratory resistance.

After methacholine inhalation, the values of spectrum curve indices, A_3/A_T, B_4/A_T, RPF_{75} and RPF_{50}, were significantly decreased (Table 2). After β_2-agonist inhalation, these values of spectrum curve indices were significantly increased, and recovered to the same level as a baseline.

Differences in the breath sound spectrum curve before, just after methacholine inhalation, and after β_2 agonist inhalation

By directly measuring the sound spectrum curve during inspiration, in every 10Hz, the median of each breath sound power was calculated (n = 57). Fig. 1 shows the sound spectrum curve of median data. The sound spectrum curve measured just after methacholine inhalation (dotted line) showed a different shape to before (black line) and after β_2 agonist inhalation (thin line) (Fig. 1). The high-pitch area showed a hump-like

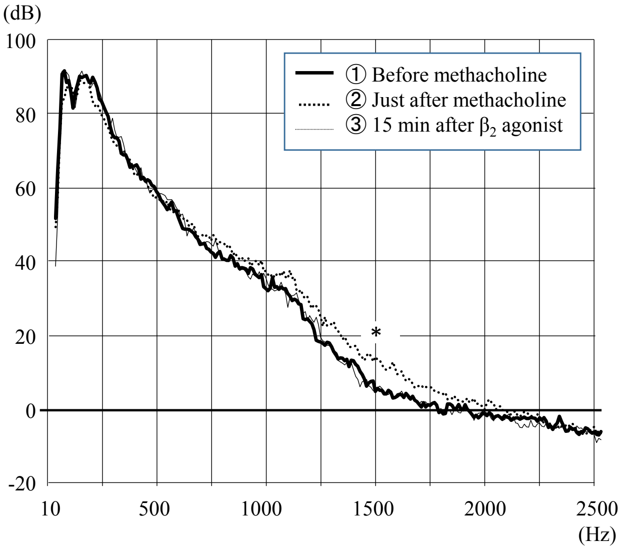

Fig. 1 Analysis of sound spectrum curve
The sound spectrum curve generated just after methacholine inhalation showed the different shape of the sound spectrum curves generated before and after β_2 agonist inhalation. In 1,500 Hz, the difference was statistically significant. *: $p < 0.001$ in comparison to the data of before β_2 agonist inhalation. Since the 95% confidence intervals were small, they have been omitted.

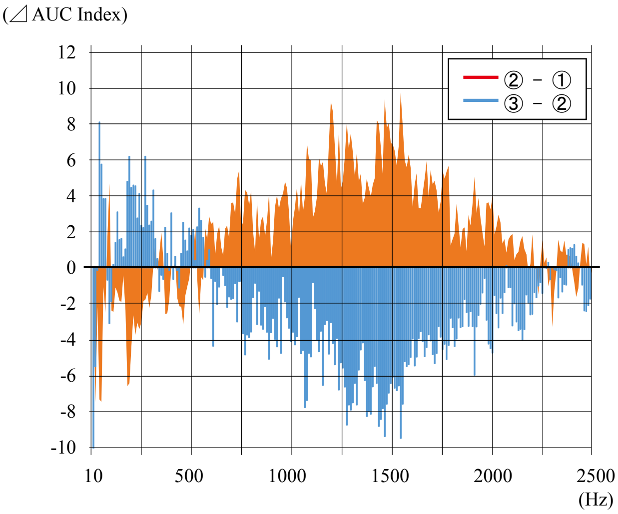

Fig. 2 Change in the AUC index during the methacholine inhalation challenge
The red bars show the subtracted values of AUC index values; the AUC index values after methacholine inhalation minus the AUC index values before methacholine inhalation, for each 10 Hz. The blue bars show the subtracted AUC index values; the AUC index values after β_2 agonist inhalation minus the AUC index values of after methacholine inhalation, for each 10 Hz.

increase. At 1,500 Hz, the difference was statistically significant ($p < 0.001$). The sound spectrum curves measured before methacholine inhalation and after β_2 agonist inhalation showed the same shape, and the hump had disappeared.

Differences in the AUC before, just after methacholine inhalation and after β_2 agonist inhalation

The result of subtracting the AUC index values is shown in Fig. 2 in order to clarify the change in the high-pitched area that is shown in Fig. 1. The red bars show the AUC index values after methacholine inhalation minus the values before methacholine inhalation, for each 10 Hz. The frequency range of the increased AUC index was 1,000 to 2,000, and maximum point was 1,500 Hz (Fig. 2). The blue bars show the AUC index values after β_2 agonist inhalation minus the values after methacholine inhalation, for each 10 Hz. The frequency range of the decreased AUC index and the maximum point of the AUC index were the opposite of the red bars.

DISCUSSION

After a patient is diagnosed with asthma, BHR is observed for long time, when there is no acute exacerbation of asthma [20]. The optimal long-term management of asthma requires objective indicators of good control [17, 21]. In this regard, it is important to regularly check for the absence of bronchoconstriction. Although older children and adults can use spirometer or peak flow meter for objective evaluation, these tests are difficult for younger children and elderly patients to perform.

In daily medical care, chest auscultation is routinely performed for children with respiratory diseases. With the progress of electronic stethoscopes in recent years, breath sound analyses have become more common [22, 23]. The breath sound analysis is useful method because it is simple, safe and no need for patients' cooperation [8, 9]. By recent technological advances, data collection using mobile phones [24] and automatic analysis by artificial intelligence [1, 3, 25] are reported.

Clinically, during an acute exacerbation of asthma, cough, dyspnea and high-pitch wheezing are usually shown, and the inhalation of β_2 agonists tends to improve this symptom. This is due to the effect of the inhaled β_2 agonist improving bronchoconstriction [26]. The evaluation of this dynamic change in breath, the quantitative change in the breath sound has not been evaluated. This evaluation is important for examining bronchial reversibility in small children.

Previously, we devised a method for analyzing breath sounds that is not easily affected by the air flow (L/sec) of respiration [4, 16, 27]. Recently, we were able to improve the accuracy of this method. That is, we added a process to adjust the zero level of the sound spectrum curve generated by individual data [19]. The new method has been found to be superior to the previous method for the analysis of the high-pitch area of lung sounds. With this method, we re-analyzed the changes in breath sounds before and after bronchoconstriction using the raw data of methacholine inhalation challenge [16]. Changes in the lung sound spectrum curve due to induced bronchoconstriction caused by methacholine inhalation seemed to be optimal because the bronchoconstriction was simple and clear [28].

In this study, the increase in respiratory resistance due to methacholine inhalation and the decrease in respiratory resistance due to β_2 agonist inhalation were confirmed in all cases. Thus, bronchoconstriction and bronchodilation occurred during the examination on this study. The values of spectrum curve indices, A_3/A_T, B_4/A_T, RPF_{75} and RPF_{50}, decreased with methacholine inhalation and following β_2 agonist inhalation, increased to the baseline values as was described in previous reports [16, 27]. Based on these results, we speculated that the constricted bronchi generated the accessory sound, and the values of spectrum curve indices decreased with the expansion of the high-pitch area of the sound spectrum curve [29].

In this study, we focused on the changes in breath sound power (dB) every 10 Hz from 100 Hz to 3000 Hz, and calculated the median of power values in every 10 Hz at three time points: before methacholine inhalation, after methacholine inhalation and after β_2-agonist inhalation. As a result, the sound spectrum curve after methacholine inhalation showed an increase in the power of the high-pitch area from 1,250 Hz to 2,000 Hz. This change clearly disappeared following β_2 agonist inhalation, suggesting that the spectral change was caused by bronchoconstriction induced by methacholine inhalation [29]. It was proved that methacholine-induced bronchoconstriction induces an increase of the high-pitch sound that could not be heard by auscultation.

It is interesting that the sound spectrum curve after methacholine inhalation returned to the original shape after the β_2-agonist inhalation. These results indicate that the change in the sound spectrum curve depend only on a transient bronchoconstriction. Although the mechanisms of bronchoconstriction and bronchodilation are not the same [13, 30], the fact that structural changes in the bronchi were demonstrated by changes in the breath sound spectrum may indicate the reliability and accuracy of this method. These findings seem to be of significant importance for the clinical application of breath sound analysis techniques.

Furthermore, in order to examine the characteristics of the spectral change generated by bronchoconstriction, we focused on the change in the AUC and calculated the AUC index (Hz·dB) for convenience [4, 27]. We devised a comparative study based on the AUC index, and the change in the AUC index at every 10 Hz was calculated at the three time points. The change in the AUC index due to methacholine inhalation was recognized as an isosceles triangle-shaped hump in the high-pitch area (Fig. 2). After β_2 agonist inhalation, a reverse hump of the same magnitude and frequency was observed, which returned the AUC to the same value as before methacholine inhalation. It was also demonstrated that the frequency of maximum power increase was around 1,500 Hz as a previous report [29]. It is unclear whether the bronchoconstriction other than the methacholine-induced bronchoconstriction produces the same spectral change, and whether infants produce similar sound during bronchoconstriction during asthma exacerbation. However, this seems to be a meaningful result in the future clinical practice.

The increased high-pitch sound discussed in this report was different from clinically audible wheezing. In previous studies of an acoustic spectrogram (time-to-sound frequency), typical wheezes could be observed as a bright wavy band from left to right, mainly in the high-pitch area of the expiratory period (400 Hz and above) [31]. During the methacholine inhalation test, respiratory resistance significantly increases and SpO_2 decreases, whereas wheezes are usually not heard with a stethoscope.

The present study was associated with some limitations. In particular, the generation of this spectral change in breath sound may depend on the peculiarity of methacholine-induced bronchoconstriction, as mentioned previously. The inhaled methacholine and β_2 agonist particles were almost the same size, as the same nebulizers were used, and deposition points of the bronchi may be same [32], which may also have resulted in the spectacular recovery of the participants. The artificial, transient airway change due to inhaled methacholine may have a different aspect from bronchoconstriction due to antigens or infections. Furthermore, we were unable to prove the part of the bronchus part from which the change came [16].

CONCLUSION

This study suggests that, in children with asthma, bronchoconstriction due to methacholine inhalation produces an increase in high-pitch sound at around 1,500 Hz. By these results, we demonstrated the possibility of detecting bronchoconstriction by analyzing breath sound spectrum curves. Our breath sound analysis technique is safe and easy to perform [8, 9], and has a sufficient clinical sensitivity and reliability to detect bronchoconstriction. This method is expected to be useful as an objective lung function test for the patients who cannot undergo standard lung function tests or perform forced oscillation techniques. In the future, we plan to stratify the target patients by age and severity.

FUNDING

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

ABBREVIATIONS

A_T: total area under the curve of 100 Hz to the highest frequency of the dBm power spectrum, **AUC**: area under the curve, **A_3**: third area under the curve of 100 Hz to the highest frequency of the dBm power spectrum, **BHR**: bronchial hyperresponsiveness, **B_4**: fourth area under the curve of 100 Hz to the highest frequency of the dBm power spectrum, **Dmin**: minimal dose of methacholine, **FEV_1**: forced expiratory flow and volume in 1 second, **FVC**: forced vital capacity, **F_{99}**: frequency limiting 99% of the power spectrum, **RPF_{50}**: ratio of power and frequency at 50% of the highest frequency of the dBm power spectrum, **RPF_{75}**: ratio of power and frequency at 75% of the highest frequency of the dBm power spectrum, **Rrs**: respiratory resistance, **Rrs.cont**: Rrs control value, **Slope**: roll-off from 600 to 1200 Hz, **St**: speed of bronchoconstriction to methacholine

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