Regional bronchodilator response assessed by computed tomography in chronic obstructive pulmonary disease

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Abstract (250 words)

Background and objective: The reliability of CT assessment of regional bronchodilation is not universally accepted. In this study, using our proprietary 3D-CT software, we first examined airway inner luminal area (Ai) before and after inhalation of SFC in a group of COPD patients and then evaluated the same parameters for two sets of CT data obtained from clinically stable subjects with no intervention.

Methods: We conducted CT at deep inspiration and pulmonary function tests before and one week after inhalation of SFC in 23 COPD patients. As a non-intervention group, we used two sets of CT data obtained with one-year interval in another group of subjects who demonstrated stable pulmonary function (n=8). We measured Ai at the mid-portions of 3^{rd} to 6^{th} generation in 8 bronchi of the right lung, a total of 32 identical sites before and after intervention.

Results: The average bronchodilation at all sites (ΔAi%: 28.2 ± 4.1 (SE)%) ($r=0.65$, $p < 0.001$) and that of each generation significantly correlated with % improvement of FEV1 (ΔFEV1%), which increased from 1.40 ± 0.10 L to 1.58 ± 0.10 L. When subjects were classified into two groups in terms of mean ΔFEV1%, even the poor responders(Δ FEV1%<14% above baseline,

n=13) displayed significantly larger ΔAi% compared with the non-intervention group (19.1 ± 4.6% vs. 2.1 ± 3.9%). Inter-observer variability for overall ΔAi% was within acceptable levels

Conclusions: CT can reliably detect the regional bronchodilation in 3rd to 6th generation airways when ΔFEV1 is as small as 180 ml on average.

This study was registered in the UMIN Clinical Trials Registry (UMIN-CTR) system (http://www.umin.ac.jp/. NO. UMIN 000002668).

Keywords: bronchodilation, pulmonary function, salmeterol/fluticasone propionate combination, computed tomography

Short Title: Regional bronchodilation assessed by CT

INTRODUCTION

Recently, computed tomography (CT) has been extensively utilized to evaluate airway remodeling in bronchial asthma and chronic obstructive pulmonary disease (COPD).[1-7] However, validation of the measurement of airway dimensions, such as airway wall area, particularly in smaller airways, has been challenging.[8,9] Airway inner luminal area (Ai) may not be suitable for assessment of airway remodeling because Ai varies with lung volume[10] and is likely affected by the balance of pressure inside and outside the airway wall.[11] Despite these physiological characteristics, Ai measurement might be superior to other CT airway indices from a couple of viewpoints. A technical merit of Ai is that it is unaffected by attachment of lung tissue and vessels, which would interfere with delineation of the outer lung edge, leading to potential errors in the measurement of airway wall parameters. Indeed, our previous study showed that the percentage predicted forced expiratory volume in 1 sec (FEV_1) correlates more closely with Ai than airway wall parameters in patients with COPD.[12] Furthermore, measurement of Ai provides accurate quantitative assessment of regional bronchodilation induced by bronchodilators.

We previously demonstrated the quantitative effects of tiotropium on the Ai of 3rd to 6th generation airways.[13] Since then, there have been several similar studies in which changes in airway dimensions, such as Ai and airway wall area, were quantitatively examined using CT following single and/or combined inhalation of tiotropium, salmeterol/fluticasone propionate, budesonide/formoterol, and indacaterol for COPD.[14-17] However, there have been no studies which attempted to examine the reproducibility and variation of the measurement of Ai when CT scans were taken twice with no intervention.

In some previous studies, airway dimensions were measured in 3rd generation airways alone, whereas in other studies, the researchers were not sure whether they really measured the same sites for comparison.[14-17] Our proprietary software for 3D-CT has the ability to assess Ai in 3rd to 6th generation airways[10,12,13,18,19], and furthermore the dual screen system enables us to simultaneously compare identical sites using two sets of CT data.

In this study, using our proprietary 3D-CT software, we first attempted to examine the magnitude of bronchodilation before and after one-week inhalation of salmeterol/fluticasone propionate combination (SFC) in a group of COPD patients and then evaluated the same

parameters for two sets of CT data obtained with one year interval from clinically stable subjects with no intervention.

METHODS

Inhalation of salmeterol/fluticasone propionate combination

Patients with clinically stable COPD (M/F, 22/1; age, 52-84 y; mean ± SD, 70.1 ± 7.6 y; GOLD[20] Stage2, n=6, Stage3, n=17) were recruited at Hokkaido University Hospital between January 2010 and February 2011. All patients were either current or former smokers. We excluded patients with bronchial asthma, pulmonary fibrosis, pulmonary cancer, giant bullae, and severe diffuse and/or local bronchiectasis. All patients provided written informed consent to participate and the Ethics Committee for Human Research at Hokkaido University Hospital approved the study. This study was registered in the UMIN Clinical Trials Registry (UMIN-CTR) system (http://www.umin.ac.jp/. NO. UMIN 000002668).

After the first visit, the subjects refrained from using any respiratory medication for one week.

At the second visit, for baseline measurement, lung CT followed by pulmonary function tests were performed if the subjects were clinically stable and had not taken any respiratory medication for the previous week. All participants commenced inhalation of SFC from the evening of the second visit and used SFC twice a day from the following day. On the seventh day after the second visit, patients inhaled SFC about 2-3 hours before they again underwent lung CT and pulmonary function tests.

Computed tomography and airway analysis

A multidetector-row spiral CT scanner with a 64-detector array (Aquilion Multi, TSX-101A/6A; Toshiba Medical Systems, Tochigi, Japan) was used. The acquisition parameters were 120 kVp, 300 mA, 64 detector × 0.5-mm collimation, slice thickness 0.5 mm, 0.5 s/rotation, and helical pitch 41. The entire lung of each patient was scanned in the supine position at full inspiration. The length of the pixel size was approximately 0.625 mm. Raw data were transferred to the workstation and reconstructed into three-dimensional chest images (Virtual place Fujin raijin 310; AZE Ltd., Tokyo, Japan). The detailed process of CT data

acquisition and reconstruction has been described previously.[13, 21] Eight bronchi were selected in the right lung: apical (B1), posterior (B2), and anterior (B3) of the upper lobe, lateral (B4) and medial (B5) of the middle lobe, and anterior basal (B8), lateral basal (B9), and posterior basal (B10) of the lower lobe. We rotate the 3D images of the bronchi to find any bifurcation, one bronchus was randomly selected at each bifurcation. If the image of the bronchus was poor or one bronchus was obstructed, the other bronchus, up to the 6th generation, was selected. These measurements were done under the condition where the examiner was unaware of which one of the two CT data had been taken earlier or later. Then, Ai was measured at the midpoint between bifurcations, from the 3rd (segmental bronchus) to 6th generation of each airway, leading to a total of 32 measurement sites per subject. We used two screens, which allowed simultaneous assessment of images before and after inhalation, so that we could compare the same point of the same bronchi in both states in a given subject (Figure 1). Average values of Ai per generation and per lobe were calculated for analysis.

We measured only airway inner luminal area and not the airway outer wall. The inner diameter

(Di) was calculated as $2\sqrt{Ai/\pi}$, assuming that the airway lumen was a true circle. The degree of

bronchodilation was expressed as % improvement of Ai (ΔAi%). To delineate the inner circle

of each airway, one was allowed to make manual plots only when the automatically obtained

outline of airway inner area was obviously out of contour.

Measurement of lung volume (LV) is described in the online data supplement.

Pulmonary Function Tests

A rolling seal type spirometer, CHESTAC-33 (CHEST M.I., Inc., Tokyo, Japan), was used.

More information is presented in the online data supplement.

Study on airway measurements at an interval of one year

We did not have genuine controls because we did not want subjects to undergo CT exams twice

within one week without any treatment. Instead, we used data from participants in the Hokkaido COPD cohort study to determine the reproducibility of Ai when the data of pulmonary function tests did not show any significant change on two occasions. Following the Hokkaido COPD cohort study protocol,[22] participants underwent CT exams yearly and respiratory function tests every 6 months. Eight pairs of CT data from moderate to severe COPD patients, obtained at an interval of 1 year during which the difference in FEV_1 was < 50 ml, were available (M/F 8/0, age, 62-86 y, mean±SD, 78.5±8.2 y; GOLD Stage 2, n=3, Stage 3, n= 5). The CT scanner and protocol for measurements of Ai were the same as in the subjects treated with SFC in the current study.

Inter-observer variability in the measurement of Ai

We examined the inter-observer variability in evaluation of bronchodilation with our methods using intra-class correlations (ICC). When the number of observers was two, null hypothesis was 0.1, alternative hypothesis was 0.8, and power was 0.9, the required number of data was

calculated as 12.[23] Then, 12 subjects were randomly selected to reflect the variability of the improvement in FEV1 from the subjects in the SFC study. Two respiratory physicians independently assessed Ai in these 12 patients.

Statistics

Data are shown as mean ± standard error of mean (SEM). We used paired Student's t-tests to analyze differences in mean values between baseline and post-bronchodilator values. Relationships between quantitative variables were examined using the Spearman test. Bland-Altman analysis and ICC between the two observers were used to assess reproducibility in ΔAi% at all sites, that is, the average value of 32 data points. For comparison, we classified SFC-treated subjects into two groups, using the mean value of ΔFEV_1, as good responders ($\Delta FEV_1 > 14\%$) and poor responders ($\Delta FEV_1 < 14\%$). When statistical significance was obtained by one-way analysis of variance in the groups, the Tukey post-hoc test was performed for quantitative continuous variables, such as ΔFEV1%, between the groups.

Dunnett's test was performed for comparison of ΔAi% at all sites and ΔAi% in the 3rd to 6th generation bronchi between the three groups. All statistical tests were 2-sided and values of $p < 0.05$ were considered statistically significant. Data were analyzed using JMP 10.0 software (SAS institute Inc., NC, USA).

RESULTS

Data of pulmonary function tests and bronchodilation assessed by CT

Twenty-seven subjects completed all visits. Four subjects were excluded because of pneumonia, atelectasis, obstructive bronchi in the middle and lower lobes, and a giant bulla. The data of 23 subjects was available.

Table 1 shows the results of pulmonary function tests before and after inhalation of SFC for one week. Vital capacity (VC), inspiratory capacity (IC), FVC, FEV_1, and FEV_1/FVC increased significantly, and RV and RV/TLC decreased significantly with treatment. LVs measured by CT before and after one week of SFC inhalation were 5294 ± 208 ml and 5219 ± 213 ml, respectively, indicating that there were no significant differences in LV with treatment ($p=0.105$).

Table 2 shows the absolute values of Ai at 32 sites and average values of ΔAi% per generation of bronchus and per lobe. We could not obtain clear images in some cases when Ai was obstructed or too small for the measurement. The numbers of data available for each site are

also shown. Di at baseline was 4.3 (0.1)(mean(SE)) mm, 3.2 (0.1) mm, 2.5 (0.1) mm, and 2.1 (0.1) mm in 3rd, 4th, 5th, and 6th generation airways, respectively. Statistically significant bronchodilation was evident at 30 of 32 sites. Generally, as the generation number increased, average ΔAi% also increased. However, differences in ΔAi% between the generations were not significant. ΔAi% was similar among the lobes.

We then examined the relationship between overall ΔAi% and Δpulmonary function variables (Table 3). We found significant correlations only between overall ΔAi% and ΔFEV_1% (r=0.638, p <0.001) and ΔFEV1/FVC% (r=0.579, p=0.004).

The correlations between ΔAi% by generation from the 3rd to the 6th generation and ΔFEV1% were statistically significant (Figure 2).

Magnitude of bronchodilation caused by SFC in comparison with that of non-intervention group

In the eight subjects with no intervention, FEV1 %predicted after inhalation of short acting

bronchodilator at baseline was 59.1 ± 3.9%(Table S1), and ΔFEV1% obtained after an interval of one year was 1.5± 0.9%. The overall ΔAi% was 2.1 ± 3.9% (Figure 3). The results of pulmonary function tests before and after inhalation in good and poor responders were shown in online supplements Table S1. In the good responders(n=10), ΔFEV_1% was 25.3 ± 7.8% and overall ΔAi% was 40.1 ± 5.2%. In poor responders (n=13), ΔFEV_1% was 5.2 ± 8.6% and overall ΔAi% was 19.1 ± 4.6%, which was significantly larger than that in non-intervention group (p=0.04) (Figure 3). We compared ΔAi% in non-intervention group with that in poor and good responders to SFC according to bronchial generation from 3rd to 6th generations(Figure S1).

Inter-observer variability

Overall ΔAi% indicated a small mean difference (-3.81%) in inter-observer variability and acceptable ICC (0.801) (Figure 4). For the data on airway generations, please see Figure S2 in the online data supplement.

DISCUSSION

In this study, we demonstrated that 3D-CT together with our proprietary software could detect the statistically significant bronchodilation induced in 3rd to 6th generation airways by one week's inhalation of SFC when the average improvement in FEV_1 was as small as 180 ml. The overall ΔAi% significantly and best correlated with ΔFEV_1% in 23 subjects with COPD. As a non-intervention group, we used the subjects in the Hokkaido cohort study, who underwent CT after an interval of one-year while their pulmonary function did not change ($\Delta FEV_1 < 50$ mL). We nevertheless found that the change in the overall ΔAi% was 2.1% on average, which was far below the value of 19.1% in poor responders to SFC whose ΔFEV1% was <14% despite that limitation. Regarding inter-observer variability, ICC was greater than 0.8 for overall ΔAi%, which is the average of all ΔAi% values obtained from 32 measurement sites in the 3rd to 6thgenerations of 8 bronchi in the right lung.

Recently, contrary opinions have been expressed regarding the accuracy and reliability of measurement of airway dimensions, largely from technical points of view, particularly for

smaller sized airways.[24,25] Oguma *et al.* have provided a detailed discussion on the limitations of measurements of airway wall area and inner luminal area. They claim that accuracy and reliability would significantly deteriorate when wall thickness of the airway is less than 1.0 mm, which they admit might be affected by the reconstruction algorithm, CT scanner types, and even the composition of airway phantoms.[25] Although we focused on Ai alone, we have to admit that we could not detect all 5th and 6th generation airways. ΔAi% in 6thgeneration bronchi showed a significant correlation with the magnitude of ΔFEV_1.but the difference in ΔAi between non-intervention group and good responders did not reach to be significant.(p=0.0857)

Several issues should be considered when assessing the bronchodilator response using CT scans. One of the most important issues is how to deal with potential heterogeneity of the response in the lung. Although we do not think that our approach would be sufficient enough to overcome this heterogeneity,[26,27] we nevertheless found statistically significant correlations between overall ΔAi% and ΔAi% values in each generation from the 3rd to 6th airway generations. However, we must admit that the more of the measurements' sites, the better as representative data at any generation. The reason for selection of only the midpoint site of airways at each generation is simply

because it is the easiest way. And we thought that taking the average at the same generation of 8 airways would provide reasonable values although not ideal ones. The second issue is that the current assessment heavily depends on well-trained and experienced personnel to identify the measurement sites and delineate the circular inner airway walls in some cases. Thus, in this study, we attempted to examine inter-observariability in measurements. We found relatively good ICC values for overall ΔAi% between two independent observers. Another issue of which we need to take note is the potential effect of differences in lung volume on Ai when CT scans are taken on different occasions.[10, 28-30] In this study, we therefore confirmed that the difference in lung volume assessed by 3D-CT at two occasions was negligible.

In our previous study using tiotropium, the slope of the regression lines between ΔAi% and ΔFEV_1% became steeper as the size of airways became smaller from the 3rd to 6th generation airways; in other words, the improvement in FEV1 with tiotropium was more marked in the more distal airways. However, we did not find any such trend with SFC in this study. We are not sure whether the difference between the two studies is due to differences in the inhaled drugs themselves or due to the different class to which each inhaled drug belongs. We cannot

comment on this because the sample size was too small in both studies and also because the subjects were different. However, it would be intriguing to pursue the potential difference in the regional bronchodilator response induced by different drugs in a future prospective, randomized, large-scaled study using 3D-CT scans.

In conclusion, CT can reliably detect the regional bronchodilation in 3rd to 6th generation airways when ΔFEV1 is as small as 180 ml on average. This study provides further support for the utility of Δ Ai% measures as a complimentary assessment of treatment effect to Δ FEV1%.

Acknowledgments

We thank Hideka Ashikaga, Ayako Kondo, and Yuko Takagi for data management, and the staff of the Division of Pulmonary Function and Department of Radiology, Hokkaido University Hospital, especially Tsukasa Sasaki, for technical assistance with this study.

Role of the Funding Source

This study was supported by a research grant from the Respiratory, Inflammation, Autoimmunity Innovative Medicines Unit, AstraZeneca, Mölndal, Sweden and a scientific research grant to the Hokkaido COPD Cohort Study from the Ministry of Education, Science, Culture and Sports of Japan (17390239 and 2139053 to M.N.); Nippon Boehringer Ingelheim; Pfizer, Inc.; and a grant to the Respiratory Failure Research Group from the Ministry of Health, Labor and Welfare, Japan. None of them concerned the design and analysis of the study and writing of the manuscript.

Table 1 Results of pulmonary function tests before and after inhalation of the salmeterol/fluticasone propionate combination for 1 week

Pulmonary function parameters	Baseline		One week after SFC inhalation		p value
VC, l (%predicted)	3.52 ± 0.13	(107.8 ± 3.5)	3.74 ± 0.15‡	(116.2 ± 3.3)	0.019
IC, l	2.36 ± 0.12		2.46 ± 0.12†		0.045
FVC, l (%predicted)	3.40 ± 0.13	(102.7 ± 3.7)	3.70 ± 0.15‡	(114.0 ± 3.3)	< 0.001
FEV_1, l (%predicted)	1.40 ± 0.10	(50.2 ± 2.6)	1.58 ± 0.10‡	(56.9 ± 2.9)	< 0.001
FEV_1/FVC, %	0.41 ± 0.02		0.43 ± 0.02‡		0.004
DL_{CO}, ml/min/mmHg (%predicted)	13.6 ± 1.3	(82.0 ± 6.2)	13.6 ± 1.3	(82.3 ± 5.6)	0.948
DL_{CO}/VA, ml/min/mmHg/l (%predicted)	3.14 ± 0.27	(70.6 ± 5.7)	3.15 ± 0.25	(71.2 ± 5.4)	0.870
TLC, l (%predicted)	6.33 ± 0.20	(114.7 ± 2.8)	6.38 ± 0.20	(115.6 ± 2.8)	0.251
FRC, l (%predicted)	3.89 ± 0.16	(118.4 ± 4.6)	3.89 ± 0.15	(118.1 ± 4.3)	0.823
RV, l (%predicted)	2.77 ± 0.14	(136.4 ± 6.9)	2.63 ± 0.11‡	(129.6 ± 5.3)	0.027
RV/TLC, %	43.6 ± 1.5		41.4 ± 1.4†		0.012

VC, vital capacity; IC, inspiratory capacity; FVC, forced vital capacity; FEV1, forced expiratory volume in 1 sec;

DL_{CO}, carbon monoxide diffusing capacity; VA, alveolar volume; TLC, total lung capacity; FRC, functional residual capacity; RV, residual volume; l, liter.

Data are shown as mean ± standard error of mean.

†$p < 0.05$, ‡$p < 0.01$ after inhalation of salmeterol/fluticasone propionate combination for 1 week versus baseline.

Table 2. Airway inner luminal areas at 32 sites before and after inhalation of the salmeterol/fluticasone propionate combination

	Generation of bronchi								
	3rd		4th		5th		6th		Averaged % increase
	Baseline	After	Baseline	After	Baseline	After	Baseline	After	in Ai% / lobe
Right upper lobe									
Apical (B1)	13.9 ± 1.4(23)	16.2 ± 1.5‡(23)	7.4 ± 0.8(21)	8.7 ± 0.8‡(21)	4.0 ± 0.4(20)	4.5 ± 0.4†(21)	2.6 ± 0.3(18)	2.9 ± 0.3‡(20)	**28.3 ± 3.8**
Posterior apical (B2)	14.2 ± 1.2(23)	17.5 ± 1.2‡(23)	8.5 ± 1.0(23)	11.0 ± 1.1‡(23)	4.7 ± 0.7(22)	6.0 ± 0.8‡(23)	2.7 ± 0.3(18)	3.8 ± 0.4‡(19)	
Anterior apical (B3)	25.3 ± 2.1(23)	28.7 ±2.1‡(23)	12.9 ± 1.1(23)	15.8 ±1.2‡(23)	7.7 ± 0.8(23)	9.2 ±0.9‡(23)	4.7 ± 0.5(23)	5.6 ±0.6†(23)	
Right middle lobe									
Medial (B4)	16.8 ± 1.3(22)	20.2 ±1.4‡(22)	8.8 ± 0.8(22)	11.3 ±0.9‡(22)	6.1 ± 0.6(21)	7.3 ±0.7‡(22)	4.1 ± 0.5(19)	4.9 ± 0.5(19)	**31.2 ± 3.6**
Lateral (B5)	12.7 ± 1.3(22)	15.5 ±1.3‡(22)	6.1 ± 0.8(21)	7.7 ± 0.8‡(22)	3.7 ± 0.6(20)	5.0 ± 0.6‡(22)	3.0 ± 0.4(14)	3.2 ± 0.3†(18)	
Right lower lobe									
Anterior basal (B8)	13.9 ± 0.9(23)	16.5 ± 1.0‡(23)	7.8 ± 0.8(22)	9.0 ± 0.7‡(23)	5.0 ± 0.5(20)	5.6 ± 0.4†(22)	3.3 ± 0.5(16)	3.8 ± 0.4†(18)	**31.2 ± 6.4**
Lateral basal (B9)	12.5 ± 1.1(21)	14.8 ± 1.0‡(22)	6.6 ± 0.8(21)	8.0 ± 0.9†(22)	4.5 ± 0.5(19)	5.5 ± 0.6‡(21)	3.5 ± 0.5(16)	4.2 ± 0.5‡(17)	
Posterior basal (B10)	14.6 ± 1.1(23)	17.9 ± 1.2‡(23)	10.5 ± 0.9(23)	12.9 ± 1.1‡ (23)	7.4 ± 0.9(23)	8.7 ± 0.8‡(23)	4.7 ± 0.7(19)	5.9 ± 0.6(22)	
Average % increase									
in Ai%/generation	**23.7 ± 3.0**		**30.5 ± 4.9**		**32.0 ± 5.0**		**34.3 ± 6.9**		**28.2 ± 4.1**

Number of available data, at both baseline and after inhalation of salmeterol/fluticasone propionate combination,
are shown for all sites. Data are shownas mean ± standard error of mean (SEM). The unit for airway inner luminal area is mm3.
†$p < 0.05$, ‡ $p < 0.01$ after one week of salmeterol/fluticasone propionate inhalation versus baseline.

Table 3. Relationships between % increase in airway inner luminal area and % improvement in pulmonary function parameters

Pulmonary function parameters	r value	p value
VC	0.389	0.066
IC	0.045	0.840
FVC	0.323	0.133
FEV1	0.638	<0.001†
FEV1/FVC	0.579	0.004†
DL_{CO}	-0.040	0.858
TLC	0.179	0.414
FRC	-0.076	0.730
RV	-0.092	0.677
RV/TLC	-0.184	0.401

†$p < 0.05$. VC, vital capacity; IC, inspiratory capacity; FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 sec; DL_{CO}, carbon monoxide diffusing capacity; TLC, total lung capacity; FRC, functional residual capacity; RV, residual volume.

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FIGURE LEGENDS

Figure 1. Short axis images of the right anterior segmental bronchus of one individual before (upper row) and after 1 week of salmeterol/fluticasone propionate combination inhalation (lower row). Generation 3 bronchi are defined as segmental.

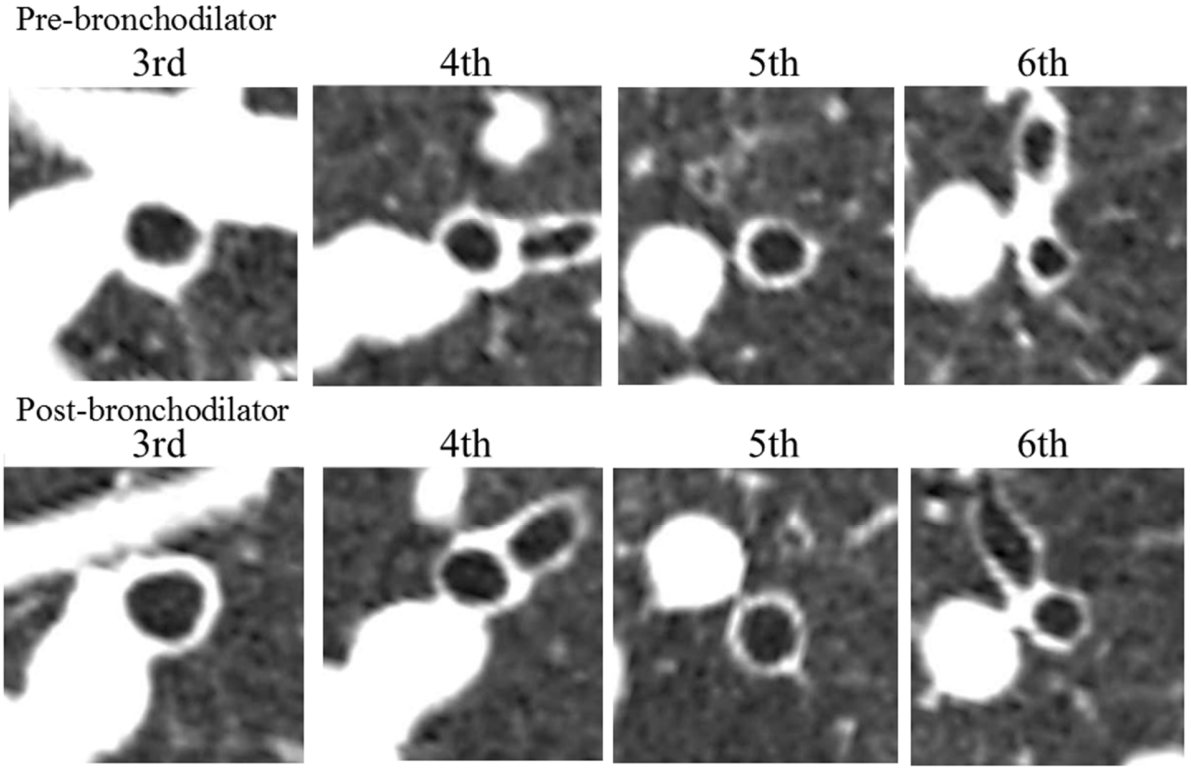

Figure 2. Relationship between % increase in airway inner luminal area and FEV1 from baseline according to airway generation after one week of salmeterol/fluticasone propionate inhalation in patients with COPD (n=23). Data of (A) 3rd, (B) 4th, (C) 5th, and (D) 6th generation bronchi are shown. Ai: airway inner luminal area, gen: generation.

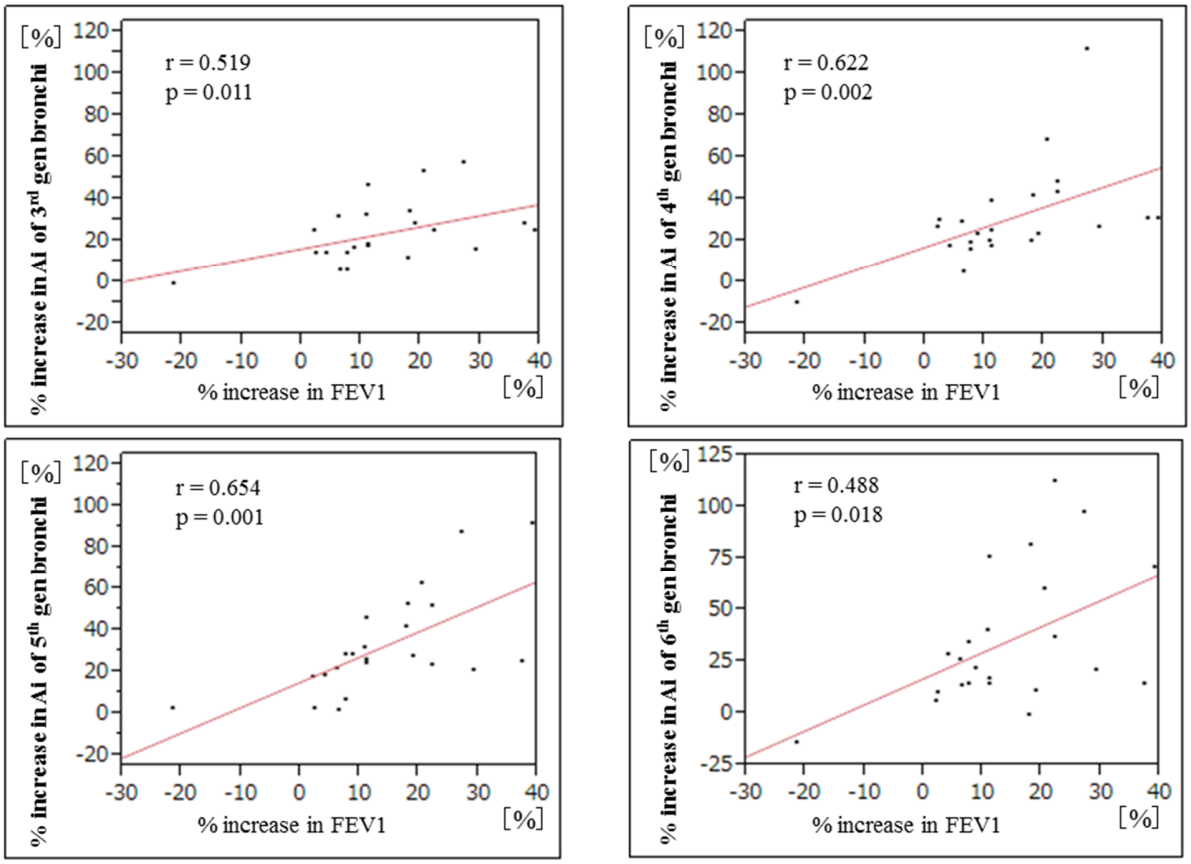

Figure 3. Comparisons between bronchodilation in a non-intervention group and subjects treated with salmeterol/fluticasone propionate combination. There was a significant difference in % increase in airway inner luminal area (ΔAi%) at the 32 points between a non-intervention group and the poor responders (19.1 ± 4.6%, p=0.04, n=13) and between a non-intervention group and good responders (40.1 ± 0.2%, p < 0.0001, n=10).

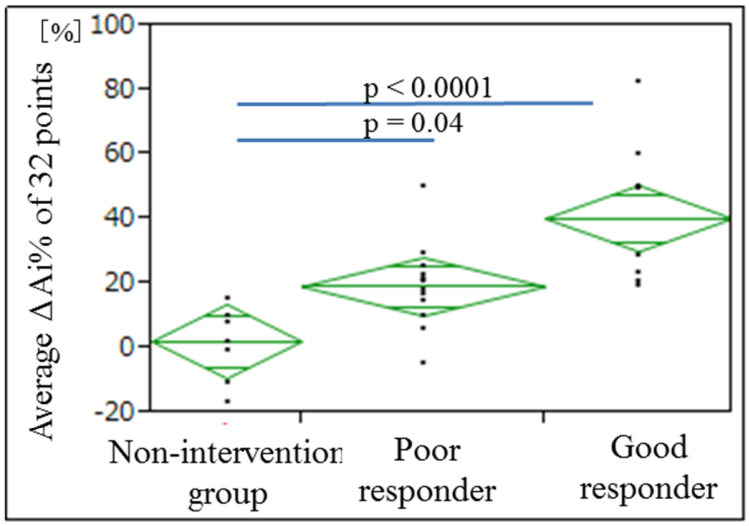

Figure 4. Inter-observer variability (IOV) in assessing % increase in airway inner luminal area (ΔAi%). Solid lines and dotted lines represent the values of mean and 2SD, respectively. A small mean difference (-3.81%) was found. Intra-Class Correlation (ICC) was 0.801.