



|                  |                                                                                                                                                                                                                                   |
|------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Title            | Regional bronchodilator response assessed by computed tomography in chronic obstructive pulmonary disease                                                                                                                         |
| Author(s)        | Shimizu, Kaoruko; Makita, Hironi; Hasegawa, Masaru et al.                                                                                                                                                                         |
| Citation         | European journal of radiology, 84(6), 1196-1201<br><a href="https://doi.org/10.1016/j.ejrad.2015.02.022">https://doi.org/10.1016/j.ejrad.2015.02.022</a>                                                                          |
| Issue Date       | 2015-06                                                                                                                                                                                                                           |
| Doc URL          | <a href="https://hdl.handle.net/2115/61986">https://hdl.handle.net/2115/61986</a>                                                                                                                                                 |
| Rights           | © 2015, Elsevier. Licensed under the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International <a href="http://creativecommons.org/licenses/by-nc-nd/4.0/">http://creativecommons.org/licenses/by-nc-nd/4.0/</a> |
| Rights(URL)      | <a href="https://creativecommons.org/licenses/by-nc-nd/4.0/">https://creativecommons.org/licenses/by-nc-nd/4.0/</a>                                                                                                               |
| Type             | journal article                                                                                                                                                                                                                   |
| File Information | manuscript.pdf                                                                                                                                                                                                                    |



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

Kaoruko Shimizu<sup>1</sup>, Hironi Makita<sup>1</sup>, Masaru Hasegawa<sup>1</sup>, Hirokazu Kimura<sup>1</sup>,  
Satoshi Fuke<sup>1</sup>, Katsura Nagai<sup>1</sup>, Takayuki Yoshida<sup>1</sup>, Masaru Suzuki<sup>1</sup>, Satoshi Konno<sup>1</sup>,  
Yoichi M. Ito<sup>2</sup> and Masaharu Nishimura<sup>1\*</sup>

<sup>1</sup> First Department of Medicine, Hokkaido University School of Medicine

N-15 W-7, Kita-Ku, Sapporo 060-8638, Japan

<sup>2</sup> Department of Biostatistics, Hokkaido University Graduate School of Medicine

N-15 W-7, Kita-Ku, Sapporo 060-8638, Japan

\*Correspondence to: Masaharu Nishimura, M.D.

First Department of Medicine, Hokkaido University School of Medicine

Fax: +81-11-706-7899; Tel: +81-11-706-5911

E-mail: [ma-nishi@med.hokudai.ac.jp](mailto:ma-nishi@med.hokudai.ac.jp)

Each author's role: MN: Study concepts: MN HM MH KS

Study design: MN HM MH KS

Data acquisition: KS HK MN HM SF KN TY

Quality control of data and algorithms: KS HK MN HM SF KN TY YI

Data analysis and interpretation: KS MN HM MS SK KN.

Statistical analysis: KS YI

Manuscript preparation: KS HM MN

Manuscript editing: KS HM MN

Manuscript review: HM MN KN MS SK

**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<sup>rd</sup> to 6<sup>th</sup> 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 ( $\Delta Ai\%$ :  $28.2 \pm 4.1$  (SE)%) ( $r=0.65$ ,  $p < 0.001$ ) and that of each generation significantly correlated with % improvement of FEV1 ( $\Delta FEV1\%$ ), which increased from  $1.40 \pm 0.10$  L to  $1.58 \pm 0.10$  L. When subjects were classified into two groups in terms of mean  $\Delta FEV1\%$ , even the poor responders ( $\Delta FEV1\% < 14\%$  above baseline,

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

**Conclusions:** CT can reliably detect the regional bronchodilation in 3<sup>rd</sup> to 6<sup>th</sup> generation airways when  $\Delta FEV1$  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).<sup>1-7</sup> However, validation of the measurement of airway dimensions, such as airway wall area, particularly in smaller airways, has been challenging.<sup>8,9</sup> Airway inner luminal area (Ai) may not be suitable for assessment of airway remodeling because Ai varies with lung volume<sup>10</sup> and is likely affected by the balance of pressure inside and outside the airway wall.<sup>11</sup> 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<sub>1</sub>) correlates more closely with Ai than airway wall parameters in patients with COPD.<sup>12</sup> 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 3<sup>rd</sup> to 6<sup>th</sup> generation airways.<sup>13</sup> 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.<sup>14-17</sup> 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 3<sup>rd</sup> generation airways alone, whereas in other studies, the researchers were not sure whether they really measured the same sites for comparison.<sup>14-17</sup> Our proprietary software for 3D-CT has the ability to assess Ai in 3<sup>rd</sup> to 6<sup>th</sup> generation airways<sup>10,12,13,18,19</sup>, 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  $\pm$  SD,  $70.1 \pm 7.6$  y;

GOLD<sup>20</sup> 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  $\times$  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 rajin 310; AZE Ltd., Tokyo, Japan). The detailed process of CT data

acquisition and reconstruction has been described previously.<sup>13, 21</sup> 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 6<sup>th</sup> 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,  $A_i$  was measured at the midpoint between bifurcations, from the 3<sup>rd</sup> (segmental bronchus) to 6<sup>th</sup> 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  $A_i$  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  $2\sqrt{A_i/\pi}$ , assuming that the airway lumen was a true circle. The degree of

bronchodilation was expressed as % improvement of  $A_i$  ( $\Delta A_i\%$ ). 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,<sup>22</sup> 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 FEV1 was < 50 ml, were available (M/F 8/0, age, 62-86 y, mean  $\pm$  SD,  $78.5 \pm 8.2$  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.<sup>23</sup> Then, 12 subjects were randomly selected to reflect the variability of the improvement in FEV<sub>1</sub> from the subjects in the SFC study. Two respiratory physicians independently assessed Ai in these 12 patients.

## **Statistics**

Data are shown as mean  $\pm$  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  $\Delta$ Ai% 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  $\Delta$ FEV<sub>1</sub>, as good responders

( $\Delta$ FEV<sub>1</sub> > 14%) and poor responders ( $\Delta$ FEV<sub>1</sub> <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  $\Delta$ FEV<sub>1</sub>%, between the groups.

Dunnett's test was performed for comparison of  $\Delta Ai\%$  at all sites and  $\Delta Ai\%$  in the 3<sup>rd</sup> to 6<sup>th</sup> 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<sub>1</sub>, and FEV<sub>1</sub>/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  $5294 \pm 208$  ml and  $5219 \pm 213$  ml, respectively, indicating that there were no significant differences in LV with treatment ( $p=0.105$ ).

Table 2 shows the absolute values of A<sub>i</sub> at 32 sites and average values of  $\Delta A_i\%$  per generation of bronchus and per lobe. We could not obtain clear images in some cases when A<sub>i</sub> 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 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup>, and 6<sup>th</sup> generation airways, respectively. Statistically significant bronchodilation was evident at 30 of 32 sites. Generally, as the generation number increased, average  $\Delta Ai\%$  also increased. However, differences in  $\Delta Ai\%$  between the generations were not significant.  $\Delta Ai\%$  was similar among the lobes.

We then examined the relationship between overall  $\Delta Ai\%$  and  $\Delta$ pulmonary function variables (Table 3). We found significant correlations only between overall  $\Delta Ai\%$  and  $\Delta FEV_1\%$  ( $r=0.638$ ,  $p < 0.001$ ) and  $\Delta FEV_1/FVC\%$  ( $r=0.579$ ,  $p=0.004$ ).

The correlations between  $\Delta Ai\%$  by generation from the 3<sup>rd</sup> to the 6<sup>th</sup> generation and  $\Delta FEV_1\%$  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,  $FEV_1\%$  predicted after inhalation of short acting

bronchodilator at baseline was  $59.1 \pm 3.9\%$  (Table S1), and  $\Delta FEV_1\%$  obtained after an interval of one year was  $1.5 \pm 0.9\%$ . The overall  $\Delta Ai\%$  was  $2.1 \pm 3.9\%$  (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 ( $n=10$ ),  $\Delta FEV_1\%$  was  $25.3 \pm 7.8\%$  and overall  $\Delta Ai\%$  was  $40.1 \pm 5.2\%$ . In poor responders ( $n=13$ ),  $\Delta FEV_1\%$  was  $5.2 \pm 8.6\%$  and overall  $\Delta Ai\%$  was  $19.1 \pm 4.6\%$ , which was significantly larger than that in non-intervention group ( $p=0.04$ ) (Figure 3). We compared  $\Delta Ai\%$  in non-intervention group with that in poor and good responders to SFC according to bronchial generation from 3<sup>rd</sup> to 6<sup>th</sup> generations (Figure S1).

### **Inter-observer variability**

Overall  $\Delta Ai\%$  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 3<sup>rd</sup> to 6<sup>th</sup> generation airways by one week's inhalation of SFC when the average improvement in FEV<sub>1</sub> was as small as 180 ml. The overall  $\Delta Ai\%$  significantly and best correlated with  $\Delta FEV_1\%$  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  $\Delta Ai\%$  was 2.1% on average, which was far below the value of 19.1% in poor responders to SFC whose  $\Delta FEV_1\%$  was  $<14\%$  despite that limitation. Regarding inter-observer variability, ICC was greater than 0.8 for overall  $\Delta Ai\%$ , which is the average of all  $\Delta Ai\%$  values obtained from 32 measurement sites in the 3<sup>rd</sup> to 6<sup>th</sup> generations 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.<sup>24,25</sup> 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.<sup>25</sup> Although we focused on Ai alone, we have to admit that we could not detect all 5<sup>th</sup> and 6<sup>th</sup> generation airways.  $\Delta Ai\%$  in 6<sup>th</sup> generation bronchi showed a significant correlation with the magnitude of  $\Delta FEV_1$ , but the difference in  $\Delta Ai$  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,<sup>26,27</sup> we nevertheless found statistically significant correlations between overall  $\Delta Ai\%$  and  $\Delta Ai\%$  values in each generation from the 3<sup>rd</sup> to 6<sup>th</sup> 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-observability in measurements. We found relatively good ICC values for overall  $\Delta A_i\%$  between two independent observers. Another issue of which we need to take note is the potential effect of differences in lung volume on  $A_i$  when CT scans are taken on different occasions.<sup>10, 28-30</sup> 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  $\Delta A_i\%$  and  $\Delta FEV_1\%$  became steeper as the size of airways became smaller from the 3<sup>rd</sup> to 6<sup>th</sup> generation airways; in other words, the improvement in FEV<sub>1</sub> 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 3<sup>rd</sup> to 6<sup>th</sup> generation airways when  $\Delta$ FEV1 is as small as 180 ml on average. This study provides further support for the utility of  $\Delta$  Ai% measures as a complimentary assessment of treatment effect to  $\Delta$  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 <sup>‡</sup>      | (116.2 ± 3.3) | 0.019   |
| IC, l                                            | 2.36 ± 0.12 |               | 2.46 ± 0.12 <sup>‡</sup>      |               | 0.045   |
| FVC, l (%predicted)                              | 3.40 ± 0.13 | (102.7 ± 3.7) | 3.70 ± 0.15 <sup>‡</sup>      | (114.0 ± 3.3) | < 0.001 |
| FEV <sub>1</sub> , l (%predicted)                | 1.40 ± 0.10 | (50.2 ± 2.6)  | 1.58 ± 0.10 <sup>‡</sup>      | (56.9 ± 2.9)  | < 0.001 |
| FEV <sub>1</sub> /FVC, %                         | 0.41 ± 0.02 |               | 0.43 ± 0.02 <sup>‡</sup>      |               | 0.004   |
| DL <sub>CO</sub> , ml/min/mmHg (%predicted)      | 13.6 ± 1.3  | (82.0 ± 6.2)  | 13.6 ± 1.3                    | (82.3 ± 5.6)  | 0.948   |
| DL <sub>CO</sub> /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 <sup>‡</sup>      | (129.6 ± 5.3) | 0.027   |
| RV/TLC, %                                        | 43.6 ± 1.5  |               | 41.4 ± 1.4 <sup>‡</sup>       |               | 0.012   |

VC, vital capacity; IC, inspiratory capacity; FVC, forced vital capacity; FEV<sub>1</sub>, forced expiratory volume in 1 sec;

DL<sub>CO</sub>, 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.

<sup>‡</sup>p < 0.05, <sup>‡</sup>p < 0.01 after inhalation of salmeterol/fluticasone propionate combination for 1 week 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   |
| FEV <sub>1</sub>              | 0.638   | <0.001† |
| FEV <sub>1</sub> /FVC         | 0.579   | 0.004†  |
| DL <sub>CO</sub>              | -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<sub>1</sub>, forced expiratory volume in 1 sec; DL<sub>CO</sub>, carbon monoxide diffusing capacity; TLC, total lung capacity; FRC, functional residual capacity; RV, residual volume.

## References

1. Nakano Y, Muro S, Sakai H, Hirai T, Chin K, Tsukino M, Nishimura K, Itoh H, Paré PD, Hogg JC, Mishima M. Computed tomographic measurements of airway dimensions and emphysema in smokers. Correlation with lung function. *Am. J. Respir. Crit. Care Med.* 2000; **162**: 1102-8.
2. Grenier PA, Beigelman-Aubry C, Fétita C, Prêteux F, Brauner MW, Lenoir S. New frontiers in CT imaging of airway disease. *Eur. Radiol.* 2002; **12**: 1022–44.
3. de Jong PA, Muller NL, Paré PD, Coxson HO. Computed tomographic imaging of the airways: relationship to structure and function. *Eur. Respir. J.* 2005; **26**: 140–52.
4. Orlandi I, Moroni C, Camiciottoli G, Bartolucci M, Pistolesi M, Villari N, Mascalchi M. Chronic obstructive pulmonary disease: thin-section CT measurement of airway wall thickness and lung attenuation. *Radiology* 2005; **234**: 604-10.

5. Aysola RS, Hoffman EA, Glarada D, Wenzel S, Cook-Granroth J, Tarsi J, Zheng J, Schechtman KB, Ramkumar TP, Cochran R, Xueping E, Christie C, Newell J, Fain S, Altes TA, Castro M. Airway remodeling measured by multidetector computed tomography is increased in severe asthma and correlates with pathology. *Chest*. 2008;**134**:1183-91.
6. Montaudon M, Berger P, de Dietrich G, Braquelaire A, Marthan R, Tunon-de-Lara JM, Laurent F. Assessment of airways with three-dimensional quantitative thin-section CT: in vitro and in vivo validation. *Radiology* 2007; **242**: 563-72.
7. Achenbach T, Weinheimer O, Biedermann A, Schmitt S, Freudenstein D, Goutham E, Kunz RP, Buhl R, Dueber C, Heussel CP. MDCT assessment of airway wall thickness in COPD patients using a new method: correlations with pulmonary function tests. *Eur. Radiol*. 2008; **18**: 2731-8.

8. Reinhardt JM, D'Souza ND, Hoffman EA. Accurate measurement of intrathoracic airways.

*IEEE Trans. Med. Imaging* 1997; **16**: 820-7.

9. King GG, Müller NL, Whittall KP, Xianq QS, Pare PD. An analysis algorithm for measuring airway lumen and wall areas from high-resolution computed tomographic data. *Am. J. Respir.*

*Crit. Care Med.* 2000; **161**: 574-80.

10. Kambara K, Shimizu K, Makita H, Hasegawa M, Nagai K, Konno S, Nishimura M. Effect of lung volume on airway luminal area assessed by computed tomography in chronic obstructive pulmonary disease. *PLoS One* 2014; **9**(2): e90040.

11. Hogg JC. Pathophysiology of airflow limitation in chronic obstructive pulmonary disease.

*Lancet* 2004; **364**: 709-21.

12. Hasegawa M, Nasuhara Y, Onodera Y, Makita H, Nagai K, Fuke S, Ito Y, Betsuyaku T,

Nishimura M. Airflow limitation and airway dimensions in chronic obstructive pulmonary disease. *Am. J. Respir. Crit. Care Med.* 2006; **173**: 1309–15.

13. Hasegawa M, Makita H, Nasuhara Y, Odajima N, Nagai K, Ito Y, Betsuyaku T, Nishimura M. Relationship between improved airflow limitation and changes in airway calibre induced by inhaled anticholinergic agents in COPD. *Thorax* 2009; **64**: 332-8.

14. Hoshino M, Ohtawa J. Effects of adding salmeterol/fluticasone propionate to tiotropium on airway dimensions in patients with chronic obstructive pulmonary disease. *Respirology* 2011; **16**: 95-101.

15. Hoshino M, Ohtawa J. Effects of tiotropium and salmeterol/fluticasone propionate on airway wall thickness in chronic obstructive pulmonary disease. *Respiration* 2013; **86**: 280-7.

16. Hoshino M, Ohtawa J. Computed tomography assessment of airway dimensions with

combined tiotropium and indacaterol therapy in COPD patients. *Respirology* 2014; **19**:

403-10.

17. Yasui H, Inui N, Furuhashi K, Nakamura Y, Uto T, Sato J, Yasuda K, Takehara Y, Suda T,

Chida K. Multidetector-row computed tomography assessment of adding

budesonide/formoterol to tiotropium in patients with chronic obstructive pulmonary disease.

*Pulm. Pharmacol. Ther.* 2013; **26**: 336-41.

18. Shimizu K, Hasegawa M, Makita, H, Nasuhara, Y, Konno S, Nishimura M Airflow limitation

and airway dimensions assessed per bronchial generation in older asthmatics. *Respir. Med.*

2010; **104**: 1809-16.

19. Shimizu K, Hasegawa M, Makita H, Nasuhara Y, Konno S, Nishimura M. Comparison of

airway remodelling assessed by computed tomography in asthma and COPD. *Respir. Med.*

2011; **105**: 1275-83.

20. Global Initiative for Chronic Obstructive Lung Disease (GOLD) (2003) Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease, updated 2006. Bethesda, MD: National Heart, Lung and Blood Institute, World Health Organization.
21. Nishimura M. Application of three-dimensional airway algorithms in a clinical study. *Proc. Am. Thorac. Soc.* 2008; **5**: 910-4.
22. Makita H, Nasuhara Y, Nagai K, Ito Y, Hasegawa M, Betsuyaku T, Onodera Y, Hizawa N, Nishimura M. Characterization of phenotypes based on severity of emphysema in chronic obstructive pulmonary disease. *Thorax* 2007; **62**: 932-7.
23. Machin D, Campbell MJ, Tan SB, Tan SH. Sample size tables for clinical studies. Third edition.

24. Coxson HO, Mayo J, Lam S, Santyr G, Parraga G, Sin DD. New and current clinical imaging techniques to study chronic obstructive pulmonary disease. *Am. J. Respir. Crit. Care Med.* 2009; **180**: 588-97.
25. Oguma T, Hirai T, Niima A, Matsumoto H, Muro S, Shigematsu M, Nishimura T, Kubo Y, Mishima M. Limitations of airway dimension measurement on images obtained using multi-detector row computed tomography. *PLoS One* 2013; 8(10): e76381.
26. Okazawa M, Müller N, McNamara AE, Child S, Verburgt L, Paré PD. Human airway narrowing measured using high resolution computed tomography. *Am. J. Respir. Crit. Care Med.* 1996; **154**: 1557-62.
27. King GG, Carroll JD, Müller NL, Whittall KP, Gao M, Nakano Y, Paré PD. Heterogeneity of narrowing in normal and asthmatic airways measured by HRCT. *Eur. Respir. J.* 2004; **24**:

211-8.

28. Matsuoka S, Kurihara Y, Yagihashi K, Hoshino M, Nakajima Y. Airway dimensions at inspiratory and expiratory multisection CT in chronic obstructive pulmonary disease: correlation with airflow limitation. *Radiology* 2008; **248**: 1042-9.
29. Bakker ME, Stolk J, Reiber JH, Stoel BC. Influence of inspiration level on bronchial lumen measurements with computed tomography. *Respir. Med.* 2012; **106**: 677-86.
30. Kundu S, Gu S, Leader JK, Tedrow JR, Sciurba FC, Gur D, Kaminski N, Pu J. Assessment of lung volume collapsibility in chronic obstructive lung disease patients using CT. *Eur. Radiol.* 2013; **23**: 1564-72.

## 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.

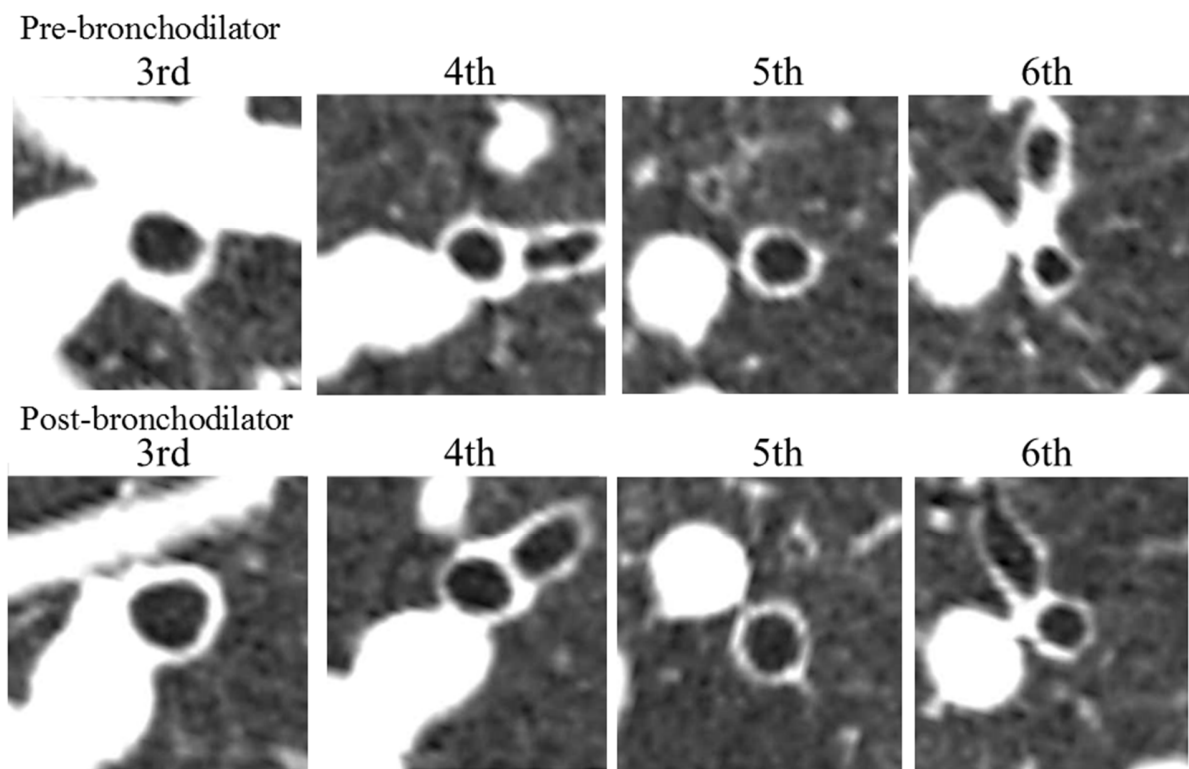


Figure 2. Relationship between % increase in airway inner luminal area and FEV1 from baseline according to airway generation after one week of salmeterol/fluticasone propionate inhalation in patients with COPD (n=23). Data of (A) 3rd, (B) 4th, (C) 5th, and (D) 6th generation bronchi are shown. Ai: airway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

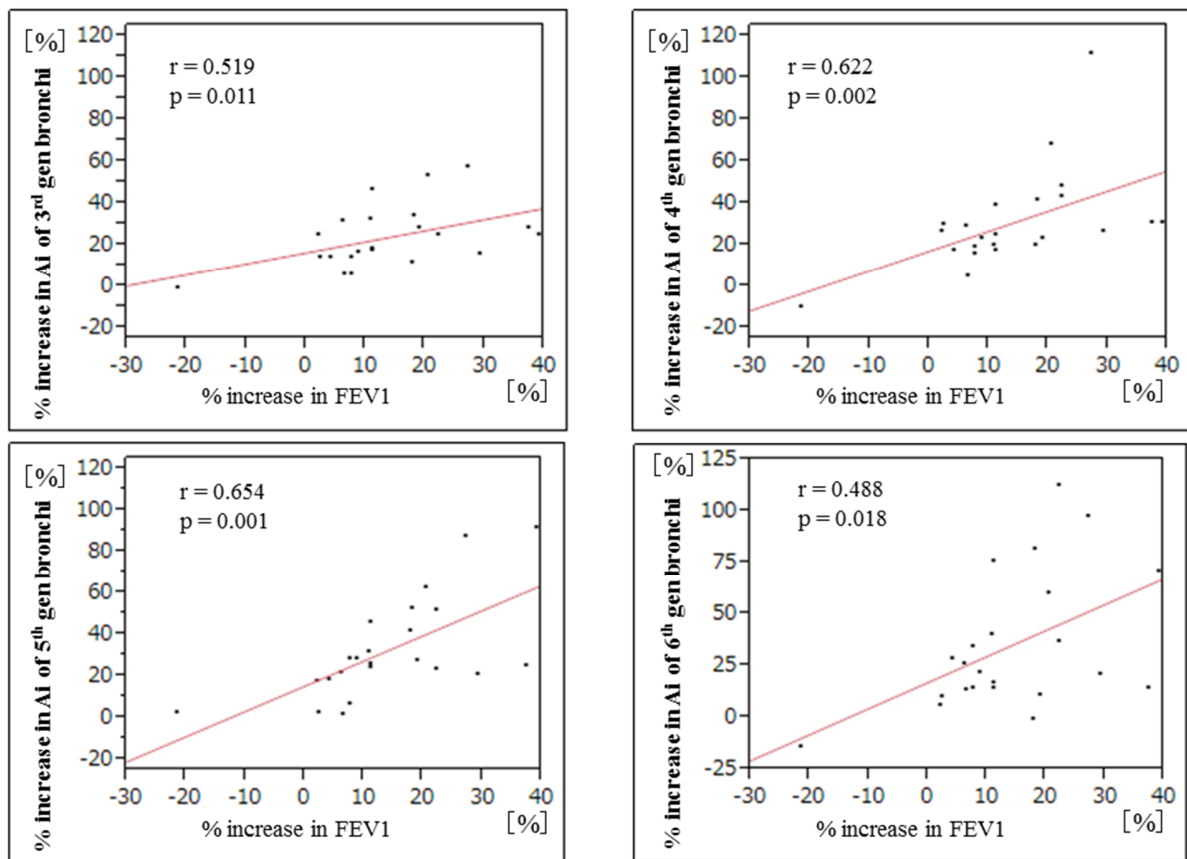


Figure 3. Comparisons between bronchodilation in a non-intervention group and subjects treated with salmeterol/fluticasone propionate combination. There was a significant difference in % increase in airway inner luminal area ( $\Delta Ai\%$ ) at the 32 points between a non-intervention group and the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

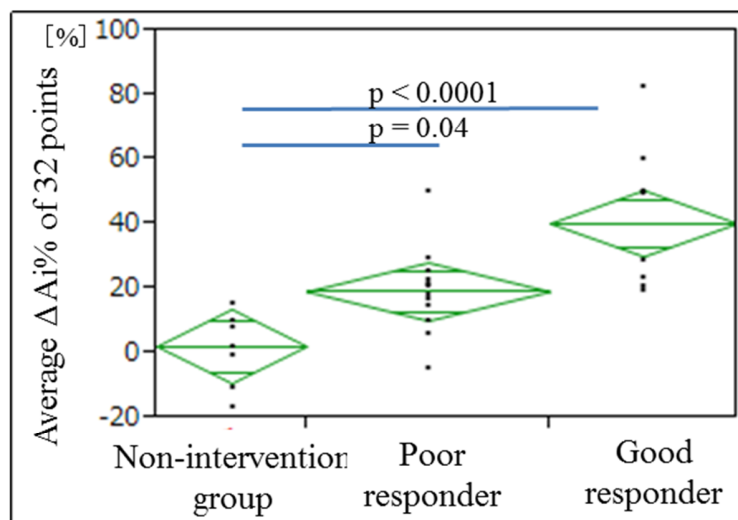


Figure 4. Inter-observer variability (IOV) in assessing % increase in airway inner luminal area

( $\Delta A_i\%$ ). 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.

