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Computed tomography (CT)-assessed bronchodilation induced by inhaled indacaterol and glycopyrronium/indacaterol in COPD

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## **Abstract (250/250 words)**

**Background:** Our previous studies suggested that the site of bronchodilation on CT might differ between inhaled  $\beta_2$  agonists and inhaled anticholinergics in COPD.

**Aim:** To assess and compare the bronchodilation effects of inhaled indacaterol and glycopyrronium/indacaterol by airway generation **in** large airways using CT.

**Methods:** CT scans at full inspiration and pulmonary function tests were done in 25 patients with moderate-severe COPD before and 4-5 weeks after daily inhalation of indacaterol and again another 4-5 weeks after inhalation of glycopyrronium/indacaterol. Airway inner luminal area ( $A_i$ ) at the 3<sup>rd</sup> (segmental) to 6<sup>th</sup> generation of 8 selected bronchi, a total of 32 sites, in the right lung was analyzed on 3 occasions. Our proprietary software enables us to select the same airways and the same measurement sites for comparison, with simultaneous confirmation using two screens on the computer.

**Results:** The overall increase of  $A_i$  ( $\Delta A_i$ , %) averaged at all 32 measurement sites induced by glycopyrronium/indacaterol had a significant correlation with FEV<sub>1</sub> improvement ( $r=0.7466$ ,  $p<0.0001$ ). Both  $\Delta A_i$ , % with indacaterol and  $\Delta A_i$ , % with additional glycopyrronium were significant at the 3<sup>rd</sup> to 6<sup>th</sup> generations. Remarkable increases in  $\Delta A_i$ , % were found at the 5<sup>th</sup> and 6<sup>th</sup> generations in several subjects with indacaterol or additional glycopyrronium. There were no significant site-differences in the bronchodilation pattern caused by indacaterol and by glycopyrronium/indacaterol at any of the 3<sup>rd</sup> to 6<sup>th</sup> generations.

**Conclusions:** Additional bronchodilation with glycopyrronium was demonstrated by CT at the 3<sup>rd</sup> to 6<sup>th</sup> generations, with no site-specific differences in bronchodilation between indacaterol and glycopyrronium/indacaterol.

This study was registered in the UMIN Clinical Trials Registry (UMIN-CTR) system (<http://www.umin.ac.jp/>. ID. UMIN000012043).

**Keywords:** bronchodilation,  $\beta_2$  agonists, anticholinergics, pulmonary function tests, three-dimensional computed tomography

Abbreviation:

Ai: airway inner luminal area

BMI: body mass index

COPD: chronic obstructive pulmonary disease

CT: computed tomography

DL<sub>co</sub>: diffusing capacity of the lung for carbon monoxide

FEV<sub>1</sub>: forced expiratory volume in 1 s

FRC: functional residual capacity

FVC: forced vital capacity

IC: inspiratory capacity

LABA: long acting  $\beta_2$  agonist

LAMA: long acting muscarinic antagonist

Pi10: the square root of the airway wall area at an internal perimeter of 10 mm

RV: residual volume

SABA: short acting  $\beta_2$  agonist

SAMA: short acting muscarinic antagonist

SFC: salmeterol/fluticasone combination inhaler

TLC: total lung capacity

$V_A$ : alveolar volume

VC: vital capacity

3D-CT: three-dimensional CT

## INTRODUCTION

The Global Initiative for Chronic Obstructive Lung Disease (GOLD) guideline provides a treatment algorithm for the management of chronic obstructive pulmonary disease (COPD), of which the mainstay is bronchodilators, long-acting  $\beta_2$  agonists (LABAs), and long acting muscarinic antagonists (LAMAs)<sup>1</sup>. The effects of bronchodilators are generally assessed by pulmonary function tests, in particular, by an increase of forced expiratory volume in 1 s (FEV<sub>1</sub>). However, COPD consists of emphysema and airway remodeling in different combinations<sup>2</sup>, and FEV<sub>1</sub> as an index of assessing bronchodilator effects may not fully address the structural heterogeneity of COPD<sup>3</sup>. A radiological approach offers a perspective on structural changes<sup>4-10</sup> and regional ventilation abnormalities<sup>11</sup>. We and others have shown that computed tomography (CT) has the potential to detect possible regional differences of bronchodilation in the airways<sup>12-16</sup>. There have been several previous studies using CT in which bronchodilation caused by salmeterol/fluticasone combination inhaler (SFC) (an inhaled corticosteroid/LABA), tiotropium (a LAMA), and indacaterol (a LABA), alone or in combination, was evaluated quantitatively<sup>17-20</sup>. However, they focused only on the right apical segmental bronchus. The square root of the airway wall area at an internal perimeter of 10 mm (Pi10)<sup>21,22</sup> has also been used and evaluated in bronchodilation<sup>23</sup>. Our proprietary software for three dimensional (3D)-airway analysis has the ability to assess Ai in the 3<sup>rd</sup> to 6<sup>th</sup> generations of airways, and its validation and reproducibility have been confirmed<sup>12</sup>. Our previous study showed that 3D-CT together with our proprietary software using dual screens could detect statistically significant bronchodilation at the identical points of the 3<sup>rd</sup> to 6<sup>th</sup> generations of airways by one week's inhalation of SFC when the average improvement in FEV<sub>1</sub> was as small as 180 ml<sup>12</sup>. Of note, in our other study using tiotropium<sup>13</sup>, more marked bronchodilation with tiotropium was seen in the more distal parts in the 3<sup>rd</sup> to 6<sup>th</sup> generations of airways, which was not found in the SFC study.

Therefore, the aims of this study were, first, to quantitatively assess the bronchodilation induced by inhaled indacaterol and a combination of glycopyrronium (a LAMA)/indacaterol by airway generation using our proprietary software and, second, to examine the possibility that site-specific patterns of bronchodilation in the 3<sup>rd</sup> to 6<sup>th</sup> generations of airways might differ between monotherapy and combination therapy.

## **METHODS**

### **Protocol of the study**

Patients with clinically stable COPD were recruited at Hokkaido University Hospital and Shiga University of Medical Science Hospital between November 2013 and April 2015. All patients with moderate to severe COPD following GOLD guideline<sup>1</sup> were ex-smokers aged 60 years or over at entry in this study. Patients with uncontrolled comorbidities and/or other respiratory diseases such as bronchial asthma, pulmonary fibrosis, pulmonary cancer, giant bullae and severe diffuse and/or local bronchiectasis were excluded. The Ethics Committee for Human Research at Hokkaido University Hospital and the Ethics Committee of the Shiga University of Medical Science Hospital approved the study, and all patients provided written, informed consent to participate. This study was registered in the UMIN Clinical Trials Registry (UMIN-CTR) system (<http://www.umin.ac.jp/>. ID. UMIN000012043).

The protocol is shown in Figure 1. The subjects did not use any long-acting bronchodilators other than short acting  $\beta_2$  agonists (SABAs) or short acting muscarinic antagonists (SAMAs) on demand for one week after the first visit. At the second visit (Visit 2), chest CT scans followed by pulmonary function tests were performed as baseline measurements when the subjects were stable. All participants started inhalation of indacaterol on the following day and used indacaterol (150 $\mu$ g) once a day during the next 4 to 5 weeks. At the third visit (Visit 3), the patients inhaled



indacaterol about 2-3 hours before they underwent the second chest CT scans and pulmonary function tests. From the following day, they started to inhale glycopyrronium (50µg) added to indacaterol and continued until the last visit (Visit 5), which was 4 to 5 weeks after the third visit. Patients took the inhalation of indacaterol and glycopyrronium by each Breezhaler® consecutively because Breezhaler® of a combination product of indacaterol and glycopyrronium was not available during the current study period in Japan. Patients visited the hospital for the prescriptions of indacaterol and glycopyrronium at Visit 4, two weeks after Visit 5. At the last visit (Visit 5), the third chest CT scans and pulmonary function tests were performed in the same way, about 2-3 hours after the last inhalation of glycopyrronium and indacaterol.

### **CT and airway, emphysema analysis**

A multidetector-row spiral CT scanner with a 64-detector array (Aquilion Multi, TSX-101A/6A; Toshiba Medical Systems, Tochigi, Japan) at Hokkaido University Hospital and one with a 320-detector array (Aquilion ONE(r); Toshiba Medical Systems, Tochigi, Japan) at Shiga University of Medical Science were 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 and standard reconstruction kernel FC03, FC52 kernel at Hokkaido University Hospital and 120 kVp, 210mA, 64 detector × 0.5 mm collimation, slice thickness 0.5 mm, 0.5 s/rotation, and helical pitch 58 and standard reconstruction kernel FC03, FC52 kernel at Shiga University of Medical Science Hospital.

Chest CT scans were performed with the patient in the supine position at full inspiration. Pixel size was 0.625 mm at Hokkaido University Hospital and 0.683 mm at Shiga University of Medical Science Hospital. Image data were transferred to the workstation and reconstructed into

3D chest images (Virtual place Fujin Raijin 310; AZE Ltd., Tokyo, Japan). Short axis images that were perpendicular to any bronchi in the lung were obtained. Manual corrections were added when the automatically obtained outline of airway walls was out of contour, resulting in a new circle, for the measurements of airway inner luminal area ( $A_i$ ).

Eight bronchi in the right lung were assessed: 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. The 3D images of the bronchi could be rotated to find any bifurcation, and one bronchus was chosen at each bifurcation unless either of the bronchi was obstructed. Then,  $A_i$  at the midpoint between bifurcations was measured from the 3<sup>rd</sup> (segmental bronchus) to the 6<sup>th</sup> generations of each airway, leading to a total of 32 measurement sites per subject. The use of two screens allowed simultaneous assessment of the same point of the same bronchi from any CT data that were taken on different occasions. Average  $A_i$  values of 32 points or average  $A_i$  values per generation were taken for analysis. The degree of bronchodilation was expressed as % improvement of  $A_i$  ( $\Delta A_i$ , %).

Total lung volume and volume of emphysema were measured. In short, whole lung containing airways (A) were extracted from the 3D image of the thorax including major vessels, heart and esophagus. Then the bronchial skeleton (B) was extracted from the whole lung. Total lung volume was defined as (A) – (B). % Low attenuation volume (%LAV) was defined as lung low attenuation volume, based on the threshold value of -950 HU, divided by total lung volume.

### **Pulmonary Function Tests**

Spirometry, the measurements of the diffusing capacity of the lung for carbon monoxide ( $DL_{co}$ ), based on the single-breath method and lung volume assessed by the helium closed-circuit method

(FUDAC-77(r) spirometer (Fukuda Denshi, Tokyo, Japan)) were performed. The rolling seal type of spirometer was used. Spirometric measurements included forced vital capacity (FVC) and FEV<sub>1</sub>, and lung volume measurements included total lung capacity (TLC), functional residual capacity (FRC) and residual volume (RV). The procedures and results of pulmonary function tests followed the pulmonary function test guidelines of the Japanese Respiratory Society Guidelines<sup>24</sup>, which are similar to those of the American Thoracic Society. DL<sub>co</sub>, DL<sub>co</sub> divided by alveolar volume (V<sub>A</sub>) was expressed as a percentage of predicted values according to the prediction equations of Burrows<sup>25</sup>. Lung volumes are expressed as percentages of predicted values according to the prediction equations of Nishida<sup>26</sup>.

## Statistics

Data are shown as means  $\pm$  standard error of the mean (SEM) except anthropometric and the data of pulmonary function tests and %LAV at baseline. Paired Student's *t*-tests were used to analyze differences in mean values between baseline and post-bronchodilator values of pulmonary function tests and Ai. T-test was used for the comparisons between good responders, who exhibited an increase in FEV<sub>1</sub> of >12% and 200 ml from baseline, and poor responders, who exhibited an increase in FEV<sub>1</sub> of <12% and 200 ml from baseline, in monotherapy or combination therapy. Relationships between quantitative variables were examined using Spearman's rank correlation test. The differences in  $\Delta$ Ai, % from the 3<sup>rd</sup> to 6<sup>th</sup> generations were ascertained using one-way analysis of variance (ANOVA). All statistical tests were 2-sided, and values of  $p < 0.05$  were considered significant. Data were analyzed using JMP 12.0 software (SAS Institute Inc., Cary, NC, USA).

## RESULTS

### Data of pulmonary function tests and bronchodilation assessed by CT

Twenty-eight subjects participated in the current study. One subject discontinued the inhalation of indacaterol because of a skin eruption, but in the opinion of a dermatologist, the skin eruption was not drug-related. Another subject dropped out because of dilated cardiomyopathy that was diagnosed for the first time during this study. Some of the CT acquisition parameters were different at the baseline measurement in one patient, so that patient was excluded from the analysis. The CT images of bronchi were too poor for assessment because of improper breath-holds at 4 to 5 weeks after inhalation of indacaterol in one patient, so CT data at baseline and at 4 to 5 weeks after inhalation of glycopyrronium/indacaterol were assessed. Eventually, the data of 25 subjects at baseline and after inhalation of glycopyrronium/indacaterol and the data of 24 subjects after inhalation of indacaterol were available for the analysis.

Table 1 shows the characteristics of the subjects and the results of pulmonary function tests at baseline, Visit 2.

Table 1. Characteristics of the subjects

| Sex        | (Male/Female) | 23 / 2        |            |      |
|------------|---------------|---------------|------------|------|
|            |               | Mean (Median) | Range      | SD   |
| Age        | yr            | 72.0 (71)     | 64-88      | 6.9  |
| Height     | cm            | 166.4 (169)   | 142-181    | 8.3  |
| Weight     | kg            | 59.7 (59)     | 40-75      | 8.9  |
| Smoking    | pack-years    | 64.4 (60)     | 33-110     | 20.8 |
| GOLD stage | ( II / III )  | 15/10         |            |      |
| VC         | L             | 3.3 (3.4)     | 1.6-4.8    | 0.7  |
| VC         | %predicted    | 94.8(93.1)    | 65.9-117.9 | 14.0 |
| IC         | L             | 2.1 (2.1)     | 0.8-3.9    | 0.6  |
| FVC        | L             | 3.2 (3.4)     | 1.6-4.6    | 0.7  |
| FVC        | %predicted    | 97 (96.6)     | 55.7-119.3 | 15.1 |

|                                  |            |               |            |      |
|----------------------------------|------------|---------------|------------|------|
| FEV <sub>1</sub> / FVC           | %          | 43.2 (42.1)   | 24.6-73.4  | 13.0 |
| FEV <sub>1</sub>                 | L          | 1.4 (1.4)     | 0.4-2.5    | 0.5  |
| FEV <sub>1</sub>                 | %predicted | 50.2 (49.6)   | 26.0-86.0  | 16.2 |
| TLC                              | %predicted | 110.6 (109.6) | 77.0-134.2 | 11.6 |
| FRC                              | %predicted | 120.2 (121.3) | 64.3-154.3 | 18.4 |
| RV                               | %predicted | 133.3 (133.0) | 56.5-183.5 | 26.6 |
| RV / TLC                         | %          | 45.2 (45.5)   | 29.1-61.4  | 7.4  |
| DL <sub>co</sub>                 | %predicted | 76.3 (73.6)   | 32.2-116.6 | 25.0 |
| DL <sub>co</sub> /V <sub>A</sub> | %predicted | 63.2 (58.7)   | 26.0-103.9 | 23.1 |
| %LAV                             | %          | 21.6 (19.7)   | 1.5-49.1   | 13.2 |

VC, vital capacity; IC, inspiratory capacity; FVC, forced vital capacity; FEV<sub>1</sub>, forced expiratory volume in 1 s; TLC, total lung capacity; FRC, forced residual volume; RV, residual volume; DL<sub>CO</sub>, diffusing capacity of the lung for carbon monoxide; VA, alveolar volume, %LAV, % lung attenuation volume = (lung attenuation volume \*100) / total lung volume.

Data are shown as mean ± standard deviation.

There are the results of pulmonary function tests at baseline (Visit 2) and at 4 to 5 weeks after inhalation of indacaterol (Visit 3) or glycopyrronium/indacaterol (Visit 5) in Table 2. Vital capacity (VC), VC, %predicted, inspiratory capacity (IC), FVC, FVC, %predicted, FEV<sub>1</sub>, FEV<sub>1</sub>, %predicted and FRC, %predicted increased significantly with indacaterol. Inhalation of glycopyrronium/indacaterol caused further increases in VC, VC, %predicted, FEV<sub>1</sub>, and FEV<sub>1</sub>, %predicted and a decrease in RV/TLC (Table 2). FEV<sub>1</sub>, %predicted, FRC, %predicted and RV, %predicted were significantly lower in the subjects who were good responders, who exhibited an increase in FEV<sub>1</sub> of >12% and 200 ml with indacaterol. (Table E1) There were no significant differences in any parameters of pulmonary function tests at visit 3 between good responders and poor responders, who exhibited an increase in FEV<sub>1</sub> of <12% or less than 200 ml with additional glycopyrronium.(Table E2) Good responders with combination therapy were

older than the poor responders but no significant differences were found in any parameters of pulmonary function tests at the baseline (Visit 2). (Table E3)

**Table 2. Results of pulmonary function tests at baseline and at 4 to 5 weeks after inhalation of indacaterol or glycopyrronium/indacaterol**

| Pulmonary function parameters | Baseline    | Indacaterol  | Glycopyrronium/<br>Indacaterol |
|-------------------------------|-------------|--------------|--------------------------------|
| VC, L                         | 3.3 ± 0.1   | 3.5 ± 0.2*   | 3.6 ± 0.1**†                   |
| VC, %predicted                | 94.8 ± 2.8  | 98.3 ± 3.1*  | 100.7 ± 2.7**†                 |
| IC, L                         | 2.1 ± 0.1   | 2.3 ± 0.1*   | 2.3 ± 0.1**                    |
| FVC, L                        | 3.2 ± 0.1   | 3.3 ± 0.2*   | 3.4 ± 0.2**                    |
| FVC, %predicted               | 97.7 ± 2.0  | 101.3 ± 3.5* | 103.9 ± 3.0**†                 |
| FEV <sub>1</sub> /FVC, %      | 43.2 ± 2.6  | 44.4 ± 2.3   | 45.8 ± 2.3*                    |
| FEV <sub>1</sub> , L          | 1.4 ± 0.1   | 1.5 ± 0.1*   | 1.6 ± 0.1**††                  |
| FEV <sub>1</sub> , %predicted | 50.2 ± 3.2  | 52.8 ± 2.8*  | 56.4 ± 3.0**††                 |
| TLC, %predicted               | 110.6 ± 2.3 | 110.8 ± 2.5  | 110.5 ± 2.3                    |
| FRC, %predicted               | 120.3 ± 3.7 | 116.1 ± 3.8* | 114.2 ± 3.7**                  |
| RV, %predicted                | 133.3 ± 5.3 | 128.6 ± 4.7  | 123.6 ± 4.5**                  |
| RV/TLC, %                     | 45.3 ± 1.5  | 43.9 ± 1.5   | 42.2 ± 1.3**†                  |
| DL <sub>CO</sub> , %predicted | 76.3 ± 5.0  | 74.5 ± 5.2   | 77.8 ± 5.4                     |
| %LAV, %                       | 21.0 ± 2.6  | 20.6 ± 2.6   | 20.3 ± 2.5                     |

VC, vital capacity; IC, inspiratory capacity; FVC, forced vital capacity; FEV<sub>1</sub>, forced expiratory volume in 1 s; TLC, total lung capacity; FRC, forced residual volume; RV, residual volume; DL<sub>CO</sub>, diffusing capacity of the lung for carbon monoxide; %LAV, % lung attenuation volume = (lung attenuation volume \*100) / total lung volume.

Data are shown as mean ± standard error of mean.

Paired *t*-test \**p*<0.05,\*\**p*<0.01 baseline vs. indacaterol, glycopirronium/indacaterol

†*p*<0.05,††*p*<0.01 indacaterol vs. glycopirronium/indacaterol

**Table 3. Computed tomography-assessed bronchodilation caused by indacaterol or glycopyrronium/indacaterol**

|                            |         | Indacaterol  | p(vs. Baseline) | Indacaterol/<br>Glycopyrronium | p(vs. Baseline) | p(vs. Indacaterol) |
|----------------------------|---------|--------------|-----------------|--------------------------------|-----------------|--------------------|
| 3 <sup>rd</sup> generation | Δ Ai, % | 9.33 ± 3.59  | p=0.0028        | 25.69 ± 4.27                   | p<0.0001        | p<0.0001           |
| 4 <sup>th</sup> generation | Δ Ai, % | 19.72 ± 3.96 | p<0.0001        | 40.56 ± 7.00                   | p<0.0001        | p=0.0004           |
| 5 <sup>th</sup> generation | Δ Ai, % | 22.37 ± 5.81 | p=0.0005        | 56.77 ± 8.23                   | p<0.0001        | p<0.0001           |
| 6 <sup>th</sup> generation | Δ Ai, % | 22.56 ± 6.10 | p=0.0019        | 48.26 ± 8.46                   | p<0.0001        | p=0.0001           |

Δ Ai, %:(postAi-preAi)\*100/preAi

The ΔAi, % with indacaterol alone was 9.33% ± 3.59%, 19.72% ± 3.96%, 22.37% ± 5.81%, and 22.56% ± 6.10% at the 3<sup>rd</sup> to 6<sup>th</sup> generations, respectively, and the ΔAi, % with glycopyrronium/indacaterol was 25.69% ± 4.27%, 40.56% ± 7.00%, 56.77% ± 8.23%, and 48.26% ± 8.46%, respectively. Indacaterol and glycopyrronium/indacaterol induced significant increases in ΔAi, % at the 3<sup>rd</sup> to 6<sup>th</sup> generations from baseline. Glycopyrronium/indacaterol added significant bronchodilation to that with indacaterol at the 3<sup>rd</sup> to 6<sup>th</sup> generations (Table 3).

Figure 2 (a) illustrates scatter plots of the relationship between CT-assessed bronchodilation caused by indacaterol and that by glycopyrronium/indacaterol. Each dot represents each individual, and one can see almost all dots over the identical line, which indicates further bronchodilation actually caused by the addition of glycopyrronium. There was high variability among the subjects in the two bronchodilator responses, and marked increases in Ai were found in several subjects at the 5<sup>th</sup> and 6<sup>th</sup> generations (Figure 2(b) ).

$\Delta Ai$ , % with indacaterol of the 3<sup>rd</sup> and 4<sup>th</sup> generations (3<sup>rd</sup> generation  $r=0.6067$ ,  $p=0.0017$ , 4<sup>th</sup> generation  $r=0.5419$ ,  $p=0.0062$ ) correlated significantly with  $\Delta FEV_1$ , % , while those correlations between  $\Delta Ai$ , % and  $\Delta FEV_1$ , % were not significant either at the 5<sup>th</sup> generation ( $r=0.3731$ ,  $p=0.0725$ ) or the 6<sup>th</sup> generation ( $r=0.3575$ ,  $p=0.0864$ ) (Figure 3). There were significant correlations of  $\Delta Ai$ , % with  $\Delta FEV_1$ , % at all of the 3<sup>rd</sup> to 6<sup>th</sup> generations with inhalation of glycopyrronium/indacaterol (3<sup>rd</sup> generation  $r=0.6585$ ,  $p=0.0003$ ; 4<sup>th</sup> generation  $r=0.7700$ ,  $p<0.0001$ ; 5<sup>th</sup> generation  $r=0.5368$ ,  $p=0.0063$ ; 6<sup>th</sup> generation  $r=0.5923$ ,  $p=0.0018$ )(Figure 4). In terms of other pulmonary function parameters,  $\Delta VC$ , %,  $\Delta FVC$ , %, and  $\Delta FEV_1$ , % showed significant correlations with  $\Delta Ai$ , % with indacaterol, while  $\Delta VC$ , %,  $\Delta FVC$ , %,  $\Delta FEV_1$ , %,  $\Delta FEV_1/FVC$ ,  $\Delta RV$ , % and  $\Delta RV/TLC$  showed significant correlations with  $\Delta Ai$ , % with glycopyrronium/indacaterol (Table 4).

**Table 4. Relationships between % increase in airway inner luminal area and % improvement in pulmonary function parameters**

| Pulmonary function parameters | indacaterol |         | glycopyrronium/indacaterol |         |
|-------------------------------|-------------|---------|----------------------------|---------|
|                               | r value     | p value | r value                    | p value |
| VC                            | 0.5129      | 0.0078  | 0.6878                     | <0.001  |
| IC                            | 0.3046      | 0.1387  | 0.3146                     | 0.1256  |
| FVC                           | 0.6746      | 0.0002  | 0.6008                     | 0.0015  |
| FEV1/FVC                      | 0.1385      | 0.5091  | 0.4377                     | 0.0287  |
| FEV1                          | 0.4709      | 0.0175  | 0.7466                     | <0.001  |
| TLC                           | 0.0515      | 0.8067  | 0.1638                     | 0.4339  |
| FRC                           | -0.2900     | 0.1597  | -0.2238                    | 0.2821  |
| RV                            | -0.2208     | 0.2889  | -0.3985                    | 0.0485  |
| RV/TLC                        | -0.3777     | 0.0627  | -0.5315                    | 0.0062  |



|                  |        |        |         |        |
|------------------|--------|--------|---------|--------|
| DL <sub>CO</sub> | 0.0246 | 0.9070 | -0.2823 | 0.1715 |
|------------------|--------|--------|---------|--------|

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r, correlation coefficient †p< 0.05. VC, vital capacity; IC, inspiratory capacity; FVC, forced vital capacity; FEV<sub>1</sub>, forced expiratory volume in 1 s; DL<sub>CO</sub>, capacity of the lung for carbon monoxide; TLC, total lung capacity; FRC, functional residual capacity; RV, residual volume.

To further examine the possible site-specific differences in bronchodilation patterns induced by monotherapy and combination therapy, only subjects who were good responders, who exhibited an increase in FEV<sub>1</sub> of >12% and 200 ml from baseline, were selected. There were 5 indacaterol and 12 glycopyrronium/indacaterol responders. There were no significant site-related differences in the magnitudes of bronchodilation caused by indacaterol and by glycopyrronium/indacaterol at any of the 3<sup>rd</sup> to 6<sup>th</sup> generations (Figure 5).

## DISCUSSION

Our proprietary software for 3D-CT analysis has an advantage in that one can detect bronchodilation at the 3<sup>rd</sup> to 6<sup>th</sup> generations of the airways and compare the bronchodilation before and after inhalation of a bronchodilator at the same sites. Thus, we anticipated in this study that we might detect different bronchodilation patterns by generation when induced by indacaterol alone and when induced by a combination of glycopyrronium and indacaterol. This concept originated from our two previous studies; in one study using tiotropium, we demonstrated that more marked bronchodilation was seen in the more distal parts in the 3<sup>rd</sup> to 6<sup>th</sup> generations of the airways, but in another study using SFC, we could not find such generation-specific differences in bronchodilation. Thus, we hypothesized that long-acting antimuscarinic agents (LAMAs) and long-acting  $\beta$  2 agonist (LABAs) might work at different generations of the airways.

However, in the present study, there were no site-specific differences in bronchodilation between monotherapy with indacaterol and a combination of glycopyrronium/indacaterol. The reasons why there were no site-specific differences in bronchodilation might be several. First, in this study, monotherapy and combination therapy were compared, and, thus, there was no direct comparison between LABA and LAMA. Such a direct comparison between two classes of bronchodilators (LABA vs. LAMA) might provide a better chance of finding any differences in site-specific bronchodilation patterns. Second, the small number of the subjects and the smaller magnitude of improvement in FEV<sub>1</sub> than expected might be reasons for no significant difference between monotherapy and combination therapy. However, this is very unlikely when one takes a look at the data of good responders either to monotherapy or to combination therapy. The third possible and most plausible explanation for negative findings in this study would be that what had been found in the previous two studies indicated the difference between the two drugs (tiotropium vs. SFC) but not a difference between two classes of bronchodilators. Such differences between the two drugs might be explained not only by pharmacological characteristics of the bronchodilators themselves but also by the particle size of the inhaled drugs and/or the characteristics of the delivery device of each drug. The disease severity and inspiratory flow, which may vary among the subjects, might also affect drugs' disaggregation from carrier and consecutive distributions in the lungs.

It is of note that our methods using 3D-CT could demonstrate the bronchodilation induced by indacaterol alone and also the additional bronchodilation induced by a combination of glycopyrronium and indacaterol. As mentioned above, the magnitude of the increase in FEV<sub>1</sub> was much smaller than had been expected before the study (90 ml with monotherapy and 180 ml with combination therapy). Despite such a small increase in FEV<sub>1</sub>, it was possible to prove that anatomical bronchodilation actually occurred at full inspiration. Another remarkable finding is

that  $\Delta FEV_1$  is the parameter with the best correlation with the magnitude of bronchodilation evaluated by CT among the many pulmonary function parameters. This agrees perfectly with our previous studies<sup>12,13</sup>. The relatively high degree of correlation between  $\Delta FEV_1$  and bronchodilation assessed by CT is of particular clinical importance because it should at least partly reflect that an increase of FEV<sub>1</sub> by spirometry is an actual indicator of bronchodilation that evidently occurs in the proximal airways.

Another point that should be emphasized in this study is the individual dots presented in Figure 2(a), where individual bronchodilator effects with addition of glycopyrronium are clearly shown. Almost all of the dots are placed over the identical line at any of the 3<sup>rd</sup> to 6<sup>th</sup> generations of the airways, indicating that additional bronchodilation is evident by 3D-CT analysis despite an increase in FEV<sub>1</sub> as small as 90 ml with glycopyrronium. One can see remarkable bronchodilation at the 5<sup>th</sup> and 6<sup>th</sup> generations in some subjects with addition of glycopyrronium who did not necessarily respond to indacaterol alone. This phenomenon reminds us of our previous study where we found that some patients with COPD are preferential responders to inhaled beta-agonists, while others are preferential responders to inhaled anticholinergics.<sup>27</sup> Furthermore, the pattern of bronchodilation in these subjects might be considered to be similar to those subjects we encountered in another 3D-CT airway analysis study<sup>13</sup>. In that study, some tiotropium responders displayed marked improvement of bronchodilation at the 5<sup>th</sup> and 6<sup>th</sup> generations of the airways.

Possible parallel heterogeneity in bronchodilation even in the same generation of airways in the lungs and/or airway generation-based longitudinal heterogeneity has been a vital issue of interest when considering the pharmacological effects of bronchodilators. Lung imaging might have greater ability to solve this issue than pulmonary function tests, which provide only overall information about the consequences of bronchodilation. Recently, a novel functional imaging

method<sup>28-32</sup> has been reported in a pilot study to assess the bronchodilator effects on the geometry and computational fluid dynamics-based resistance of the central and distal airways. Such a novel approach together with our proprietary software might provide new perspectives in the treatment strategy with bronchodilators, as well as the development of new drugs. Detailed analysis of bronchodilation at various regions of the lung could provide information beyond the assessment of pulmonary function alone.

There are, of course, several limitations in our current methods of 3D airway analysis. Although we can select the same sites for the measurement of bronchodilation before and after, we assess only one bronchial tree of the 3<sup>rd</sup> to 6<sup>th</sup> generations per segment, which could result in a selection bias. Our current method also precludes an investigation of heterogeneity in bronchodilation, which could be caused by different lung pathologies of bronchial walls and/or heterogeneous distribution of inhaled bronchodilators due partly to improper inhalation. We did not use body plethysmography in this study so that we could not examine the airway resistance caused by bronchodilators. It remains possible that any parameters derived from body plethysmography could have better correlations with CT-assessed bronchodilation in comparison with spirometric data or lung volumes measured by the helium closed-circuit method. Finally, with use of any imaging modalities, we cannot get to the small airways in vivo, which should be the most vital and responsible site causing airflow limitation in COPD.

In conclusion, using our proprietary 3D-CT airway analysis, significantly more bronchodilation was demonstrated with a combination of indacaterol/glycopyrronium compared with indacaterol monotherapy in the 3<sup>rd</sup> to 6<sup>th</sup> generations of the airways. However, there were no generation-specific differences in bronchodilation patterns between monotherapy and combination therapy.

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## FIGURE LEGENDS

Figure 1.

At the second visit (Visit 2), chest CT scans followed by pulmonary function tests were performed as baseline measurements. All participants started inhalation of indacaterol on the following day and used indacaterol (150µg) once a day during the next 4 to 5 weeks.

At the third visit (Visit 3), the patients inhaled indacaterol about 2-3 hours before they underwent the second chest CT scans and pulmonary function tests. From the following day, they started to inhale glycopyrronium (50µg) added to indacaterol and continued until the last visit, which was 4 to 5 weeks after Visit 3. Patients visited the hospital for the prescriptions of indacaterol and glycopyrronium at Visit 4, two weeks after Visit 5. At the last visit (Visit 5), the third chest CT scans and pulmonary function tests were performed in the same way, about 2-3 hours after the last inhalation of glycopyrronium and indacaterol.

Figure 2

(a) Relationships between bronchodilation induced by indacaterol and that induced by glycopyrronium/indacaterol

(b) Relationships between bronchodilation induced by indacaterol and that induced by additional glycopyrronium

Most of the scatter plots are over the identity line, which may show further bronchodilation caused by glycopyrronium (Figure 1(a)), and an evident increase in Ai is found in several subjects at the 5<sup>th</sup> and 6<sup>th</sup> generations ( Figure1(b) ).

Figure 3. Correlations between bronchodilation induced by indacaterol and improvement of FEV<sub>1</sub> at the 3<sup>rd</sup> to 6<sup>th</sup> generations

There are significant correlations of  $\Delta A_i$ , % with  $\Delta FEV_1$ , % at the 3<sup>rd</sup> and 4<sup>th</sup> generations with indacaterol.

Figure 4. Correlations between bronchodilation induced by glycopyrronium/indacaterol and improvement of FEV<sub>1</sub> at the 3<sup>rd</sup> to 6<sup>th</sup> generations

There are significant correlations of  $\Delta A_i$ , % with  $\Delta FEV_1$ , % from the 3<sup>rd</sup> to 6<sup>th</sup> generations with glycopyrronium/indacaterol.

Figure 5. Bronchodilation per generation caused by indacaterol and glycopyrronium/indacaterol in responders

Responders are defined as those who exhibited an increase in FEV<sub>1</sub> of >12% and 200 ml from the baseline. Site-specific differences in bronchodilation are not detected by CT between

indacaterol and glycopyrronium/indacaterol.

|                                                            | Visit 1               |                   | Visit 2               |                                 | Visit 3               | Visit 4                                    | Visit 5               |
|------------------------------------------------------------|-----------------------|-------------------|-----------------------|---------------------------------|-----------------------|--------------------------------------------|-----------------------|
|                                                            |                       | Wash out<br>1week |                       | Inhalation of<br>bronchodilator |                       | Inhalation of<br>bronchodilators           |                       |
| Informed consent                                           | <input type="radio"/> |                   |                       |                                 |                       |                                            |                       |
| Inhalation of indacaterol or<br>indacaterol/glycopyrronium |                       |                   |                       | Indacaterol<br>4-5weeks         |                       | Indacaterol/<br>glycopyrronium<br>4-5weeks |                       |
| Pulmonary function tests                                   |                       |                   | <input type="radio"/> |                                 | <input type="radio"/> |                                            | <input type="radio"/> |
| CT                                                         |                       |                   | <input type="radio"/> |                                 | <input type="radio"/> |                                            | <input type="radio"/> |

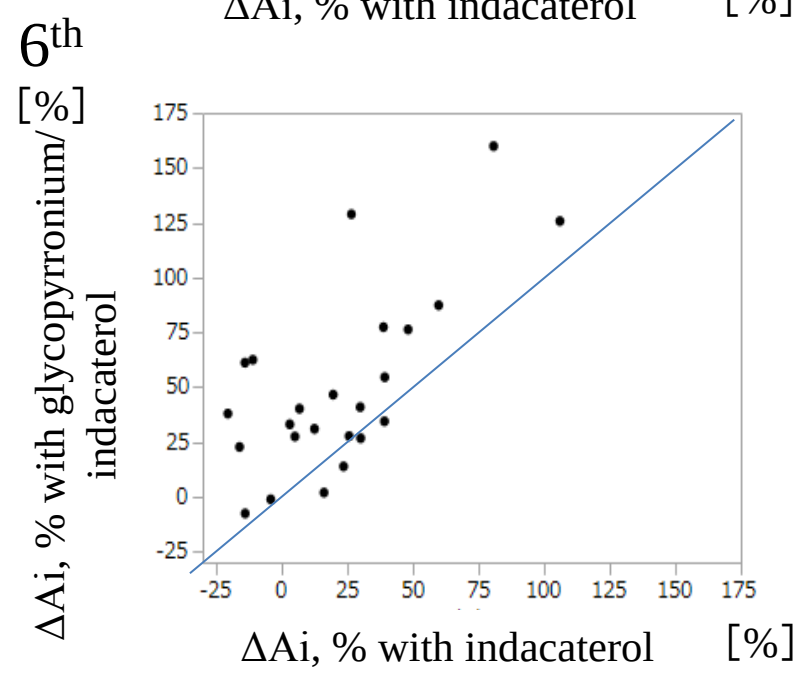
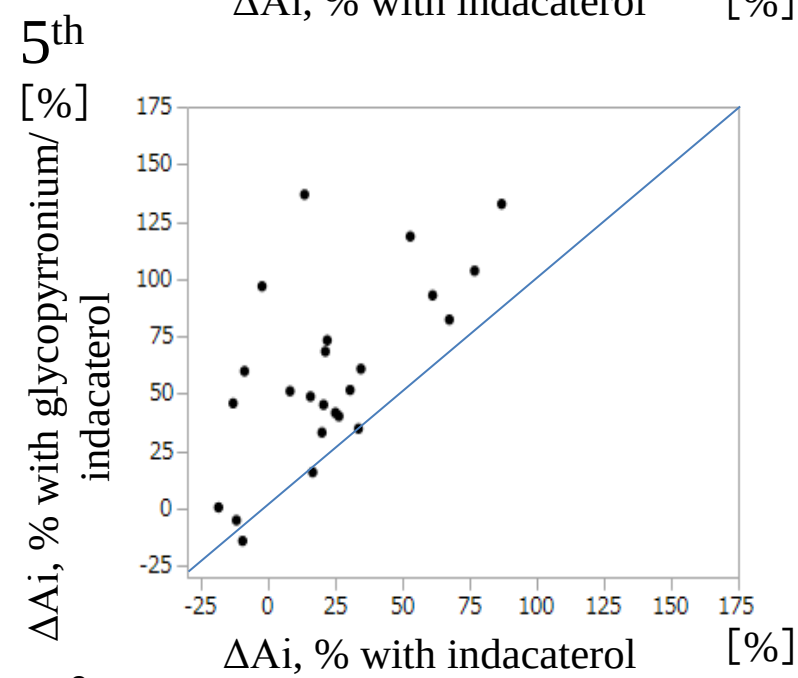
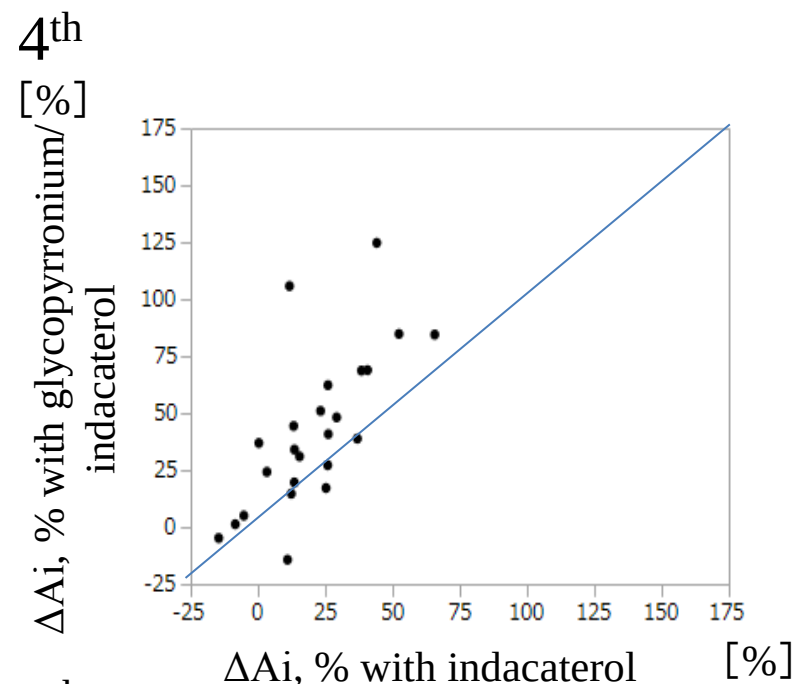
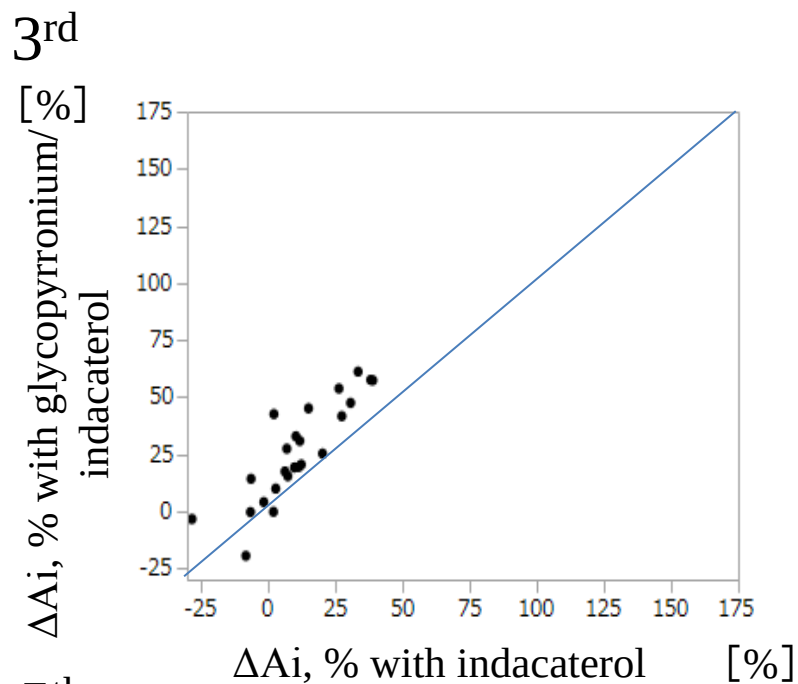


Figure 2a



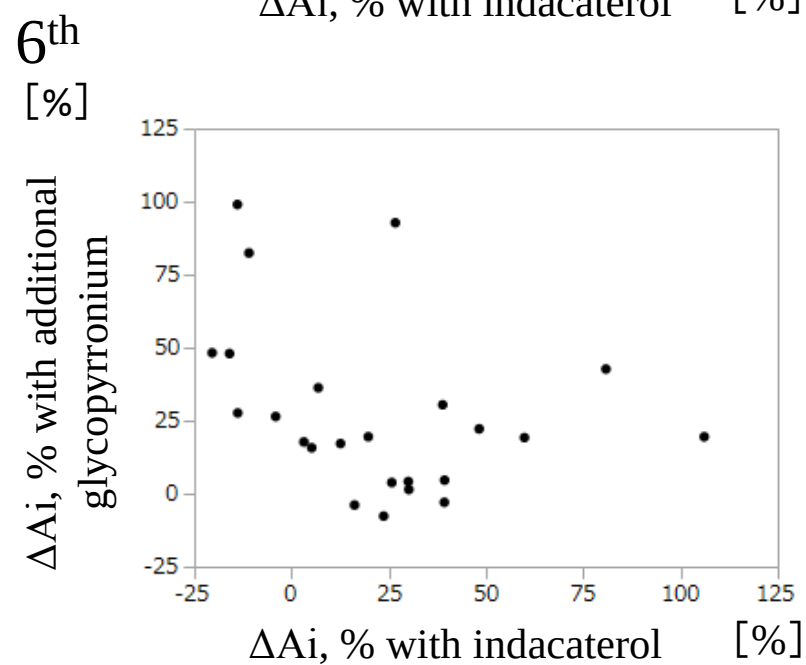
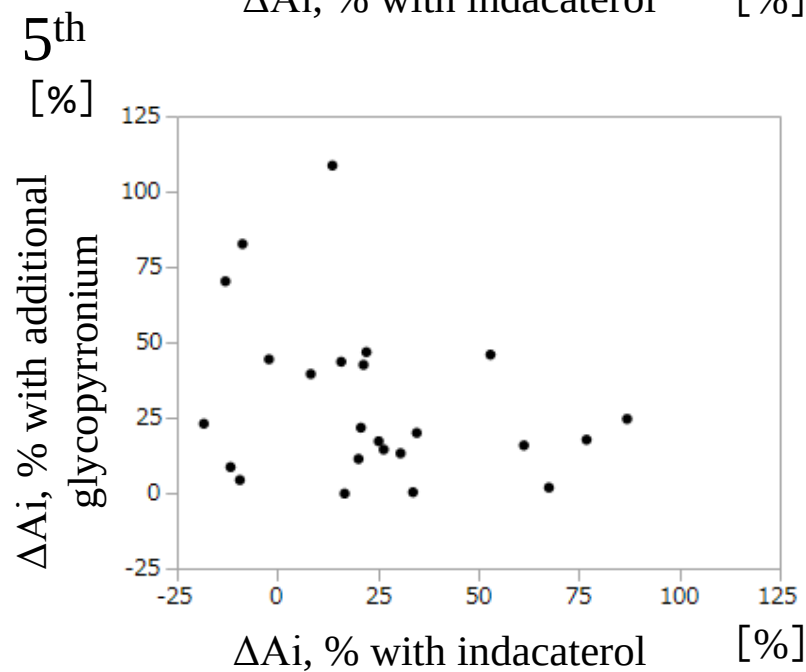
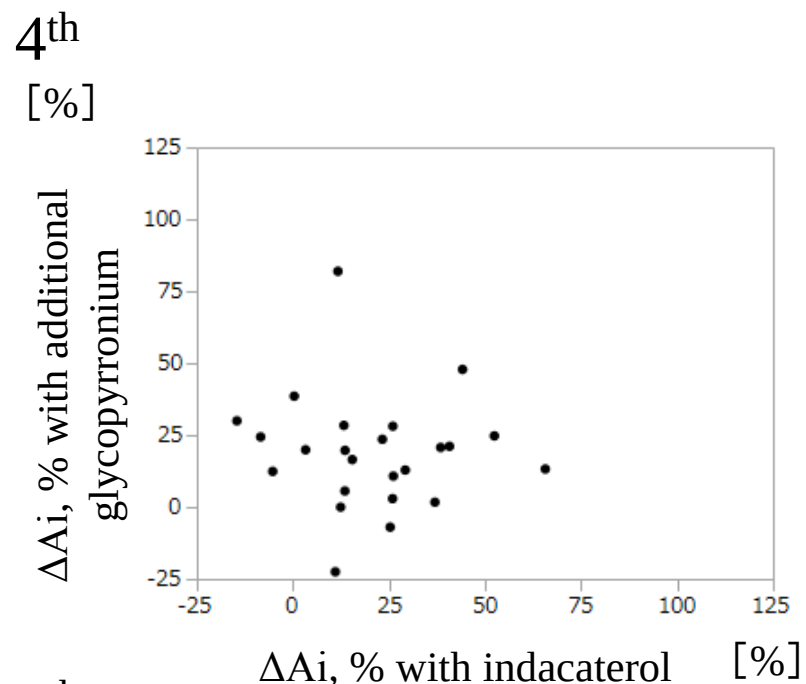
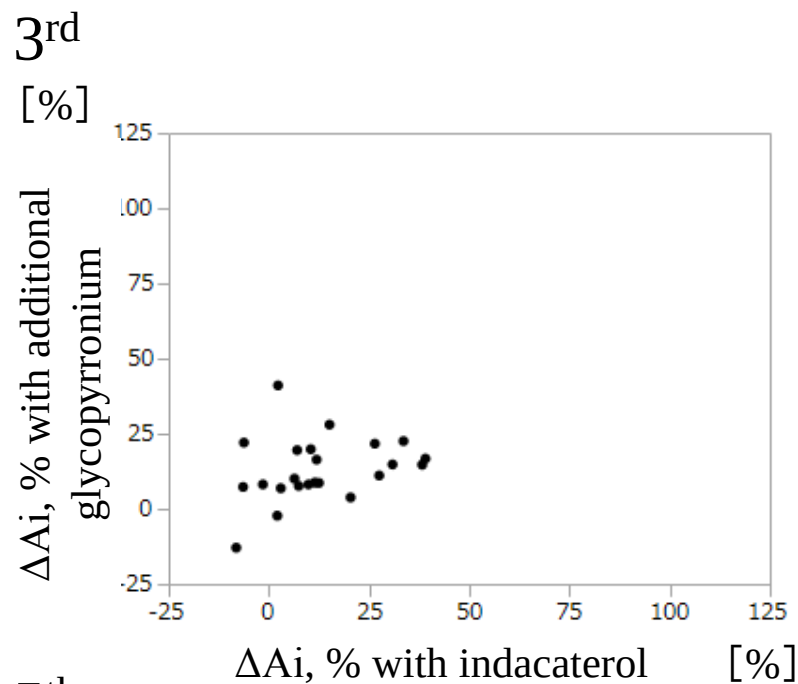


Figure 2b

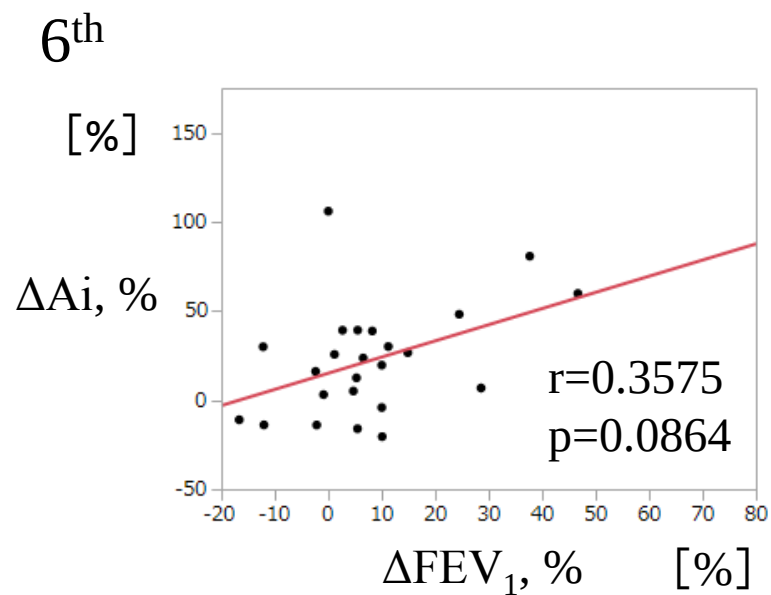
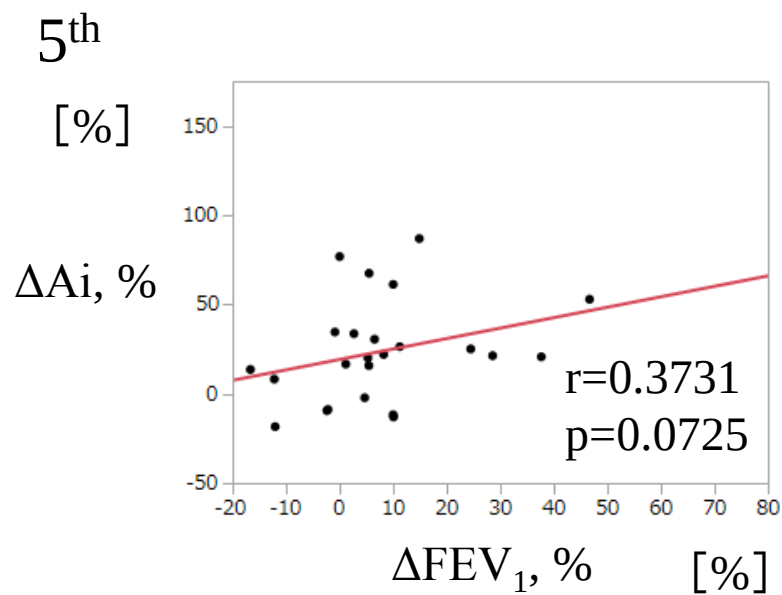
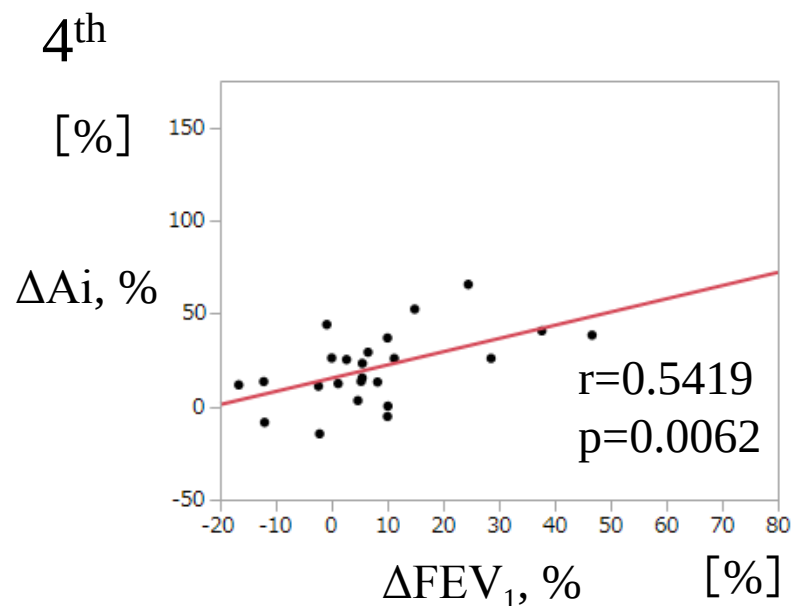
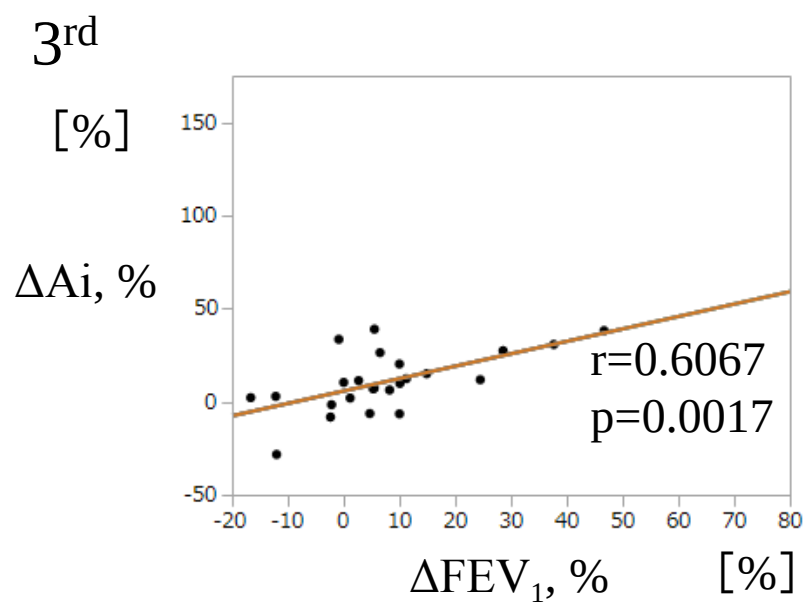


Figure 3

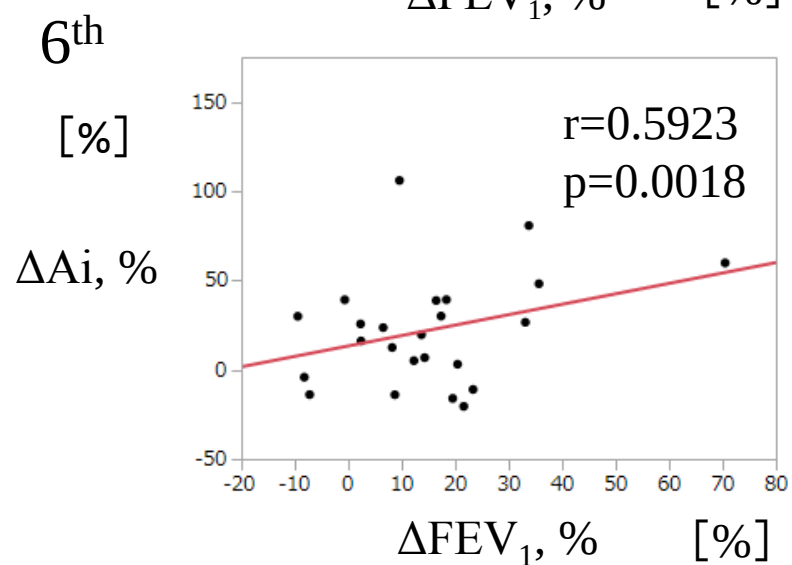
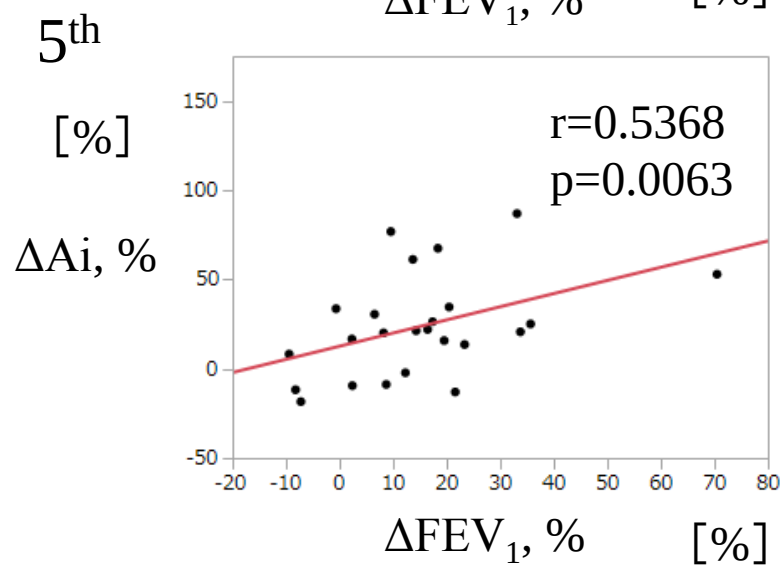
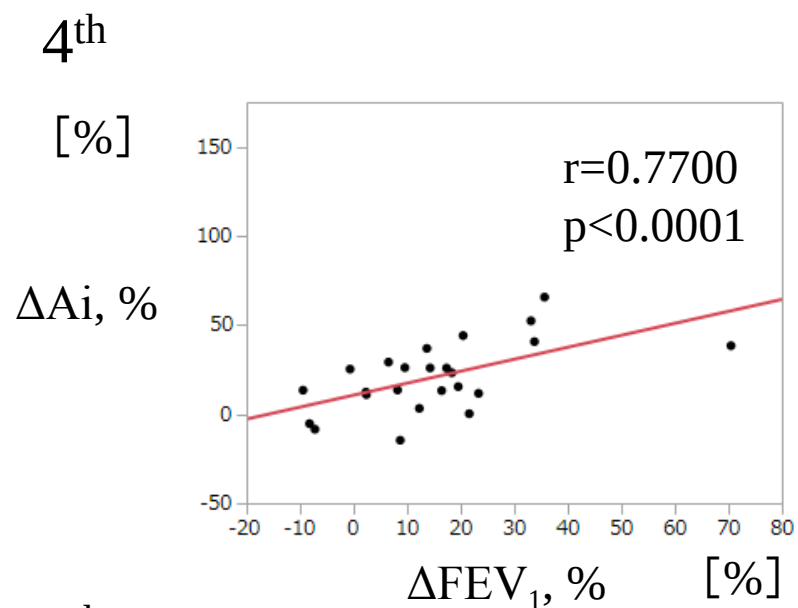
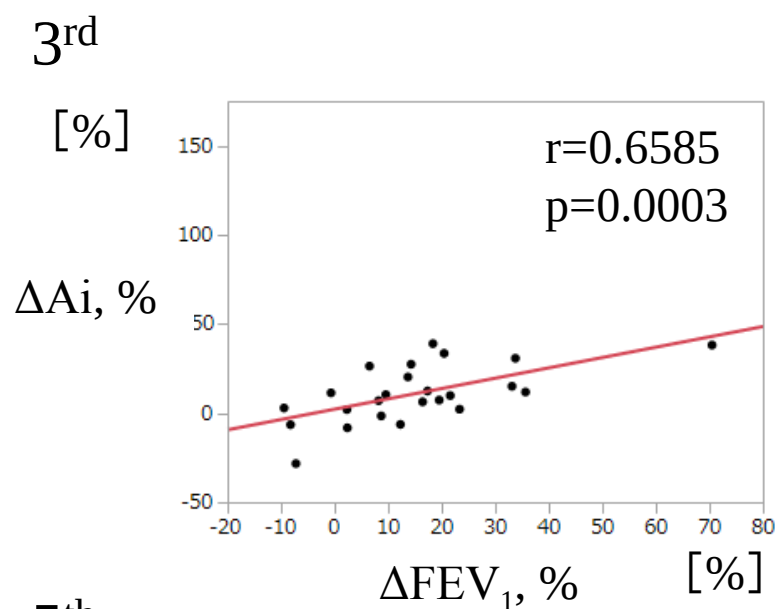


Figure 4

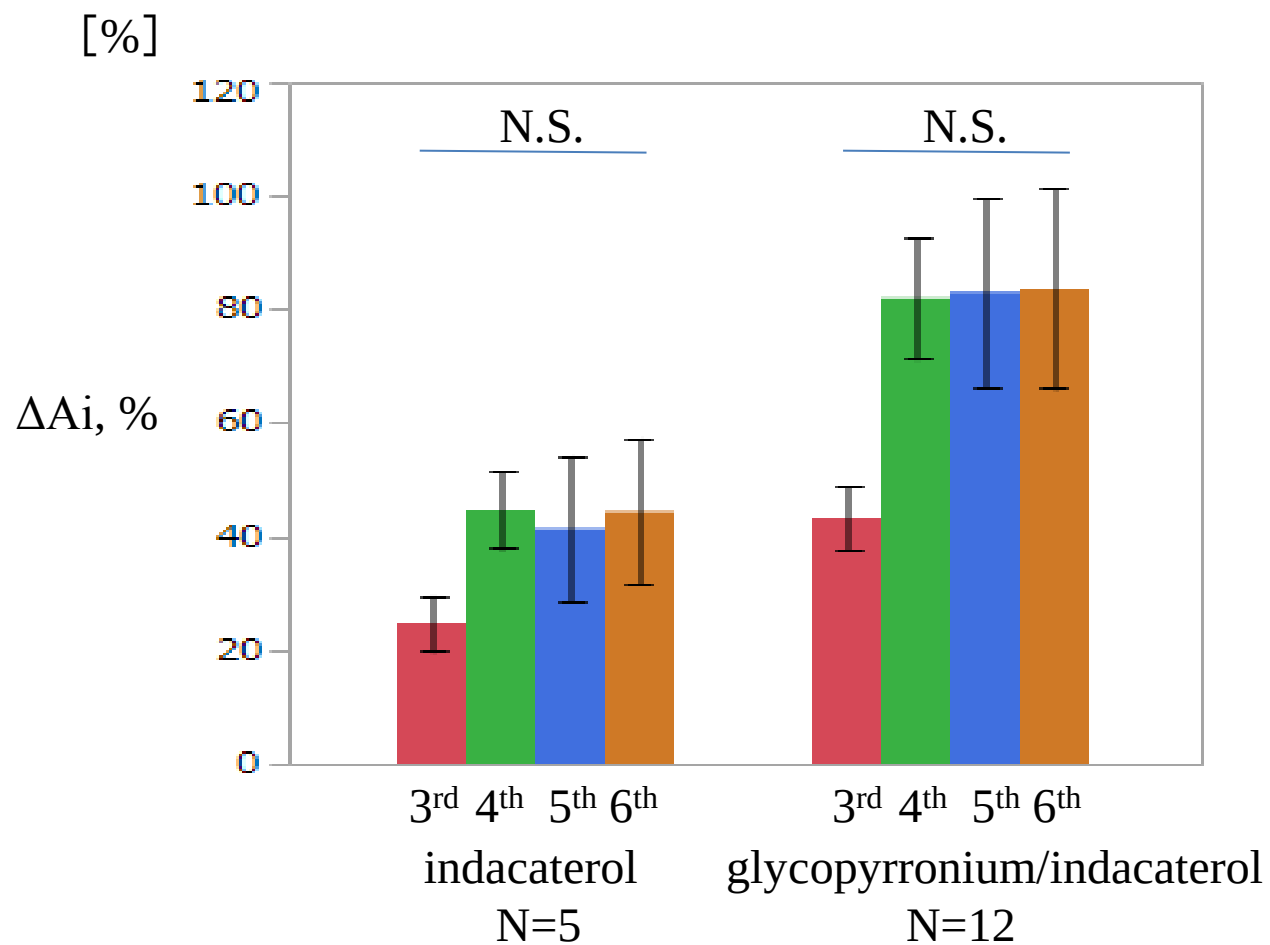


Figure 5