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Characterization of phenotypes based on severity of emphysema in chronic obstructive pulmonary disease

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See end of article for all contributors to the Hokkaido COPD cohort study.
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ABSTRACT

Background: Airflow limitation in chronic obstructive pulmonary disease (COPD) is caused by a mixture of small airway disease and emphysema, the relative contributions of which may vary among patients. Phenotypes of COPD classified purely based on severity of emphysema are not well defined and may be different from classical phenotypes, pink puffers and blue bloaters.

Methods: To characterize clinical phenotypes based on severity of emphysema, we recruited 274 subjects with COPD, excluding physician-diagnosed bronchial asthma. For all subjects, we conducted a detailed interview of disease history and symptoms, quality of life (QOL) measurement, blood sampling, pulmonary function tests before and after inhalation of salbutamol (0.4 mg), and high-resolution computed tomography.

Results: Severity of emphysema visually evaluated varied widely even among subjects with the same stage of disease. No significant differences were noted among three groups of subjects classified by severity of emphysema in any of age, smoking history, chronic bronchitis symptoms, blood eosinophil count, serum IgE level or bronchodilator response. However, subjects with severe emphysema displayed significantly lower body-mass index (BMI) and poorer QOL scores, evaluated using St. George's respiratory questionnaire (SGRQ), compared to subjects with no/mild emphysema (BMI: 21.2±0.5 vs. 23.5±0.3, respectively; SGRQ total score: 40±3 vs. 28±2, respectively; $p<0.001$ for both). These characteristics held true even if subjects with the same degree of airflow limitation were chosen.

Conclusions: Severity of emphysema is widely varied even in the same stage of COPD, and chronic bronchitis symptoms are equally distributed irrespective of emphysema severity. The phenotype with emphysema in predominance has lower BMI and poorer health-related QOL.

Chronic obstructive pulmonary disease (COPD) is the fourth leading cause of death worldwide,[1] and morbidity and mortality has been increasing in Japan,[2] as in many other Western countries.[3] COPD is described as a disease state characterized by airflow limitation that is not fully reversible according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines and the American Thoracic Society / European Respiratory Society consensus guidelines.[1,4,5] This airflow limitation is progressive and caused by a mixture of abnormal inflammatory responses in small airways and parenchymal destruction of the lungs, the relative contributions of which vary from person to person.[6]

Historically, typical phenotypes of COPD used to be known as “pink puffer” and “blue bloater”,[7,8] or A, B and X types.[9] This is because COPD had been recognized as a disease that is a mixture of chronic bronchitis and emphysema, with predominantly bronchitis and predominantly emphysema as the two extreme phenotypes.[10] However, a number of studies over the last three decades have revealed small airways as the most important site causing airflow limitation in COPD,[11-14] and parenchymal destruction (emphysema) is certainly a contributing factor to a variable extent through the loss of elastic recoil pressure.[1,4,5] Hogg et al. recently re-demonstrated how important inflammatory changes in small airways are as a determinant of progression and severity in COPD.[15] The narrowing of small airways caused by inflammation and scarring and the blocking of small airway lumens with mucous secretions are thought to represent the primary pathology of airflow limitation.[6]

In this study, we are thus interested in the clinical phenotype purely based on severity of emphysema, which could be quantitatively evaluated using high-resolution computed tomography (HRCT). If severity of emphysema varies despite the same degree of airflow limitation, we could compare subjects with COPD where relative contributions of small airway disease and emphysema varied. For instance, subjects displaying little evidence of emphysema despite severe airflow limitation could be considered as showing a phenotype with small airway disease in predominance, but not necessarily that of chronic bronchitis. This is the first report from the Hokkaido COPD cohort study, which was primarily designed to evaluate the natural history and prognosis of COPD, as classified by severity of emphysema.

METHODS

Subjects

A total of 307 subjects with physician-diagnosed COPD were recruited at Hokkaido University Hospital and 9 affiliated hospitals from May 2003 through April 2005. All study protocols were approved by the ethics committees of all hospitals, and all subjects provided written informed consent. All were either current or former smokers with a smoking history of at least 10 pack-years. We carefully excluded the subjects when respiratory physicians diagnosed them as having bronchial asthma or bronchiectasis at the entry of this study. Subjects were also excluded when they had active tuberculosis, any history of lung cancer, any history of lung resection, and any history of cystic fibrosis, allergic alveolitis or pulmonary fibrosis. Those who would compromise 5-year follow-up or accurate assessment of pulmonary function or who were receiving long-term supplemental oxygen therapy for >12 h/day were also excluded. To avoid interference with bronchodilator reversibility testing, those who had been taking non-selective beta-blockers for treatment of hypertension were excluded. On the

first visit, diagnoses were reconfirmed by pulmonologists and established based on the spirometric criteria of the GOLD guidelines.[1] While 33 subjects were excluded from this study because the post-bronchodilator ratio of forced expiratory volume in 1 s (FEV_1): forced vital capacity (FVC) was ≥ 0.7, these subjects were encouraged to join the subsequent follow-up study.

Chronic bronchitis symptoms

Well-trained clinical research coordinators (CRCs) elicited disease history, smoking history and other information about all treatments. Chronic cough and sputum expectoration were considered to be present when they occurred on most days for ≥ 3 months/year and for ≥ 2 consecutive years.[16,17] To avoid a bias by patient reports about the presence of chronic sputum symptoms, the amount of sputum should be > 10 ml/day for the definition described above and this was confirmed by clinical research coordinators for all subjects.

Pulmonary Function Tests

Rolling seal spirometers, Chestac (Chest M.I., Inc., Tokyo) or Fudac (Fukuda Denshi CO., Ltd., Tokyo), were used for the measurement of spirometry and diffusing capacity of carbon monoxide (D_{LCO}) at all hospitals. Further details of procedures are provided in the online supplement. Predicted values of spirometric measurements were derived from the guidelines for pulmonary function tests issued by Japanese Respiratory Society (JRS).[18] DL_{CO} was measured by the single breath method. Results were corrected by alveolar volume (V_A) and hemoglobin concentration. DL_{CO} / V_A were compared to the predicted normal values.[18]

We evaluated the reversibility of airflow limitation by measuring spirometry before and 30 min after inhalation of salbutamol (0.4 mg). Bronchodilator response (BDR) was expressed in three ways, as an absolute change in FEV_1, as a percentage change from baseline FEV_1, and as a percentage change from predicted FEV_1. Reversibility of airflow limitation was considered to be significant if an increase in FEV_1 was both >200 ml and 12% above pre-bronchodilator FEV_1 according to GOLD guidelines.[1]

HRCT

Chest HRCT scans were performed under the supine position, holding their breaths at full inspiration. CT scanners used in 9 hospitals are described in the online supplement. Technical parameters were as follows; 1 mm collimation, 120-140 kV, 75-350 mA, 0.75-1 s for scanning time, and 1-2 mm thickness. HRCT images were selected at 3 levels, including the aortic arch, carina, and 1-2 cm above the highest hemi-diaphragm. Image interpretations were performed under -600 to -900 Hounsfield units (HU) window levels and 800 to 1500 HU window widths based on the best condition for detecting emphysema at each hospital.

The severity of emphysema was visually assessed by three independent pulmonologists according to the modified Goddard scoring system.[19] We analyzed 6 images in 3 slices in the lungs and an average score of all images was considered as a representative value of severity of emphysema in each person. Each image was classified as normal (score 0), up to 5 percent affected (score 0.5), up to 25 percent affected (score1), up to 50 percent affected (score 2), up to 75 percent affected (score 3), more than 75 percent affected (score 4), giving a minimum score of 0 and maximum of 24. When the three independent pulmonologists split in their evaluation, we took only the score assessed by the majority.

Three dimensional CT analyses were performed only in Hokkaido University hospital (n=137) to confirm the accuracy and the reliability of visual assessment.

How we did computerized assessment of emphysema for the whole lung is described in detail in the online supplement.

The St. George's Respiratory Questionnaire (SGRQ)

SGRQ was used for assessment of health-related QOL. The SGRQ is a supervised, self-administered measure designed specifically for use in respiratory disease, and contains three domains: symptoms (relating to cough, sputum, wheeze and shortness of breath); activity (relating to physical activity limited by breathlessness); and impact (relating to control, panic, medication and expectations).[20] A total score was calculated from all three domains.

Blood samplings

Blood was sampled from all subjects for the measurement of α_1-antitrypsin, leukocyte count, eosinophil count and immunoglobulin (Ig) E level.

Statistical analysis

Data are shown as means ± standard error of the mean (SEM) unless otherwise specified. Skewed data were either transformed to logarithmic data or expressed as medians with interquartile ranges. Univariate analysis employed χ^2 tests for categorical variables and one-way analysis of variance for quantitative variables with Scheffe's test as a post-hoc test for multiple comparisons. Relationships between two variables of quantitative data were examined using Spearmen's tests. For BMI and SGRQ scores, the Jonckheere-Terpstra test was used to examine trends for three groups of subjects. All statistical tests were two-sided and values of $p<0.05$ were considered statistically significant. Data were analyzed using SPSS for Windows version 12 software (SPSS Japan, Tokyo).

RESULTS

Regarding assessment of severity of emphysema, there was a high correlation between subjective visual score for 3 CT images and an objective computerized quantification for the whole lung ($n=137$, $r=0.835$, $p<0.0001$), which was done only for the subjects in Hokkaido University Hospital. This would justify the use of visual assessment of emphysema for this multi-site study.

Figure 1 shows the relationship between emphysema score and post-bronchodilator value of FEV_1 % predicted in all subjects with COPD. A weak but significant overall correlation was seen between the two parameters ($n=274$, $r=-0.302$, $p<0.001$). On the other hand, a better correlation was noted between emphysema score and DL_{CO} / VA ($n=273$, $r=-0.577$, $p<0.001$). An extremely wide variation in severity of emphysema was seen in any stage of COPD. In other words, severity of emphysema varied widely from none / mild to very severe even for subjects with the same level of airflow limitation. A similar finding was noted even if those subjects showing significant reversibility of airflow limitation ($n=86$) as defined by the GOLD guidelines were excluded (Data not shown).

To emphasize the characterization of phenotypes in COPD, all subjects were then classified into three groups based on severity of emphysema. The first group comprised subjects with no / mild emphysema (emphysema score < 1, a percentage of a low attenuation area (LAA) in the assessed lung was less than 12.5% on average). Subjects in the second group displayed moderate emphysema ($1 \leq$ emphysema score < 2.5, a percentage of a LAA in the assessed lung was less than 50% on average). The third group displayed severe emphysema (emphysema score ≥ 2.5). Table 1 shows characteristics of the three groups. Although indices of airflow limitation and DL_{CO} deteriorated as emphysema became more severe,

no significant differences in age, sex, smoking history, blood eosinophil count or serum IgE levels were found. There was no α_1 anti-trypsin deficiency in any subjects, and no significant difference was noted in the average level of serum α_1 anti-trypsin among the three groups. In terms of medication, anti-cholinergic agents or theophyllines were more prescribed as emphysema became more severe, however β_2-agonists and inhaled corticosteroids were given to similar extents to the three groups of subjects. Prevalence of chronic cough and / or sputum was remarkably similar among the three groups, indicating that the prevalence of chronic bronchitis is equal, regardless of severity of emphysema.

In terms of BDR to salbutamol, wide inter-subject variation was seen, but a clear relationship was apparent between baseline FEV_1 and post-bronchodilator increase in FEV_1 when expressed as a percent change from baseline FEV_1 (Figure 2A). BDR was then compared among the three groups classified based on severity of emphysema. No significant differences were seen in any of absolute change in FEV_1 (no / mild emphysema, 173 ± 13 ml, n = 105; moderate emphysema, 163 ± 12 ml, n = 123; severe emphysema,150 ± 20 ml, n = 45), percent change from baseline FEV_1 (12.6 ± 1.2%, 14.1 ± 1.3%, 14.1 ± 2.0%; respectively, Figure 2B) or percent change from predicted FEV_1 (6.5 ± 0.5%, 6.1 ± 0.4%, 5.6 ± 0.7%, respectively). Significant differences between groups were still absent even if we chose either only subjects without reversibility of airflow limitation, or subjects with FEV_1 < 60% predicted, indicating that airflow limitation was perfectly comparable among the three groups (post-bronchodilator FEV_1 in no / mild emphysema: 46.9 ± 1.6 % predicted, n = 32; moderate emphysema: 44.8 ± 1.3 % predicted, n = 63; severe emphysema: 43.5 ± 1.7 % predicted, n = 35, NS).

Figure 3 showed data for BMI and health-related QOL. BMI was significantly lower as emphysema became more severe in overall subjects (Table 1). Of particular note is the fact that this feature held true even if we compared the subjects separately based on the COPD stage (Figure 3A) or only subjects with FEV_1 < 60% predicted, indicating that airflow limitation was perfectly comparable among the three groups classified by the severity of emphysema (no / mild emphysema: 23.7 ± 0.6 kg/m^2, moderate emphysema: 21.1 ± 0.4 kg/m^2, severe emphysema 21.1 ± 0.6 kg/m^2, respectively). All dimensions of SGRQ scores became significantly higher as emphysema became more severe (Figure 3B). Statistical differences remained present in activity and total scores of SGRQ even for subjects with FEV_1 < 60% predicted (activity score: 51 ± 4%, 52 ± 3%, 64 ± 3%, respectively; total score: 37 ± 3%, 40 ± 2%, 45 ± 2%, respectively). Consequently, there was significant relationships of severity of emphysema with BMI overall ($r = -0.293$, $p < 0.001$) and also with total score of SGRQ ($r = 0.231$, $p < 0.001$).

DISCUSSION

This study first demonstrated that severity of emphysema varies widely despite the same disease stage in COPD. For instance, even in patients with moderate or severe COPD, some show very little evidence of emphysema, while others show marked emphysema. Furthermore, some patients retain relatively normal pulmonary function despite the presence of severe emphysema. These observations support the findings of several past studies, which argued against emphysema as the major cause of airflow limitation in COPD.[1,21-23]

All subjects were then classified into three groups based on severity of emphysema. Although small airways were not directly evaluated in this study, the

extremely wide variation in severity of emphysema observed among subjects with the same degree of airflow limitation appeared to indicate that the three groups might well represent differences in relative contributions of small airway disease and emphysema to airflow limitation. The most important findings in this study were that patients with severe emphysema show significantly lower BMI and worse QOL compared with patients with no / mild emphysema (small airway disease in predominance), despite similarities in age, smoking history, blood eosinophil count and IgE levels. Activity score, which includes dyspnea on exertion, was particularly significant. These differences remained significant even if subjects with the same degree of airflow limitation were compared. Celli et al. recently proposed the BODE index, a simple multidimensional grading system, for predicting risk of death in subjects with COPD.[24] They demonstrated the importance of BMI, dyspnea and exercise capacity index for assessment of subjects with COPD in addition to airflow limitation index. Several other reports support the notion that BMI and QOL, including dyspnea, represent independent factors for the prognosis of COPD.[25-28] Phenotyping of COPD described in the present study may thus have some clinical relevance in the management of patients with COPD. Another important consideration is that more attention should be paid to these phenotypes when studying the epidemiology, genetic background and pathogenesis of COPD.[29,30] In fact, an interesting study from Japan recently demonstrated that body weight loss in COPD is associated with a novel polymorphism in secretary A_2-IID, an enzyme responsible for mobilization of fatty acids, including arachidonic acid, from phospholipids, thereby potentially influencing systematic inflammation in COPD.[31] The reasons why severity of emphysema rather than airflow limitation itself is associated with lower BMI may be due to exaggerated systemic inflammatory response or increased work load of breathing in the emphysematous type of COPD, however, these speculations are not the scope of this study and need further investigation.

Historically, patients with COPD used to be classified as “pink puffer” or “blue bloater”,[7,8] or A, B and X types.[9] The phenotypic classification of COPD that we propose here definitely differs from classical phenotypes, as the prevalence of chronic bronchitis symptoms was almost equal among the three groups in this study. Indeed, we used to see far more subjects with COPD who were suffering from chronic bronchitis in the past compared to nowadays. However, at least in Japan, we have seen a dramatic decrease in the number of subjects diagnosed with COPD and chronic bronchitis over the last 3 decades.[32] Our data indicate that the decreasing frequency of chronic bronchitis in COPD over recent years does not reflect an increase of subjects with severe emphysema, but rather the manifestation of subjects with small airways disease in predominance, which is not necessarily associated with bronchitis symptoms.

The reversibility of airflow limitation in COPD has long been a subject of debate.[33-35] The present study found a wide inter-subject variation in BDR to salbutamol, but no statistical differences according to phenotype based on severity of emphysema. These data led to two important speculations. First, conclusions of the present study were unlikely to be biased by the inadvertent inclusion of patients with bronchial asthma, particularly in the group with no / mild emphysema. Secondly, what is occurring in large or proximal airways (chronic bronchitis symptoms and reversibility of airflow limitation) may be independent of what is occurring in peripheral sites in the lungs (small airways disease and emphysema).

Drawbacks in this study are twofold. Firstly, we used subjective visual scoring for assessment of emphysema severity rather than objective quantification. This is because we had to use various kinds of CT scanners for this study and could not obtain the Digital Imaging and Communications in Medicine (DICOM) images from all affiliate hospitals. However, all HRCT images were thin-slice less than 2 mm and we carefully optimized the data acquisition parameters as well as the parameters for data analysis at each hospital so as to obtain ideal images for assessment of emphysema. In addition, we showed that visual emphysema score only for 3 CT images was highly correlated with objective volume-based computerized assessment for the whole lung in almost a half of subjects, and also found the significant correlation between visual emphysema score and DLco / V_A as was nicely described in the past study.[36] Secondly, this study did not directly evaluate small airways disease, so we could not measure the real contribution of small airways disease to airflow limitation in any subjects. In parallel with this study, we attempted to develop new computer software using curved multiplanar reconstruction, to obtain longitudinal images and accurately analyze short-axis images of airways with inner diameter $\geq$ 2 mm located anywhere in the lungs. We recently published a paper demonstrating that airflow limitation in COPD is more closely related to dimensions of the distal airways (sixth generation) than proximal airways (third generation) in both the upper and lower lobes.[37] The observed high correlation coefficients between FEV_1 % predicted and the dimensions of such distal airways are in sharp contrast with the weak but significant relationship between FEV_1 % predicted and severity of emphysema observed in this study. These data suggest that the site of small airways contributes more significantly to airflow limitation than emphysema in COPD. In other words, contribution of small airways may be vitally important in COPD regardless of the phenotype of COPD based on severity of emphysema.

In conclusion, this study demonstrated that severity of emphysema is highly variable, even among subjects with the same stage of COPD, and that COPD phenotypes based on severity of emphysema clearly differ from the classical phenotypes of pink puffer and blue bloater. Prevalence of bronchitis symptoms and average bronchodilator responses to inhaled β_2-agonist were similar among the three groups classified according to severity of emphysema. However, BMIs were significantly lower and SGRQ scores were significantly worse in the phenotype with severe emphysema compared to that with no/mild emphysema. Accordingly, classification of COPD based on computed tomography may provide distinct phenotypes and display great clinical relevance in the management of COPD. Further studies are ongoing in an attempt to examine possible differences in natural history of the disease according to phenotypes based on the severity of emphysema.

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Competing interests
No

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Table 1. Clinical characteristics of phenotypes based on severity of emphysema*

Characteristic	No / Mild (n=105)	Moderate (n=124)	Severe (n=45)	Total (n=274)
Age (years)	70 (64-76)	72 (64-75)	71 (68-77)	71 (64-76)
Sex (male / female)	96 / 9	119 / 5	43 / 2	258 / 16
BMI (kg / m^2)	23.5 ± 0.3	21.7 ± 0.3 †	21.2 ± 0.5 †	22.3 ± 0.2
Smoking (pack-years)	58 ± 3	65 ± 3	66 ± 4	63 ± 2
Current smoker (%)	39/105 (37)	26/124 (21)	10/45 (22)	75/274 (27)
Clinical symptoms (%)				
Chronic cough	11 / 105 (11)	18 / 124 (15)	5 / 45 (11)	34 / 274 (12)
Chronic sputum	19 / 105 (18)	23 / 124 (19)	7 / 45 (16)	49 / 274 (18)
Chronic cough and sputum	8 / 105 (8)	16 / 124 (13)	5 / 45 (11)	29 / 274 (11)
Pulmonary function tests				
Pre-bronchodilator				
FVC (% predicted)	91.4 ± 2.0	94.7 ± 2.0	88.3 ± 3.1	92.4 ± 1.3
FEV_1 (% predicted)	63.0 ± 2.0	56.9 ± 2.1 †	46.5 ± 3.0 †,‡	57.5 ± 1.4
Post-bronchodilator				
FVC (% predicted)	98.9 ± 1.8	102.6 ± 1.8	98.4 ± 2.9	100.5 ± 1.2
FEV_1 (% predicted)	69.5 ± 1.9	62.7 ± 2.0	52.0 ± 3.0 †,‡	63.5 ± 1.3
FEV_1 / FVC	0.56 ± 0.01	0.48 ± 0.01 †	0.42 ± 0.02 †,‡	0.50 ± 0.01
Diffusing capacity				
DL_{CO} / VA (% predicted)	78.1 ± 2.2	58.4 ± 1.8 †	41.6 ± 2.5 †,‡	63.3 ± 1.5
Blood analysis				
Eosinophils (log cells / µl)	2.26 ± 0.03	2.16 ± 0.03	2.19 ± 0.05	2.20 ± 0.02
Serum total IgE (log IU / ml)	1.87 ± 0.07	1.72 ± 0.06	1.74 ± 0.09	1.78 ± 0.04
α1-antitrypsin (mg / dl)	128 ± 2	129 ± 2	135 ± 3	129 ± 1
Medications, no. of patients / total (%)				
Anti-cholinergic reagents	41 / 105 (39)	64 / 124 (52)	32 / 45 (71) †,‡	137 / 274 (50)
β_2-agonists	29 / 105 (28)	43 / 124 (35)	18 / 45 (40)	90 / 274 (33)
Theophyllines	36 / 105 (34)	59 / 124 (48) †	27 / 45 (60) †	122 / 274 (45)
Inhaled corticosteroid	12 / 105 (11)	10 / 124 (8)	9 / 45 (20)	31 / 274 (11)

BMI, body mass index; FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 s; DL_{CO}, carbon monoxide diffusing capacity; VA, alveolar volume; No / Mild, no or mild emphysema (emphysema score < 1); Moderate, moderate emphysema (1 ≤ emphysema score < 2.5); Severe, severe emphysema (emphysema score ≤ 2.5).

Data are shown as means ± standard error of the mean (SEM). Skewed data were expressed as medians with interquartile ranges. Univariate analysis employed χ^2 tests for categorical variables and one-way analysis of variance for quantitative variables with Scheffe's test as a post-hoc test for multiple comparisons.

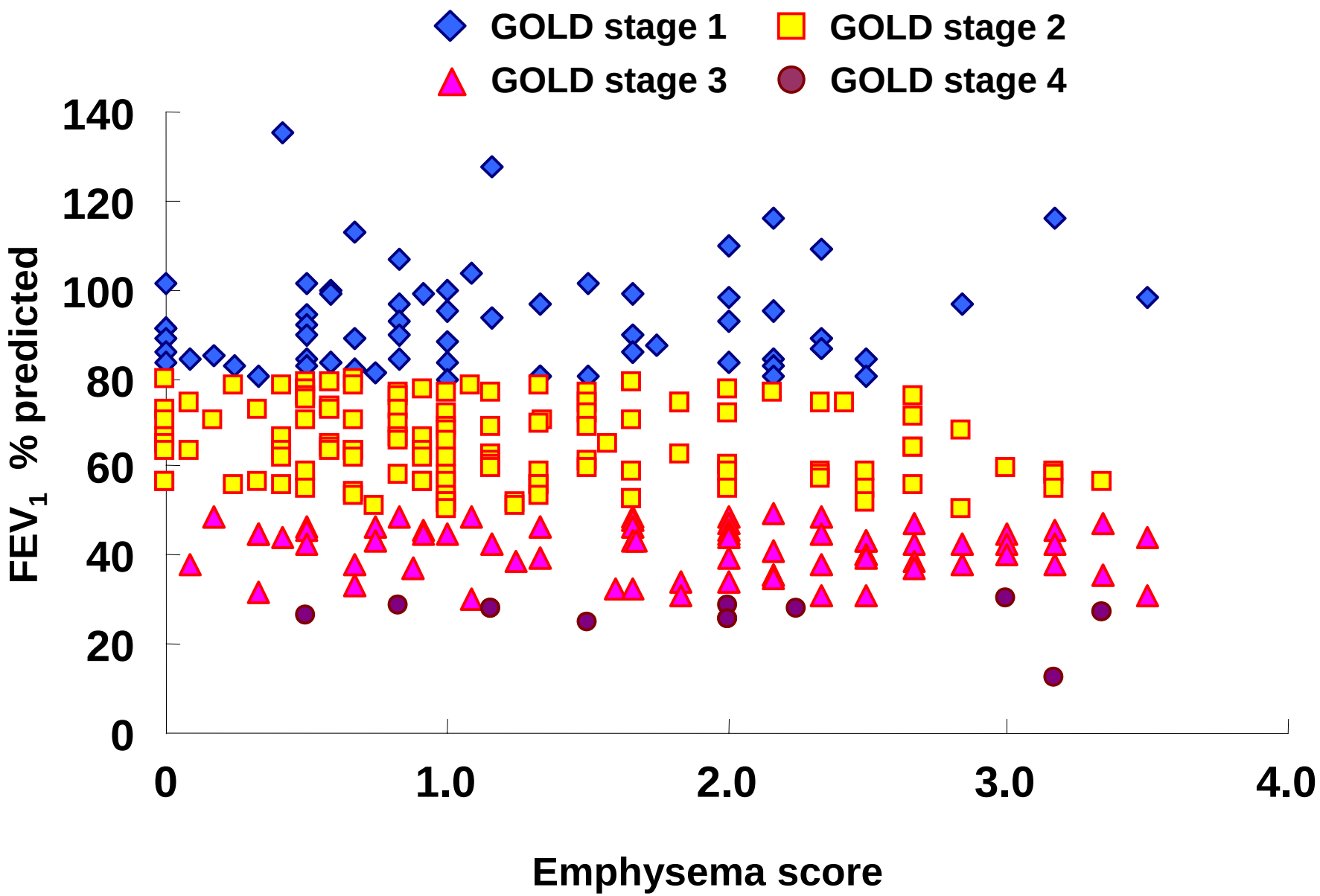
†: vs. No / Mild, $P < 0.05$, ‡: vs. Moderate, $P < 0.05$.

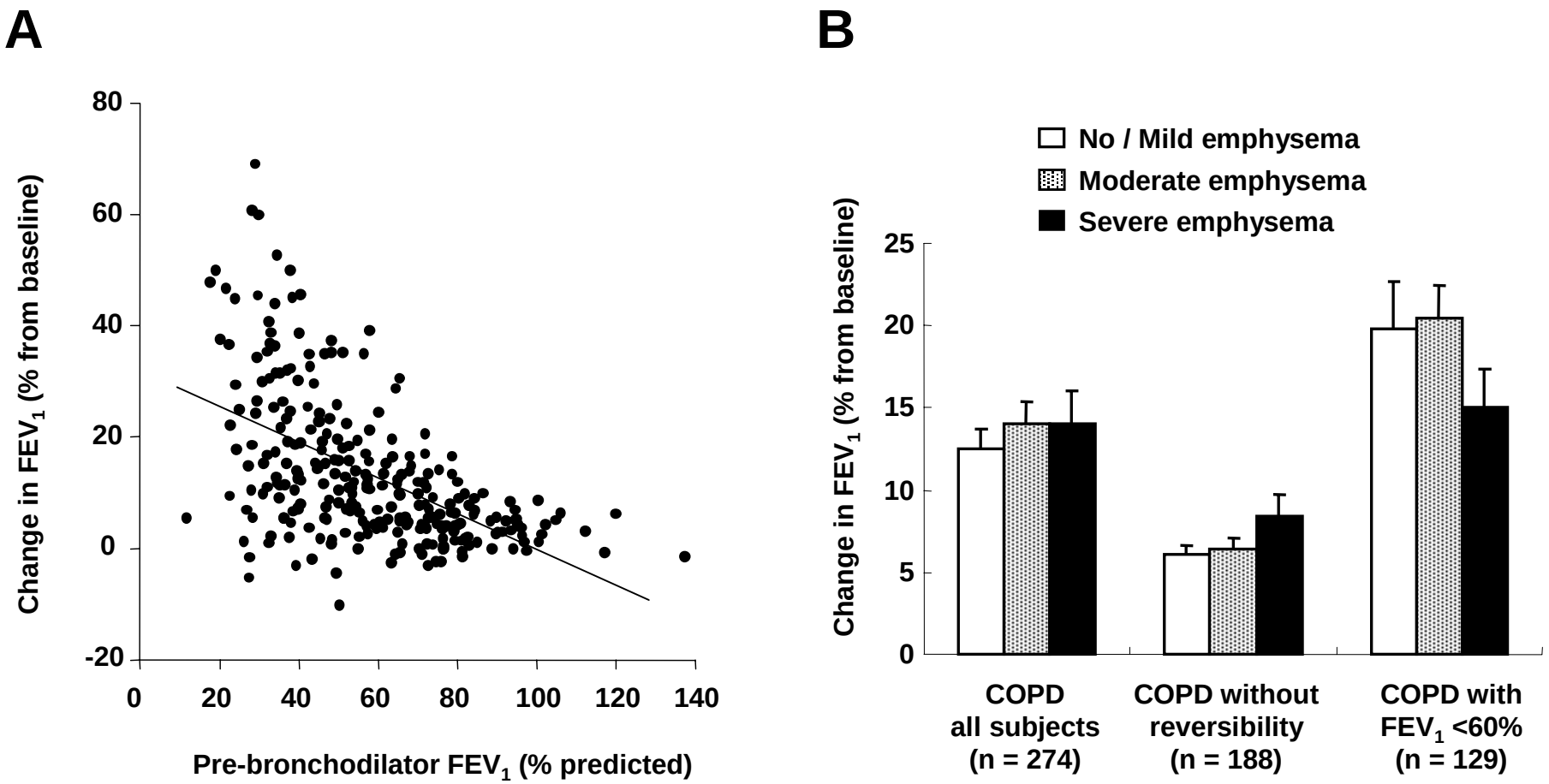
FIGURE LEGENDS

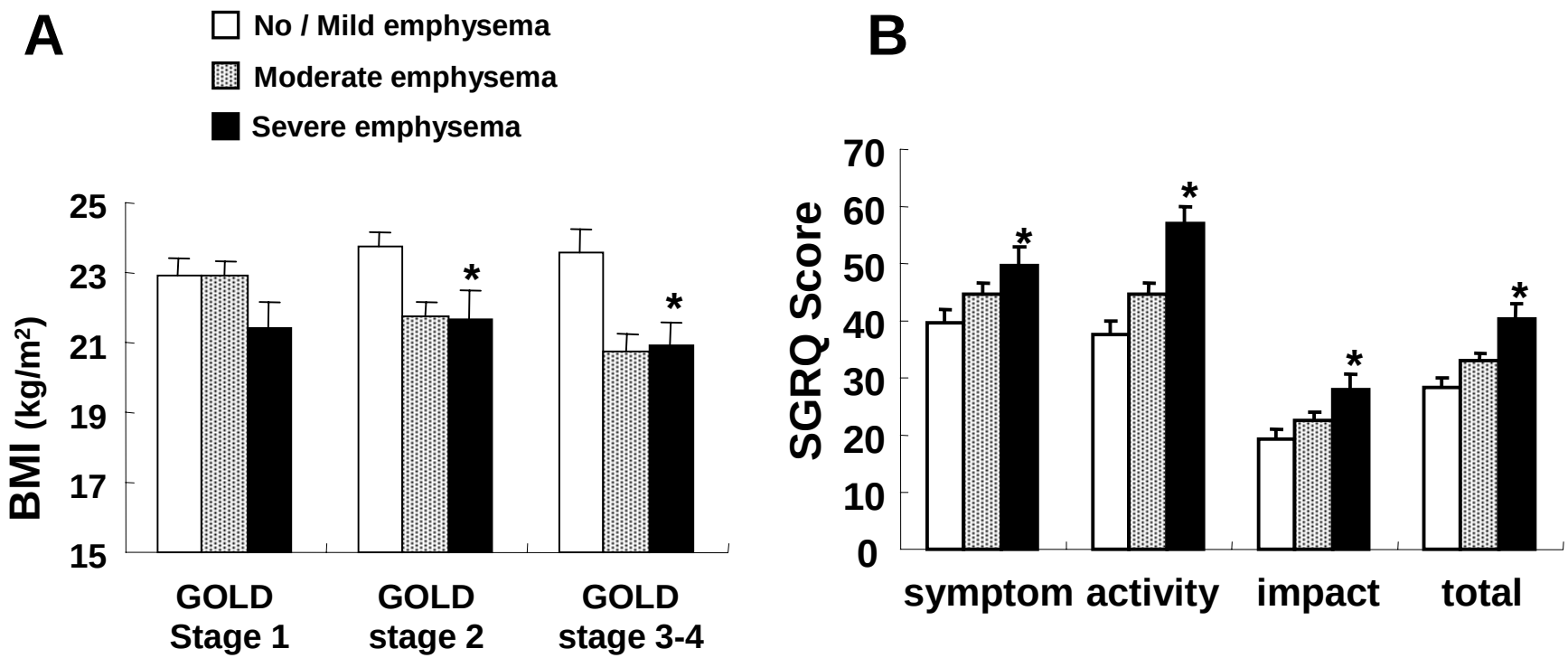
Figure 1. Relationship between emphysema score and forced expiratory volume in 1 s (FEV_1) % predicted in all subjects with COPD(n=274). They were classified into 4 stages according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines (◆: stage 1, n=64; □: stage 2, n=127; ▲: stage 3, n=71; and ●: stage 4, n=12). Severity of emphysema was highly variable despite the same stage of COPD although the relationship between emphysema score and post-bronchodilator FEV_1 % predicted was significant overall ($r = -0.302$, $p<0.001$).

Figure 2. Left panel (A): Relationship between pre-bronchodilator FEV_1 and bronchodilator response (BDR) to β_2 agonist in all subjects with COPD. Right panel (B): Comparison of BDR among three groups classified by severity of emphysema. Open columns indicate no / mild emphysema, gray columns indicate moderate emphysema, solid columns indicate severe emphysema. Values are mean [SEM].

Figure 3. Left panel (A): Body mass index (BMI) and Right panel (B): St George's Respiratory Questionnaire (SGRQ) among three groups classified by severity of emphysema. Open columns indicate no/mild emphysema, gray columns indicate moderate emphysema, solid columns indicate severe emphysema. Values are mean [SEM]. Severity of emphysema is associated with lower BMI regardless of GOLD stages. See text for the details of SGRQ data. The Jonckheere-Terpstra test was used to examine trends for three groups of subjects (*: $p < 0.05$).







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Chronic bronchitis symptoms

Well-trained clinical research coordinators (CRCs) elicited disease history, smoking history and other information about all treatments. Chronic cough and sputum expectoration were considered to be present when they occurred on most days for ≥ 3 months/year and for ≥ 2 consecutive years.[E2,E3] To avoid a bias by patient reports about the presence of chronic sputum symptoms, the amount of sputum should be > 10 ml/day for the definition described above and this was confirmed by clinical research coordinators for all subjects.

Pulmonary Function Tests

Rolling seal spirometers, Chestac (Chest M.I., Inc., Tokyo) or Fudac (Fukuda Denshi CO., Ltd., Tokyo), were used for the measurement of spirometry and diffusing capacity of carbon monoxide (DLco) at all hospitals. The accuracy of spirometers had been examined everyday by trained technicians. Volume calibration using a calibration syringe had been performed by technicians in each hospital before examination, and maintained to acceptable standards for spirometry testing according to Japanese Respiratory Society (JRS) guidelines ,[E4] which are compatible with ATS recommendations.[E5] A quality-control protocol was developed based on the criteria used in the Lung Health Study to increase accuracy and decrease intra-individual variability.[E6] All technicians in each hospital were trained by a central coordinator, although they had extensive prior experience in pulmonary function testing. On the test day, we measured body mass index (BMI) and confirmed that subjects were in a stable condition and free from any medication that might influence Pulmonary Function Tests using following methods. The CRCs asked subject condition and confirmed freedom from respiratory medication by telephone on the previous day. All subjects had undergone a physical check by a respiratory physician just before spirometry to confirm subjects were stable and free from

respiratory infection. Finally, a technician reconfirmed to make sure omitting respiratory medicine. Inhalation of short-acting bronchodilators was prohibited in the 12 h preceding measurement, and long-acting β_2 adrenergic agonists or sustained-release theophyllines were prohibited in the previous 24 h. Only 2 subjects were using long-acting anticholinergic inhaled agents, as these agents had been not available in Japan until December 2004, and these subjects were asked to omit long-acting anticholinergic inhaled agents for 48 h before measurements. Acceptable maneuvers and acceptable measurements followed the guidelines for pulmonary function tests issued by JRS.[E4] We checked all the spirometric tracings from different centers in the central office. Predicted values of spirometric measurements were derived from the guidelines for pulmonary function tests issued by JRS.[E4]

We evaluated the reversibility of airflow limitation by measuring spirometry before and 30 min after inhalation of salbutamol (0.4 mg). Bronchodilator response (BDR) was expressed in three ways, as an absolute change in FEV_1, as a percentage change from baseline FEV_1, and as a percentage change from predicted FEV_1. Reversibility of airflow limitation was considered to be significant if an increase in FEV_1 was both > 200 ml and 12% above pre-bronchodilator FEV_1 according to GOLD guidelines.[E1]

DL_{CO} was measured by the single breath method. Results were corrected by alveolar volume (V_A) and hemoglobin concentration. DL_{CO} / V_A were compared to the predicted normal values.[E4]

HRCT

The following commercially available scanners were used in 9 affiliate hospitals: Somatom plus Volume Zoom (Siemens, Berlin); Aquilion Multi TSX-101A/2A, TSX-101A/4E, TSX-101A/6A, X Force TSX-011A/6A, X Vigor SS TSX-012A/4B (Toshiba Medical Systems, Japan); MX-8000/ID16 (Philips, Netherlands). Chest HRCT scans were performed under the supine position, holding their breaths at full inspiration. No patient received intravenous contrast medium. Other technical parameters were as follows; 1 mm collimation, 120-140 kV, 75-350 mA, 0.75-1 s for scanning time, and 1-2 mm thickness. HRCT images were selected at 3 levels, including the aortic arch, carina, and 1-2 cm above the highest hemi-diaphragm. Image interpretations were performed under -600 to -900 Hounsfield units (HU) window levels and 800 to 1500 HU window widths based on the best condition for detecting emphysema at each hospital.

Severity of emphysema was visually assessed by three independent pulmonologists according to the modified Goddard scoring system.[E7] We analyzed 6 images in 3 slices in the lungs and an average score of all images was considered as a representative value of severity of emphysema in each person. Each image was classified as normal (score 0), up to 5 percent affected (score 0.5), up to 25 percent affected (score1), up to 50 percent affected (score 2), up to 75 percent affected (score 3), more than 75 percent affected (score 4), giving a minimum score of 0 and maximum of 24, and the average score of 6 images was considered as a representative value of the severity of emphysema in the lungs. When the three independent pulmonologists split in their evaluation, we took only the score assessed by the majority.

Three dimensional CT analyses were performed only in Hokkaido University hospital (n=137) to confirm the accuracy and the reliability of visual assessment. All

CT row data sets were reconstructed to isometric voxel data using both soft-tissue and bone algorithms. The length of the 1 voxel side was 0.625 mm. Reconstructed data were transferred to the workstation, and then reconstructed into three-dimensional chest images (AZE Ltd, Tokyo, Japan). On this workstation, using original software with volume rendering technique, three dimensional lung objects for analyses were obtained at 3steps as follows; First, only trachea, bronchus and entire lung were extracted by using opacities under -740 HU. Second, trachea and central bronchus until second order bronchus were extracted from the object after the first step by using opacities from -740 to -975 HU. Third, only the entire lung object was obtained by subtraction of the second object from the first object. In these steps, the software automatically removed central pulmonary arteries and veins until the second order branch, which had similar opacities of lung, using original algorithm recognizing morphology. As in some previous reports, low attenuation volume (LAV) of the entire lung was measured as a volumes of the lung with attenuation coefficients lower than -960 HU.[E8,E9] LAV represents relative values in the total lung volume.[E10,E11]

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