significantly associated with the diffusing capacity for carbon monoxide, alveolar-arterial oxygen pressure difference, 6-min walk distance and end-exercise oxygen saturation. Additionally, circulating levels of intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 were inversely correlated with RHI.

Conclusions: We confirmed a possible link between pulmonary fibrosis and cardiovascular disease by demonstrating an impairment of endothelium-dependent vasodilator response, which was significantly associated with the severity of pulmonary fibrosis and circulating levels of adhesion molecules.

Introduction

A growing body of epidemiological evidence indicates an association between cardiovascular diseases and pulmonary fibrosis.[1–4] Although most deaths in idiopathic pulmonary fibrosis (IPF) are due to a respiratory cause, cardiovascular events are also reported to be an important cause of mortality.[5,6] There are several possible mechanisms linking pulmonary fibrosis to cardiovascular disease. For example, several cytokines including interleukin-1β (IL-1β), IL-8, tumor necrosis factor-α (TNF-α) and intercellular adhesion molecule-1 (ICAM-1), all of which have been reported to be involved in the pathogenesis of pulmonary fibrosis,[7–9] also play a key role in the process of atherosclerosis.[10] In addition, the clotting cascade is activated in pulmonary fibrosis and has been implicated in its pathogenesis.[11] Furthermore, chronic hypoxic stress, to which patients with pulmonary fibrosis are often exposed, results in irreversible remodeling of the vasculature and surrounding tissues, characterized by smooth muscles proliferation and fibrosis.[12]

The vascular endothelium acts to maintain vascular homeostasis through multiple mechanisms, and alteration in its function precedes the development of morphological atherosclerotic changes.[13] Endothelial dysfunction results in impaired regulation of vascular tone, a prothrombotic state, and increased production of inflammatory cytokines and adhesion molecules, and hence, it is associated with the risk of future cardiovascular events.[13] Endothelial function has been assessed based on the endothelium-dependent vasodilator response,[13] and measurements of this response using digital pulse volume amplitude has emerged as a noninvasive, automated quantitative test for endothelial function.[14,15]

Based on the epidemiological association between pulmonary fibrosis and cardiovascular disease, we hypothesized that endothelial function would be more impaired among patients with pulmonary fibrosis and that the impairment would not be explained by the presence of classical cardiovascular risk factors. In the present study, we assessed endothelium-dependent vasodilator response in patients with pulmonary fibrosis using digital pulse amplitude tonometry (PAT) and evaluated the relationship between disease-related factors and endothelium-dependent vasodilator response.

Methods

Study subjects

We consecutively recruited 39 newly-diagnosed idiopathic chronic interstitial pneumonitis/fibrosis patients who visited Kyoto University Hospital. Patients diagnosed as having any collagen vascular disease or vasculitis, and patients whose lung diseases were potentially caused by drug or occupational-environmental exposures were excluded. None of the patients had other pulmonary diseases or a history of cardiovascular events such as myocardial infarction or stroke, and none were receiving insulin, corticosteroids or supplementary oxygen therapy. IPF was diagnosed on the basis of the current official joint statement on IPF from the American Thoracic Society (ATS), European Respiratory Society (ERS), Japanese Respiratory Society and Latin American Thoracic Association.[16] Age-, sex- and body mass index (BMI)-matched subjects without lung disease or any previous history of cardiovascular events were also recruited as controls. This study was approved by the Ethics Committee of Kyoto University and informed consent was obtained from all patients.

Arterial blood gas analysis, including arterial partial pressure of oxygen (PaO_2) and arterial partial pressure of carbon dioxide ($PaCO_2$), was performed while patients were breathing room air at rest in the supine position. The alveolar-arterial oxygen pressure difference ($A\text{-}aDO_2$) was calculated according to the standard formula, using a respiratory exchange ratio of 0.8. The degree of nocturnal hypoxemia was assessed by oxygen desaturation index (ODI) using a pulse oximeter (Pulsox-300i, Konica Minolta Inc., Osaka, Japan). ODI was calculated by dividing the total number of oxygen desaturations by the total recording time, with desaturation defined as a decrease in SpO_2 to 4% or more below the baseline level. Six-minute walk testing was performed as recommended by ATS guidelines.[17]

Digital PAT

Endothelium-dependent vasodilator response was assessed by digital PAT in the fasting state, the principle of which has been described previously.[14,15] Briefly, digital pulse amplitude was continuously recorded with the Endo-PAT 2000 device (Itamar Medical Inc., Caesarea, Israel) placed on the tip of each index finger and a blood pressure cuff was placed on one upper arm (study arm), while the contralateral arm served as a control (control arm). After a 5-min equilibration period, the cuff was inflated to 60 mmHg above the systolic blood pressure or 200 mmHg for 5-min and then deflated to induce reactive hyperemia. Pulse amplitude was recorded electronically in both fingers and analyzed by a computerized, automated algorithm that provided the ratio of the average amplitude over a 1-min time interval starting 1.5-min after cuff deflation divided by the average amplitude of a 2.5-min time period before cuff inflation. The calculated ratio reflects the reactive

hyperemia index (RHI), with a higher index indicating a higher flow-mediated hyperemic response.

Blood sample collection and laboratory assessments

Samples of peripheral venous blood were collected in the morning after an overnight fast. Total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), triglycerides, HbA1c and fasting glucose were measured as classical cardiovascular risk factors. C-reactive protein (CRP) and fibrinogen were also measured as conventional systemic inflammatory biomarkers.

Serum concentrations of IL-1β, IL-6, IL-8, TNF-α, ICAM-1 and vascular cell adhesion molecule-1 (VCAM-1) in patients with chronic interstitial pneumonitis/fibrosis were determined using the Bio-Plex Pro Human Cytokine Assay (Bio-Rad Laboratories, Hercules, CA, USA) according to the manufacturer's instructions.[18] Cytokine-specific antibody-coated beads were used for these experiments. Beads were read on the Bio-Plex 200 suspension array system, and cytokine concentrations were automatically calculated with Bio-Plex Manager Software by a standard curve derived from a recombinant cytokine standard.

Pulmonary function tests

Pulmonary function tests were performed using a CHESTAC system (Chest M.I. Inc., Tokyo, Japan). The diffusing capacity for carbon monoxide (DL_{CO}) was measured using the single-breath technique. Percent-predicted values were used for analyses.

Visual scoring of high-resolution computed tomography (HRCT)

Two independent observers (AK and TK) reviewed the three HRCT images taken at the level of the aortic arch, the carina and right inferior pulmonary venous confluence. Each lobe of the lung was scored on a scale of 0–5 for the extents of ground-glass opacity (ground-glass score) and fibrotic opacity (fibrosis score).[19] The two observers' scores for each lobe were averaged for data analyses.

Statistics

All statistical analyses were performed using JMP version 9 software (SAS Institute, Cary, NC, USA). Continuous variables are expressed as mean ± standard deviation (SD). Comparisons of variables between two groups were made by chi-square tests or unpaired *t*-tests. Relationships between pairs of variables were analyzed by Pearson's correlation coefficient tests. A *p* value less than 0.05 was considered to indicate statistical significance.

Results

Characteristics of patients and controls

The clinical characteristics of all subjects are summarized in Table 1. Among 39 patients with idiopathic chronic interstitial pneumonitis/fibrosis, 11 had a typical usual interstitial pneumonia (UIP) pattern on HRCT scan. In the remaining 28 patients with HRCT results that were atypical for UIP, 12 patients underwent surgical lung biopsy: 6 had UIP, 1 had nonspecific interstitial pneumonia, and 5 did not meet ATS/ERS histopathologic criteria for a specific diagnosis of idiopathic interstitial pneumonias.[20] There was no statistically significant difference between groups with respect to sex, age, BMI, smoking, blood pressure, heart rate, ODI, prevalence of diabetes and usage rate of antihypertensive agents or statins.

Table 1 Characteristics of patients and controls.

	Patients (n = 39)	Controls (n = 30)	p value
Sex, male/female	24/15	20/10	0.80
Age, years	65.0 ± 8.5	62.7 ± 7.6	0.25
BMI, kg/m^2	24.2 ± 3.4	23.2 ± 3.2	0.23
Smoking history, current/ex/never	7/22/10	5/11/14	0.17
Smoking, pack years	29.4 ± 27.3	17.5 ± 24.3	0.07
Systolic blood pressure, mmHg	123.7 ± 12.2	126.5 ± 13.2	0.36
Diastolic blood pressure, mmHg	73.5 ± 9.8	77.5 ± 11.0	0.12
Heart rate, bpm	61.2 ± 9.4	58.4 ± 5.9	0.16
ODI	3.8 ± 4.0	5.1 ± 2.6	0.14
Diabetic patients	6	2	0.45
Antihypertensive treatment	9	10	0.42
Statins use	6	2	0.45

Data are presented as number or mean ± SD.
BMI, body mass index; ODI, oxygen desaturation index.

Table 2 shows pulmonary function, arterial blood gas data and laboratory cardiovascular risk factors in patients and control subjects. Forced vital capacity (FVC) and DL_{CO} were significantly lower in chronic interstitial pneumonitis/fibrosis patients than in control subjects (both $p < 0.001$) but there were no significant differences in PaO_2, $PaCO_2$ or A-aDO_2. Although total cholesterol and triglycerides levels were similar in both groups, HDL levels were significantly lower and LDL levels were significantly higher in idiopathic chronic interstitial pneumonitis/fibrosis patients than in control subjects (p = 0.002 and 0.01, respectively). With regard to indices of diabetes, HbA1c and fasting glucose levels were comparable between groups.

RHI and its related factors

As shown in Fig. 1, RHI of idiopathic chronic interstitial pneumonitis/fibrosis patients was significantly lower than that of control subjects (1.8 ± 0.4 vs 2.1 ± 0.6, p = 0.02). To identify predisposing factors for impaired endothelium-dependent vasodilator response in idiopathic chronic interstitial pneumonitis/fibrosis patients, we investigated

Table 2 Pulmonary function, arterial blood gas data and laboratory cardiovascular risk factors.

	Patients (n = 39)	Controls (n = 30)	p value
Pulmonary function			
FVC, % predicted	94.9 ± 18.5	115.0 ± 18.0	<0.001
DL_{CO}, % predicted	51.2 ± 13.0	87.3 ± 13.2	<0.001
Arterial blood gas data			
$PaCO_2$, mmHg	41.0 ± 3.3	41.5 ± 3.5	0.62
PaO_2, mmHg	83.5 ± 10.7	83.4 ± 11.0	0.98
A-aDO_2, mmHg	15.2 ± 11.7	14.8 ± 12.1	0.88
Laboratory cardiovascular risk factors			
Total cholesterol, mg/dl	208.4 ± 34.2	207.0 ± 32.6	0.86
HDL, mg/dl	47.1 ± 11.1	57.6 ± 16.3	0.002
LDL, mg/dl	133.7 ± 30.6	116.7 ± 20.8	0.01
Triglycerides, mg/dl	127.8 ± 47.9	131.9 ± 92.1	0.81
HbA1c, %	5.7 ± 0.8	5.5 ± 0.5	0.15
Fasting glucose, mg/dl	105.8 ± 24.9	97.1 ± 20.6	0.13

Data are presented as mean ± SD.
FVC, forced vital capacity; DL_{CO}, diffusing capacity for carbon monoxide; $PaCO_2$, arterial partial pressure of carbon dioxide; PaO_2, arterial partial pressure of oxygen; A-aDO_2, alveolar-arterial oxygen pressure difference; HDL, high density lipoprotein; LDL, low density lipoprotein.

Table 3 Correlation coefficients of reactive hyperemia index with clinical characteristics and laboratory cardiovascular risk factors.

	All subjects (n = 69)		Patients (n = 39)	
	r value	p value	r value	p value
Patient characteristics				
Age, years	−0.07	0.58	0.12	0.48
BMI, kg/m^2	−0.24	0.045	−0.22	0.19
Smoking, pack years	−0.21	0.09	−0.03	0.87
ODI	0.01	0.91	−0.08	0.64
Laboratory cardiovascular risk factors				
Total cholesterol, mg/dl	0.18	0.13	0.31	0.06
HDL, mg/dl	0.18	0.13	0.21	0.21
LDL, mg/dl	0.09	0.48	0.26	0.11
Triglycerides, mg/dl	0.03	0.81	−0.18	0.26
HbA1c, %	−0.12	0.34	−0.05	0.77
Fasting glucose, mg/dl	−0.05	0.71	0.06	0.73

BMI, body mass index; ODI, oxygen desaturation index; HDL, high density lipoprotein; LDL, low density lipoprotein.

the associations of RHI with clinical characteristics, laboratory cardiovascular risk factors and disease-related factors such as pulmonary function, arterial blood gas data, 6-min walk testing data and HRCT visual scores. In the entire study population (n = 69), RHI was significantly correlated with BMI [r (correlation coefficient) = −0.24, p = 0.045], but not with other variables (Table 3). Within the patient group (n = 39), statistically significant relationship with RHI was not found in age, BMI, smoking, ODI or indices of dyslipidemia and diabetes. Regarding disease-related factors, RHI was significantly correlated with DL_{CO} (r = 0.42, p = 0.008), A-aDO_2 (r = −0.34, p = 0.04), 6-min walk distance (r = 0.38, p = 0.02), and end-exercise oxygen saturation (r = 0.37, p = 0.03) (Fig. 2), but not with $PaCO_2$ (r = 0.26, p = 0.13), PaO_2 (r = 0.27, p = 0.11), FVC (r = 0.17, p = 0.29), HRCT ground-glass score (r = −0.27, p = 0.10) or HRCT fibrosis score (r = −0.02, p = 0.89) (Table 4).

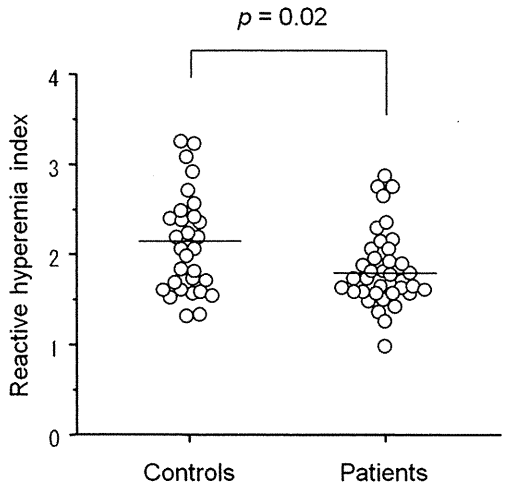

Figure 1 Comparison of reactive hyperemia index in controls (2.1 ± 0.6) and patients with idiopathic chronic interstitial pneumonitis/fibrosis (1.8 ± 0.4). Horizontal bars indicate mean values.

Next, we assessed whether a significant relationship between RHI and disease severity also exists in the homogeneous subgroup of IPF-confirmed patients. Pulmonary function, arterial blood gas data, 6-min walk distance and end-exercise oxygen saturation were not significantly different between IPF-confirmed patients and other patients. IPF-confirmed patients had less ground-glass and more fibrosis scores on HRCT (Supplementary Table 1). Among IPF-confirmed patients, RHI was significantly correlated with FVC (r = 0.58, p = 0.02), DL_{CO} (r = 0.64, p = 0.006), A-aDO_2 (r = −0.50, p = 0.04), end-exercise oxygen saturation (r = 0.53, p = 0.03) and HRCT fibrosis score (r = −0.54, p = 0.03), but not with $PaCO_2$ (r = 0.41, p = 0.10), PaO_2 (r = 0.40, p = 0.12), 6-min walk distance (r = 0.36, p = 0.16) or HRCT ground-glass score (r = −0.37, p = 0.14) (Table 4).

Relationships between RHI and circulating inflammatory biomarkers

To assess the possible role of systemic inflammation in the impairment of endothelium-dependent vasodilator response in patients with idiopathic chronic interstitial pneumonitis/fibrosis, we investigated the associations between RHI and

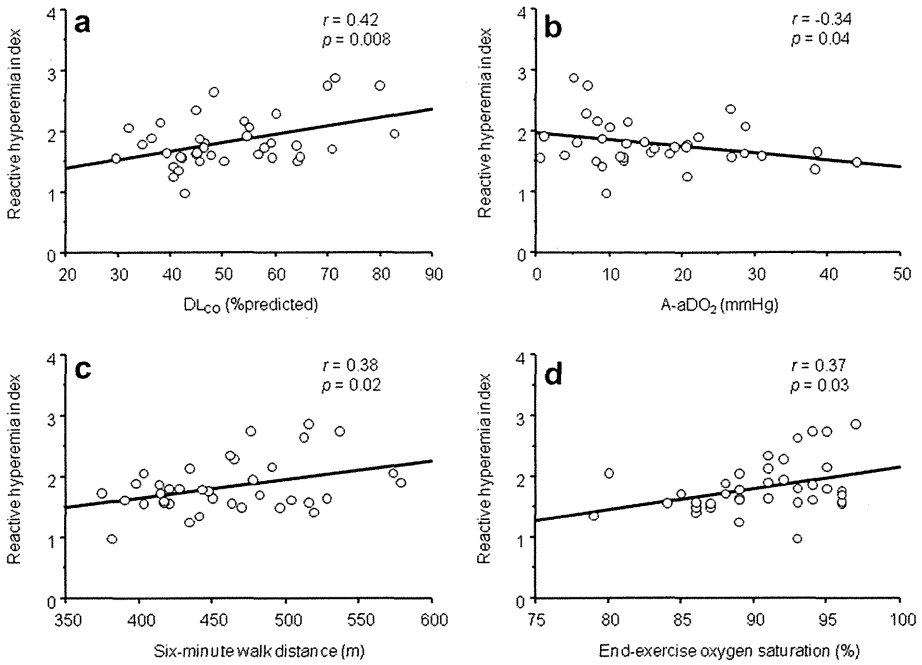

Figure 2 Scatter diagrams showing the correlation of reactive hyperemia index with DL_{CO} (a), A-aDO_2 (b), 6-min walk distance (c) and end-exercise oxygen saturation (d) in patients with idiopathic chronic interstitial pneumonitis/fibrosis. The *r* value indicates the correlation coefficient. Lines indicate regression lines.

circulating levels of inflammatory biomarkers. Since the concentrations of IL-1β, IL-6 and TNF-α were below detectable levels (<1 pg/ml) in more than half of the samples, we conducted further analyses only for CRP (0.4 ± 0.7 mg/dl), fibrinogen (344.9 ± 73.0 mg/dl), IL-8 (5.4 ± 2.6 pg/ml), ICAM-1 (237.3 ± 60.8 ng/ml) and VCAM-1 (135.7 ± 25.3 ng/ml) levels. Significant and near significant negative correlations were found between RHI and serum levels of ICAM-1 ($r = -0.30$, $p = 0.07$) and VCAM-1 ($r = -0.42$, $p = 0.008$), whereas CRP ($r = -0.27$, $p = 0.10$), IL-8 ($r = 0.01$, $p = 0.94$) and fibrinogen ($r = 0.02$, $p = 0.92$) levels were not significantly associated with RHI.

Table 4 Correlation coefficients of reactive hyperemia index with disease-related factors.

	All patients ($n = 39$)		IPF-confirmed ($n = 17$)	
	r value	*p* value	*r* value	*p* value
Pulmonary function				
FVC, % predicted	0.17	0.29	0.58	0.02
DL_{CO}, % predicted	0.42	0.008	0.64	0.006
Arterial blood gas data				
$PaCO_2$, mmHg	0.26	0.13	0.41	0.10
PaO_2, mmHg	0.27	0.11	0.40	0.12
A-aDO_2, mmHg	−0.34	0.04	−0.50	0.04
Six-minute walk test				
Six-minute walk distance, m	0.38	0.02	0.36	0.16
End-exercise oxygen saturation, %	0.37	0.03	0.53	0.03
HRCT scores				
HRCT ground-glass score	−0.27	0.10	−0.37	0.14
HRCT fibrosis score	−0.02	0.89	−0.54	0.03

IPF, idiopathic pulmonary fibrosis; FVC, forced vital capacity; DL_{CO}, diffusing capacity for carbon monoxide; $PaCO_2$, arterial partial pressure of carbon dioxide; PaO_2, arterial partial pressure of oxygen; A-aDO_2, alveolar-arterial oxygen pressure difference; HRCT, high-resolution computed tomography.

Discussion

The present study demonstrated a significant impairment of digitally-recorded endothelium-dependent vasodilator response in patients with idiopathic chronic interstitial pneumonitis/fibrosis. This impairment was significantly related to DL_{CO}, A-aDO_2, 6-min walk distance and end-exercise oxygen saturation, all of which were major physiologic indices for the severity of pulmonary fibrosis. Additionally, circulating levels of ICAM-1 and VCAM-1 correlated inversely with RHI.

The impairment of endothelium-dependent vasodilator response in patients with pulmonary fibrosis and its correlation with several functional parameters of disease status not only support a possible link between pulmonary fibrosis and cardiovascular disease[1–4] but also imply potential mechanisms for the impairment of vascular function in pulmonary fibrosis. Reduced exercise performance and systemic oxygen desaturation with exercise may lead to sedentary lifestyle or chronic exposures to hypoxia. Suvorava et al. showed that forced physical inactivity in young healthy mice induces reduction of endothelium-dependent vasorelaxation and vascular endothelial nitric oxide synthase (eNOS) expression.[21] In addition, diminished eNOS expression and NO release in chronic hypoxic human

endothelial cells were also noted.[22] Thus, reduced exercise performance and systemic oxygen desaturation with exercise in pulmonary fibrosis can potentially elicit the impairment of endothelium-dependent vasodilator response via reduced expression of eNOS in the absence of other cardiovascular risk factors.

Dysfunctional endothelial cells can induce increased local production of endothelin-1,[23] angiotensin-II[24] and plasminogen activator inhibitor-1,[25] all of which are involved in the pathogenesis of pulmonary fibrosis.[26–28] Hence, conversely, local endothelial dysfunction in the lung could be associated with the development or progression of fibrosis, thereby possibly resulting in gas change derangement. Although we evaluated endothelial function in the extrapulmonary systemic circulation, which does not necessarily reflect changes in pulmonary capillaries, it is plausible that, in patients with pulmonary fibrosis, local endothelial dysfunction in the pulmonary microcirculation is present and perpetuates the progression of fibrosis via mediators released by injured endothelial cells.

The recruitment, adhesion and subsequent transendothelial migration of circulating leukocytes are important processes involved in atherosclerosis.[29] These processes are mediated by inflammatory cytokines and adhesion molecules,[10] the expression of which is upregulated in dysfunctional endothelial cells.[13] In our population, the concentrations of IL-1β, IL-6 and TNF-α were below detectable levels in more than half of the samples and circulating levels of CRP, fibrinogen and IL-8 were not significantly associated with RHI. These results suggest that systemic inflammation is less prominent and may play a minor role in the impairment of endothelium-dependent vasodilator response in patients with pulmonary fibrosis. However, we found significant and near significant negative correlations between circulating adhesion molecules and endothelial function, indicating the presence and association of dysfunction and inflammation in the vascular endothelium, although the magnitude might not be great.

Notably, while circulating levels of total cholesterol, triglycerides, HbA1c and fasting glucose were similar, patients with chronic interstitial pneumonitis/fibrosis had significantly lower HDL levels and higher LDL levels than did control subjects. Although these factors were not significantly related to RHI, they might contribute at least in part to the development of endothelial dysfunction. It is unclear whether impaired lipid metabolism is an outcome of pulmonary fibrosis itself or of comorbid conditions associated with pulmonary fibrosis. Local alterations in the lipid composition of bronchoalveolar lavage fluid were reported in animal models of bleomycin-induced pulmonary fibrosis,[30] as well as in patients with IPF.[31,32] In addition, recent studies showed that obstructive sleep apnea was prevalent in patients with IPF[33] and that chronic intermittent hypoxia can cause circulating lipid levels to increase in relation to the severity of the hypoxic stimulus.[34] Thus, pulmonary fibrosis, comorbid conditions and metabolic alterations may be interrelated, but the underlying mechanisms remain to be elucidated and further studies are needed.

We should mention some of the limitations of the present study. Firstly, our cohort of patients was relatively heterogeneous because it also included some patients other than IPF or those who could not be diagnosed as IPF due to the lack of surgical lung biopsy. Although further studies involving more patients with IPF are necessary, RHI was also significantly correlated with disease severity including FVC, DL_{CO}, A-aDO_2, end-exercise oxygen saturation and HRCT fibrosis score even in the subgroup of IPF-confirmed patients. Secondly, the sample size was small and the impact of pulmonary hypertension, which commonly complicates the course of IPF,[6] was not investigated. Although the relationships between RHI and several classical cardiovascular risk factors were not statistically significant in the present study, there remains a need for further studies with larger samples to demonstrate that impaired endothelium-dependent vasodilator response in pulmonary fibrosis patients is unlikely to be the result of confounders.

In conclusion, we confirmed a possible link between pulmonary fibrosis and cardiovascular disease by demonstrating an impairment of endothelium-dependent vasodilator response in patients with pulmonary fibrosis. Further knowledge of the presence and pathophysiological relevance of endothelial dysfunction in pulmonary capillaries may provide a more integrated understanding of the mechanisms of pulmonary fibrosis.

Conflict of interest disclosure

This study was supported by grants from the Respiratory Failure study group and Diffuse Lung Disease study group from the Japanese Ministry of Health, Labor and Welfare.

Appendix A. Supplementary material

Supplementary material related to this article can be found on line at http://dx.doi.org/10.1016/j.rmed.2012.10.005.

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Circulation Journal
Official Journal of the Japanese Circulation Society
http://www.j-circ.or.jp

ORIGINAL ARTICLE
Pulmonary Circulation

Safety and Efficacy of Epoprostenol Therapy in Pulmonary Veno-Occlusive Disease and Pulmonary Capillary Hemangiomatosis

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Background: Pulmonary veno-occlusive disease (PVOD) and pulmonary capillary hemangiomatosis (PCH) are rare causes of pulmonary hypertension. There is no proven medical therapy to treat these diseases, and lung transplantation is thought to be the only cure. Administration of vasodilators including epoprostenol sometimes causes massive pulmonary edema and could be fatal in these patients.

Methods and Results: Eight patients were treated with epoprostenol for 387.3±116.3 days (range, 102–1,063 days), who were finally diagnosed with PVOD or PCH by pathological examination. The maximum dose of epoprostenol given was 55.3±10.7 ng·kg^{-1}·min^{-1} (range, 21.0–110.5 ng·kg^{-1}·min^{-1}). With careful management, epoprostenol therapy significantly improved the 6-min walk distance (97.5±39.2 to 329.4±34.6 m, P<0.001) and plasma brain natriuretic peptide levels (381.3±136.8 to 55.2±14.4 pg/ml, P<0.05). The cardiac index significantly increased from 2.1±0.1 to 2.9±0.3 L·min^{-1}·m^{-2} (P<0.05). However, pulmonary artery pressure and pulmonary vascular resistance were not significantly reduced. For 4 patients, epoprostenol therapy acted as a bridge to lung transplantation. For the other patients who had no chance to undergo lung transplantation, epoprostenol therapy was applied for 528.0±216.6 days and the maximum dose was 63.9±19.0 ng·kg^{-1}·min^{-1}.

Conclusions: This study data suggest that cautious application of epoprostenol can be considered as a therapeutic option in patients with PVOD and PCH. (*Circ J* 2012; **76:** 1729–1736)

Key Words: Epoprostenol; Pulmonary capillary hemangiomatosis; Pulmonary hypertension; Pulmonary veno-occlusive disease

Pulmonary veno-occlusive disease (PVOD) and pulmonary capillary hemangiomatosis (PCH) are rare causes of pulmonary hypertension, and their categories have been changed at every World Symposium on Pulmonary Hypertension.[1,2] The latest clinical classification of pulmonary hypertension categorized these diseases as Group 1' considering the similarity of risk factors and the genetic mutations in idiopathic pulmonary arterial hypertension (IPAH).[3,4] Continuous intravenous infusion of epoprostenol decreases pulmonary vascular resistance and improves the prognosis of IPAH,[5,6] and it has become a standard therapy for IPAH. However, the indication of epoprostenol for other subgroups of pulmonary hypertension including PVOD and PCH is controversial. A few patients with PVOD have been reported to show amelioration by application of epoprostenol.[7,8] In contrast, other reports have warned that epoprostenol precipitates severe pulmonary edema in patients with PVOD or PCH,[9,10] which never occurs in patients with IPAH. This is why epoprostenol is not widely accepted as a standard therapy for PVOD and PCH.

Montani et al reported the possible efficacy of epoprostenol for PVOD as a bridge to lung transplantation.[11] They successfully treated 12 patients (10 patients with PVOD proven by pathological studies and 2 patients with a clinical diagnosis of PVOD) for 210 days with a maximal dose of 13 ng·kg^{-1}·min^{-1} of epoprostenol. This was the first report to show the clinical application of epoprostenol therapy in a series of patients with PVOD. However, no reports have described the successful

Received August 29, 2011; revised manuscript received February 20, 2012; accepted March 7, 2012; released online April 5, 2012
Time for primary review: 28 days

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ISSN-1346-9843 doi:10.1253/circj.CJ-11-0973

Table 1. Baseline Data, Histological Diagnosis and Outcome

Patient no.	Age (years)	Sex	WHO FC	Mean PAP (mmHg)	%DLco (%)	Histological diagnosis	Outcome
1	42	M	III	39	24	PVOD	Death
2	26	M	IV	60	31	PVOD	Death
3	29	M	IV	114	NA	PVOD	Death
4	11	M	IV	52	64	PCH	Death
5	25	F	IV	55	36	PCH	LDLLT
6	28	F	III	65	81	PVOD	LDLLT
7	16	F	III	63	61	PVOD	LDLLT
8	32	F	III	44	23	PVOD	LDLLT

Age, age at diagnosis; WHO FC, World Health Organization classification of functional status of patients with pulmonary hypertension; PAP, pulmonary artery pressure; %DLco, diffusion capacity of the lung for carbon monoxide expressed as % predicted; M, male; F, female; PVOD, pulmonary veno-occlusive disease; PCH, pulmonary capillary hemangiomatosis; LDLLT, living-donor lobar lung transplantation.

application of epoprostenol for PCH. We report on 8 patients (6 patients with PVOD and 2 with PCH) whose diagnoses were confirmed by pathological examination, and who were treated with a higher dose of epoprostenol and for a longer period than previously reported. With great caution, epoprostenol was safely applied and improved the clinical status in all patients. Careful application of long-term epoprostenol therapy appears to be a safe option and results in a favorable therapeutic outcome in patients with PVOD and PCH.

Methods

We treated patients with pulmonary hypertension with epoprostenol at 2 institutions (Okayama University Hospital and National Hospital Organization Okayama Medical Center, Okayama, Japan) between April 1999 and April 2010. Diagnosis of pulmonary hypertension was made according to a standard diagnostic algorithm including physical examination, chest radiograph, blood tests including screening for the cause of secondary pulmonary hypertension, pulmonary function testing, transthoracic Doppler echocardiography, and right heart catheterization.[12]

Eight patients had the clinical diagnosis of pulmonary hypertension, which was finally determined to be PVOD or PCH, in this study period. We performed a standardized chart review from the medical records to extract clinical data from them retrospectively. We compared clinical, hemodynamic, and radiographic data before and after application of epoprostenol. Data after epoprostenol treatment were obtained at the time when patients achieved the best values for the cardiac index by right heart catheterization.

Seven patients underwent pulmonary function tests when first admitted to our hospital. Vital capacity and forced expiratory volume at 1 s were calculated by using standard formulas. Diffusion capacity of the lung for carbon monoxide (DLco) was measured by the single-breath method and expressed as %DLco (% predicted). Cardiac catheterization was routinely performed at baseline before starting epoprostenol therapy and then repeatedly after starting epoprostenol therapy according to the patients' condition. Chest radiographs were obtained from all patients at the initial visit and were repeatedly taken according to their status. All patients underwent high-resolution computed tomography (CT) of the chest to define coexisting conditions, including pulmonary venous congestion, pulmonary arterial enlargement, atelectasis, or pleural effusion.

Titration of Epoprostenol Therapy

Epoprostenol therapy was initiated at a dose of 0.25–0.5 $ng \cdot kg^{-1} \cdot min^{-1}$, and the dose was gradually titrated upward in increments of 0.5–1.0 $ng \cdot kg^{-1} \cdot min^{-1}$, based on adverse effects and tolerance. When the cardiac index was below 2.0 $L \cdot min^{-1} \cdot m^{-2}$, continuous intravenous catecholamines were added to epoprostenol therapy. On adjusting the dose of epoprostenol, we paid careful attention to hypotension and signs of deterioration of heart failure and pulmonary edema. When the patients' chest radiographs showed deterioration, we stopped increasing the dose of epoprostenol and added diuretics or intravenous infusion of catecholamines, depending on the severity of pulmonary edema. After improvement, titration of the dose of epoprostenol was resumed.

Pathological Examination

No open or thoracoscopic lung biopsy was performed in any of the patients, because all patients were severely ill and they were considered intolerable to a lung biopsy. Lung specimens were obtained by living-donor lobar lung transplantation (LDLLT) or autopsy. Lung tissue was fixed in 10% formalin. Histological sections were stained with hematoxylin and eosin stain and elastica-Masson's trichrome stain.

Statistical Analysis

Results are reported as mean±standard error of the mean. Differences between groups in variables measured at baseline and after epoprostenol therapy were tested by the paired t-test. Differences were considered statistically significant at a P value of <0.05.

Results

Baseline Data, Pathological Findings and Outcome

Eight patients undergoing epoprostenol therapy had the histological diagnosis of PVOD or PCH (Table 1). The patients included 4 males and 4 females with a mean age of 26.0±3.4 years at the time of diagnosis of pulmonary hypertension. At baseline, 4 patients with PVOD were in the World Health Organization (WHO) functional class III and the other 4 patients (PVOD, n=2; PCH, n=2) were in the functional class IV. All patients showed a high mean pulmonary artery pressure (PAP) and 4 patients showed a marked decrease in %DLco as low as below 40%.

Two patients (patients 4 and 5) were finally diagnosed with PCH and the other cases were diagnosed with PVOD. Representative histology is shown in Figure 1. In all cases, foci of

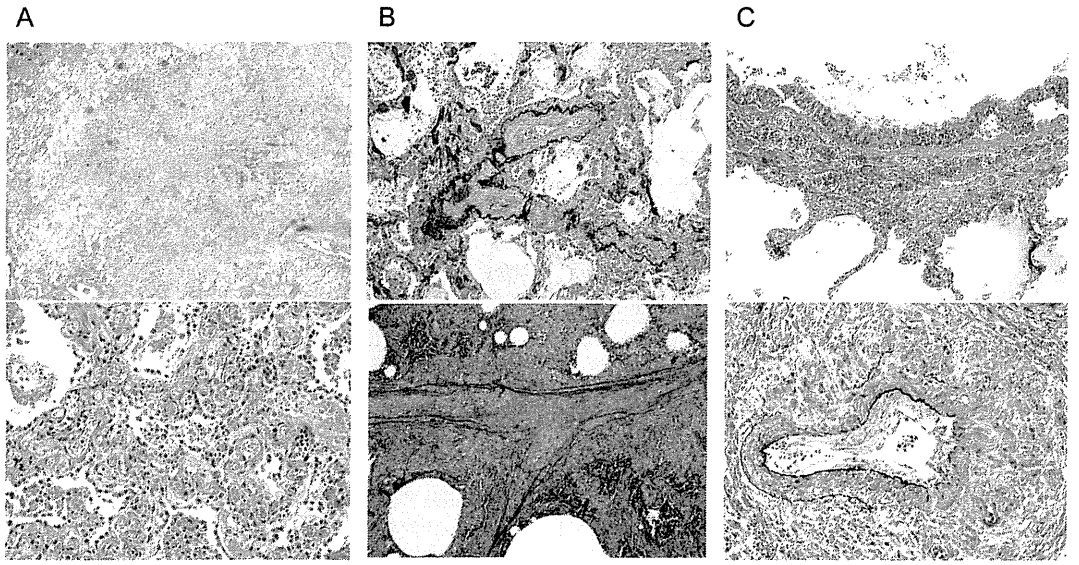

Figure 1. Pathological findings of lung specimens. (**A**) Specimens of pulmonary veno-occlusive disease (PVOD) show centrilobular congestion at low magnification (**Upper panel**) and characteristic alveolar capillaries at a higher magnification (**Lower panel**). These foci are seen in both PVOD and pulmonary capillary hemangiomatosis (PCH) (hematoxylin and eosin stain). (**B**) Venous vessel walls are thickened by intimal fibrous proliferation. Markedly stenosed (**Upper panel**) and completely obliterated (**Lower panel**) veins can be seen in PVOD (elastica-Masson's trichrome stain). (**C**) Proliferating capillaries are shown in the walls of bronchi (**Upper panel**) and arteries (**Lower panel**) in PCH (elastica-Masson's trichrome stain).

Table 2. Clinical and Hemodynamic Data Before and After Epoprostenol Therapy

	Baseline	After epoprostenol therapy	P value
WHO FC (n)			
II	0	5	
III	4	3	
IV	4	0	
6MWD (m)	97.5±39.2	329.4±34.6	<0.001
BNP (pg/ml)	381.3±136.8	55.2±14.4	<0.05
Hemodynamics			
Systolic PAP (mmHg)	89.4±11.0	90.9±4.9	NS
Diastolic PAP (mmHg)	44.1±7.2	43.4±4.0	NS
Mean PAP (mmHg)	61.5±8.1	61.5±3.9	NS
PCWP (mmHg)	7.0±1.3	11.8±3.6	NS
RAP (mmHg)	6.9±2.2	7.6±1.5	NS
SvO_2 (%)	59.6±5.3	64.9±4.8	NS
CI ($L \cdot min^{-1} \cdot m^{-2}$)	2.1±0.1	2.9±0.3	<0.05
PVR ($dyne \cdot s \cdot cm^{-5}$)	1,449.3±194.9	1,096.3±199.5	NS
Epoprostenol therapy			
Duration (days)		164.1±79.7	
Dose ($ng \cdot kg^{-1} \cdot min^{-1}$)		24.4±5.6	
Associated therapy (n)			
Anticoagulation	8	6	
Digitalis	4	3	
Bosentan	2	2	
Sildenafil	2	2	

After epoprostenol therapy, at the time when patients achieved the best values for cardiac index; 6MWD, 6-min walk distance; BNP, plasma concentrations of brain natriuretic peptide; PCWP, pulmonary capillary wedge pressure; RAP, right atrial pressure; SvO_2, mixed venous oxygen saturation; CI, cardiac index; PVR, pulmonary vascular resistance; duration, time from initiation of epoprostenol; NS, not significant; dose, dose of epoprostenol. All other abbreviations are as per Table 1.

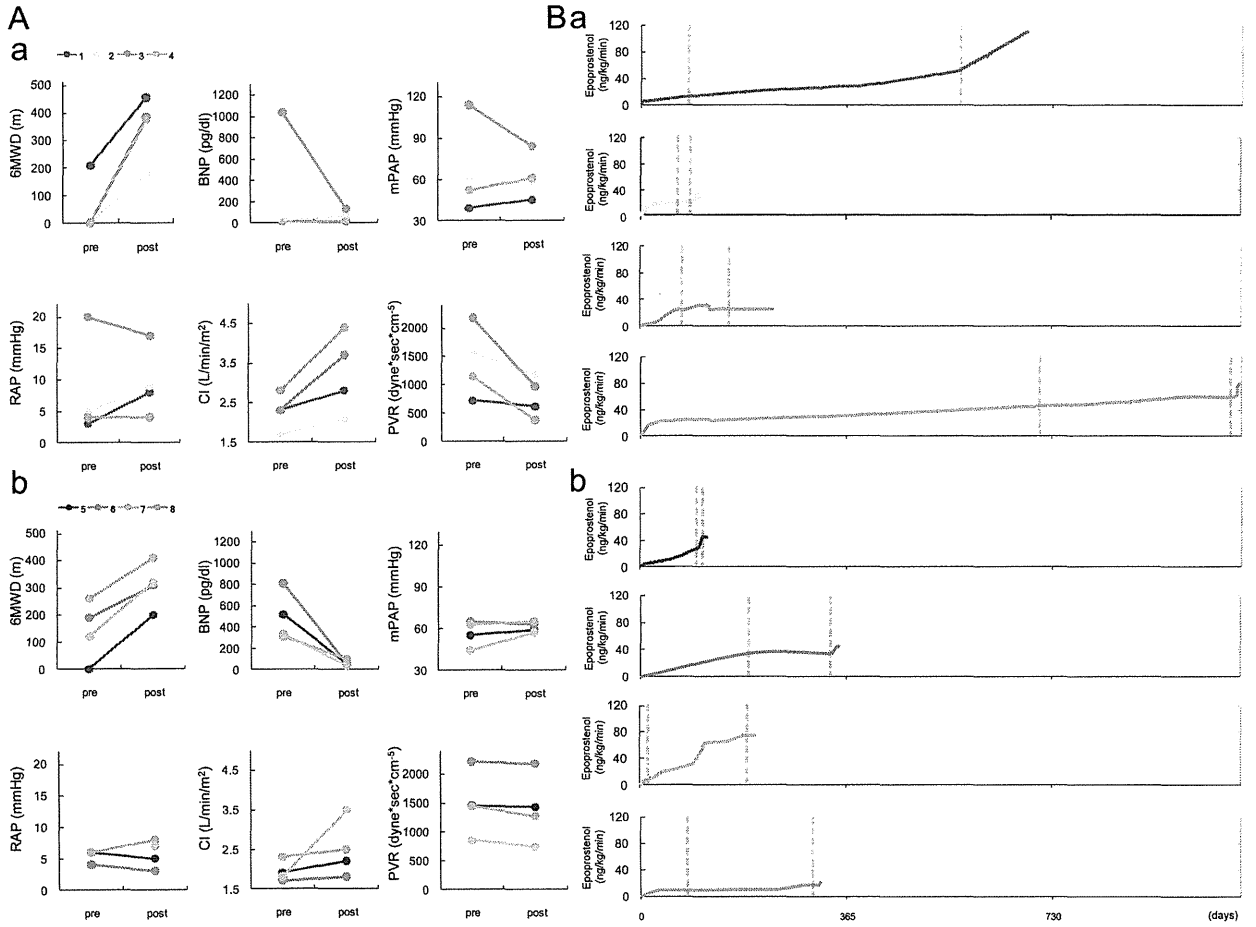

Figure 2. Changes in parameters and dose titration of epoprostenol. (A) Parameters before and after epoprostenol therapy were compared in patients without lung transplantation (a) and patients who underwent living-donor lobar lung transplantation (LDLLT) (b). Data after epoprostenol treatment were obtained at the time when patients achieved the best values for the cardiac index (CI) by right heart catheterization. The 6-min walk distance (6MWD), plasma concentrations of brain natriuretic peptide (BNP), and CI were significantly improved by epoprostenol therapy. mPAP, mean pulmonary artery pressure; RAP, right atrial pressure; PVR, pulmonary vascular resistance. (B) Dose titration of epoprostenol of each patient is shown (patients without lung transplantation (a) and patients who underwent LDLLT (b)). Red dotted lines indicate the time when patients achieved the best values for CI. Gray dotted lines indicate the time when patients showed deterioration.

Table 3. Epoprostenol Therapy and Associated Therapy

Patient no.	After epoprostenol therapy				Final	
	Time from initiation (days)	Dose ($ng \cdot kg^{-1} \cdot min^{-1}$)	Bosentan (mg/day)	Sildenafil (mg/day)	Time from initiation (days)	Dose ($ng \cdot kg^{-1} \cdot min^{-1}$)
1	82	12.5	–	–	685	110.5
2	66	15.0	–	–	102	33.7
3	70	24.9	–	60	234	32.0
4	708	46.3	–	–	1,063	79.2
Mean of patients 1–4	231.5±158.9	24.7±7.7			528.0±216.6	63.9±19.0
5	98	45.0	–	–	115	46.0
6	193	34.9	–	–	351	45.4
7	14	7.5	125	40	202	75.2
8	82	9.0	250	–	318	21.0
Mean of patients 5–8	96.8±36.9	24.1±9.4			246.5±54.2	46.7±11.1

After epoprostenol therapy, at the time when patients achieved the best values for cardiac index; final, at the time of lung transplantation or death; time from initiation, time from initiation of epoprostenol therapy; dose, dose of epoprostenol.

centrilobular congestion were observed at low magnification, and characteristic dilatation of alveolar capillaries was observed at a higher magnification (Figure 1A). Hemosiderin-laden macrophages were often observed in the alveolar space. PVOD was characterized by marked stenosis and occlusion of small intrapulmonary veins (Figure 1B). Vessel walls were thickened by intimal fibrous proliferation. In patients 4 and 5, invasive proliferation of capillaries were also observed in the walls of bronchi and arteries, leading to the diagnosis of PCH (Figure 1C). These capillaries were engorged and tortuous.

Four patients successfully underwent LDLLT and the remaining 4 patients had no suitable living donors of the lung and finally died while awaiting cadaveric lung transplantation. The causes of death were respiratory failure or concomitant respiratory infection. No patient died from adverse effects of epoprostenol itself.

Patient Characteristics Before Epoprostenol Therapy

Patient characteristics before epoprostenol therapy are shown in Table 2. All patients were in WHO functional class III and IV. The 4 patients who were in WHO functional class IV could not walk because of severe oxygen desaturation at baseline. The other 4 patients in WHO functional class III could only walk approximately 200 m (Figure 2A). Plasma BNP levels were not always elevated. Three patients showed low BNP levels in spite of the severity of their general condition and inability to walk. For the pulmonary function test, 2 patients showed mild restrictive defects (62% and 72%), and another patient showed a mild obstructive defect (65%). Overall, lung function was within normal limits (%vital capacity: 86.4±6.3%; forced expiratory volume at 1 s: 77.4±3.1%) except for low %DLco (45.8±8.6%). All patients manifested pulmonary hypertension with a mean PAP of 61.5±8.1 mmHg on right heart catheterization. Pulmonary capillary wedge pressure and right atrial pressure were within the normal range in all patients. In 4 patients, the cardiac index was lower than 2.0 $L \cdot min^{-1} \cdot m^{-2}$.

Table 4. Radiographic Findings at Baseline and After Epoprostenol Therapy

Radiographic findings	PVOD and PCH (n=8)
Baseline	
Dilated pulmonary arteries	8
Kerley B lines	2
Interstitial infiltrates	8
Ground-glass opacities	7
Pleural effusion	2
Interlobular thickening	8
Lymphadenopathy	3
After epoprostenol therapy	
Increase in pleural effusion	3
Thickened interlobular septae	8
Deterioration of ground-glass opacities	8

Data indicates the number of patients.
PVOD, pulmonary veno-occlusive disease; PCH, pulmonary capillary hemangiomatosis.

Efficacy of Epoprostenol Therapy

Patients were cautiously treated with epoprostenol for 387.3±116.3 days (range, 102–1,063 days) (Table 3; Figure 2B). The maximum dose of epoprostenol given was 55.3±10.7 $ng \cdot kg^{-1} \cdot min^{-1}$ (range, 21.0–110.5 $ng \cdot kg^{-1} \cdot min^{-1}$). Patients who had no chance to undergo a lung transplantation had epoprostenol therapy applied for 528.0±216.6 days and the maximum dose was 63.9±19.0 $ng \cdot kg^{-1} \cdot min^{-1}$. The best value for cardiac

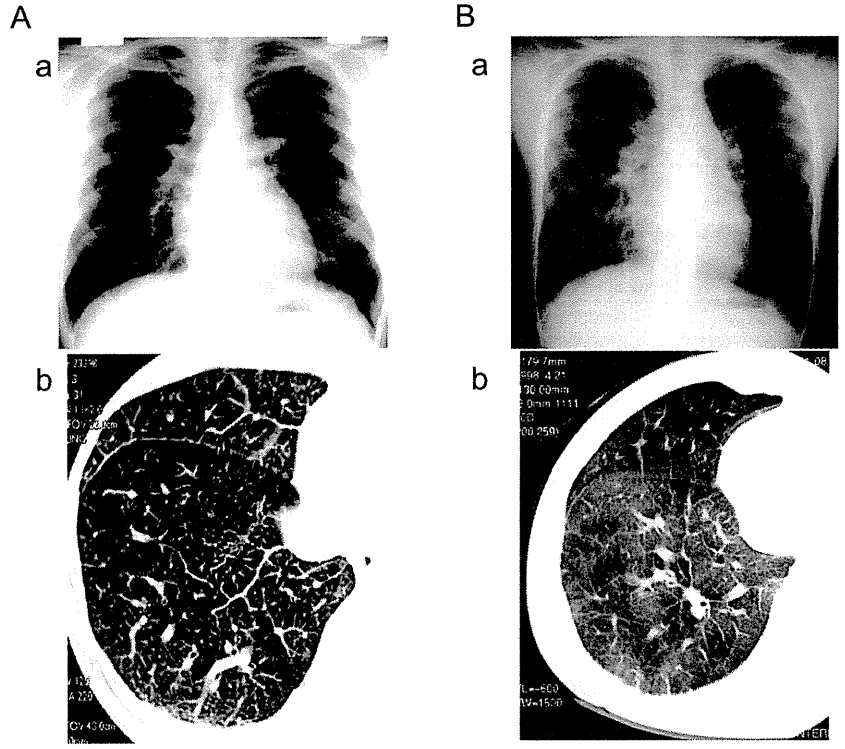

Figure 3. Representative radiographic findings. (A) Radiographic findings of pulmonary veno-occlusive disease (patient 1). (a) A chest radiograph shows a prominent main pulmonary artery and interstitial infiltrates. (b) A chest computed tomography (CT) scan of the right lobe shows multiple thickened interlobular septal lines, ground-glass opacities, and pleural effusion. (B) Radiographic findings of pulmonary capillary hemangiomatosis (patient 4). (a) A chest radiograph shows a prominent main pulmonary artery and main truncus of the right pulmonary artery. Faint nodular opacities and numerous Kerley B lines are also shown. (b) A chest CT scan shows diffuse, ill-defined, ground-glass opacities, and interlobular septal thickening is less prominent.

Table 5. Flow of Supplemental Oxygen Required Before and After Starting Epoprostenol Therapy

Patient no.	Baseline	Best	Later
1	3	2	9
2	2	5	8
3	3	2	15
4	4	4	10
5	2	3	12
6	NA	3	10
7	3	3	12
8	3	4	10
P value		NS	<0.01

Data indicate the flow of supplemental oxygen (L/min).
Repeated-measures analysis of variance with Bonferroni correction was performed. P values indicate "best" and "later" values compared with the "baseline" value.
Baseline, before starting epoprostenol therapy; best, at the time when patients achieved the best values for cardiac index; later, maximum oxygen flow required while the dose of epoprostenol was increased later.

index was obtained at 164.1±79.7 days after initiation of epoprostenol with a dose of 24.4±5.6 ng·kg^{-1}·min^{-1}.

After the application of epoprostenol, the WHO functional class improved at least temporarily to class II or III in all patients. The mean 6-min walk distance significantly increased from 97.5±39.2 to 329.4±34.6 m (P<0.001) (Table 2; Figure 2A). As mentioned above, plasma levels of BNP were not always elevated at baseline. In patients who had high BNP levels prior to epoprostenol therapy, BNP levels were significantly reduced after therapy. In total, the mean BNP levels were significantly reduced from 381.3±136.8 to 55.2±14.4 pg/ml (P<0.05). The mean cardiac index significantly improved from 2.1±0.1 to 2.9±0.3 L·min^{-1}·m^{-2} (P<0.05). However, the mean PAP and right atrial pressure did not change between before and after epoprostenol therapy. Although mixed venous oxygen saturation was increased and pulmonary vascular resistance was decreased after epoprostenol therapy, these differences were not statistically significant.

Associated Therapy

Associated therapy before and after epoprostenol therapy is shown in Tables 2 and 3. At baseline, anticoagulation and diuretics were used in all patients, digitalis was given in 4 patients (patients 2, 5, 6, and 7), and no calcium channel blockers were used in any of these patients. An endothelin receptor antagonist, bosentan, was used in 2 patients (patient 7: 125 mg/day; patient 8: 250 mg/day) and a phosphodiesterase 5 inhibitor, sildenafil, was used in 2 patients (patient 3: 60 mg/day; patient 7: 40 mg/day). The doses of bosentan and sildenafil were unchanged during epoprostenol therapy. Catecholamines were not used at the time when patients achieved the best values for the cardiac index. Anticoagulation was discontinued in 2 patients (patients 3 and 4) based on our previous report regarding the risk of alveolar hemorrhage induced by concomitant use with epoprostenol.[13] Digitalis was stopped in patient 5 who manifested bradycardia. All other medications were unchanged after epoprostenol therapy.

Radiographic Changes and Oxygen Supplementation During Epoprostenol Therapy

All 8 patients manifested atypical radiographic features as IPAH at baseline (Table 4; Figure 3). Their chest radiographs revealed not only dilated pulmonary arteries and enlargement of the heart, but also peripheral interstitial infiltrates in both lung fields, and sometimes prominent septal lines. High-resolution CT scans showed pleural effusion, thickened interlobular septa, bilateral ground-glass opacities, and a mosaic pattern of lung attenuation. Lymphadenopathy in the mediastinum, which is sometimes observed as a reactive adenopathy in PVOD, was detected in 1 patient with PVOD and 2 patients with PCH. After initiation of epoprostenol therapy, all patients' chest X-rays or CTs showed thickened interlobular and intralobular septae and an increased density of interstitial opacities. Three of them also showed an increase in pleural effusion. At that time, we temporarily stopped increasing the dose of epoprostenol and added diuretics and/or intravenous infusion of catecholamines. After congestion improved, we started to titrate the dosage of epoprostenol again.

Before epoprostenol therapy, patients required oxygen supplementation with 2.9±0.3 L/min (Table 5). At the time when patients achieved the best values for cardiac index, patients needed 3.3±0.4 L/min of supplemental oxygen. As the dose of epoprostenol was increased, patients showed deterioration of oxygen desaturation and an increase in interstitial infiltrates on chest X-rays. They finally needed an oxygen supplement at a significantly higher flow (10.8±0.8 L/min) than they did before epoprostenol therapy (P<0.01).

Discussion

Among a variety of diseases that can lead to pulmonary hypertension, PVOD and PCH are especially rare, and their classification has been changed at all the World Symposiums on Pulmonary Hypertension. In the previous classification of pulmonary hypertension, they were categorized in a subgroup of pulmonary arterial hypertension, termed "pulmonary arterial hypertension associated with significant venous or capillary involvement".[2] In the most recent Dana Point classification, these diseases are classified as Group 1', similar to but with some differences from Group 1, because of their similarities in histological changes, clinical presentations, risk factors and having shared mutations in the BMPR2 gene, similar to that for IPAH.[3]

The prognosis of PVOD and PCH is still unknown because of the rareness of the disease. It is believed to be poor, with most patients with PVOD dying within 2 years from the initial presentation.[7] Most PCH patients rapidly progress to death over several months of the clinical disease.[14] In the last decade, PAH-targeted drugs have improved the survival of patients with PAH.[6,15,16] However, no medical treatment has been proven to improve the survival of patients with PVOD and PCH. Therefore, patients with PVOD and PCH have a higher mortality and a lower chance of survival compared with patients with IPAH.

Currently, lung transplantation is the only method to cure these diseases and patients who desire it are placed on the list for lung transplantation as soon as possible.[4] However, there are few organ donors available to undergo cadaveric lung transplantation. In Japan, where organ transplantation has been recently introduced, chances of transplantation are very limited and the mean waiting time for lung transplantation is reported to be approximately 3 years. In most cases, it is difficult for patients to survive for this long period of time considering their poor prognosis. Although LDLLT is expected to be an alternative for cadaveric lung transplantation, there are more strict criteria for donors of LDLLT.[17,18] Not all patients and their families who desire to receive lung transplantation can

undergo LDLLT. A therapeutic option is required for patients waiting for a suitable donor or for those who are not candidates for lung transplantation.

Continuous intravenous infusion of epoprostenol has been reported to improve the prognosis of IPAH.[6,19] However, its indication for PVOD and PCH is still controversial. Some reports have cautioned against the possibility of causing massive pulmonary edema by application of epoprostenol for patients with PVOD or PCH.[9,10] Application of epoprostenol for PVOD or PCH might be unsuccessful because when the pulmonary arterioles dilate and resistance of the pulmonary veins remains fixed, transcapillary hydrostatic pressure might increase and pulmonary edema might occur.[20] In contrast, some patients with PVOD have been reported to show temporary amelioration by application of epoprostenol.[7,8] There is 1 case report that showed that long-term epoprostenol therapy improved exercise capacity and pulmonary hemodynamics in PVOD.[8] The authors concluded that in this case, the administration of epoprostenol played a role in the regulation of vascular tone in pulmonary venules rather than in the pulmonary arteries. Detailed hemodynamic measurements showed that microvascular pressures initially increased during an infusion of no more than $6\,ng \cdot kg^{-1} \cdot min^{-1}$ of epoprostenol, but at higher doses, cardiac output increased and the calculated pulmonary vascular resistance decreased.[21] To the best of our knowledge, there are no reports that have described patients with PCH being successfully treated with epoprostenol.

We administered epoprostenol to 8 patients with PVOD or PCH because they had no other therapeutic option besides lung transplantation. In our cases, we cautiously administered epoprostenol, starting with a low dose. When we increased the dose of epoprostenol too quickly, an imbalance of dilatation between pulmonary arterioles and veins occurred. However, if we slowly increased the dose in a step-wise manner and used diuretics or inotropes as necessary, the transcapillary hydrostatic pressure decreased and we could avoid severe pulmonary congestion.

For the successful treatment of PVOD and PCH with epoprostenol, early recognition and diagnosis of PVOD/PCH are essential in addition to the careful application of epoprostenol. A lung biopsy is the only method of definitively diagnosing PVOD and PCH. However, in most cases, it is difficult to perform a lung biopsy because of the severity of the patients' condition. This is why it is important to clinically diagnose PVOD/PCH with available data and results of examinations. It is vital to be aware of poor oxygenation, low DLco, and distinct radiographic findings to diagnose or suspect PVOD and PCH.[20,22] In the present study, all patients presented with marked oxygen desaturation on exertion and a severe decrease in DLco. Their chest radiographs and high-resolution CT scans revealed radiographic findings that were characteristic for PVOD and PCH, but not IPAH (**Table 4**; **Figure 3**).[14,23] Early recognition of PVOD/PCH in patients with pulmonary hypertension is possible based on these clinical and radiographic characteristics. This might lead to careful introduction and dose adjustment of epoprostenol and to successful treatment of these complicated diseases.

The present study showed that as a result of epoprostenol therapy, clinical and hemodynamic data were improved (**Table 2**; **Figure 2**), at least temporarily. All patients were critically ill before starting epoprostenol therapy. The mean 6-min walk distance, which is reported to correlate well with the prognosis in IPAH, was significantly increased after therapy. Our data showed that epoprostenol significantly improved exercise capacity and increased cardiac output of patients with PVOD or PCH, but did not decrease PAP and right atrial pressure, which are known to determine the survival of IPAH.[24] This might be one of the reasons why patients eventually showed deterioration. Most patients showed maximal improvement within half a year after starting epoprostenol therapy. In some cases, with cautious control of epoprostenol therapy, there is a possibility of longer survival than previously reported. The dose of epoprostenol given at the time when patients showed maximal improvement in clinical status was $24\,ng \cdot kg^{-1} \cdot min^{-1}$, regardless of whether they could undergo LDLLT. Although they could walk further in the 6-min walk test because of increased cardiac output with epoprostenol therapy, patients showed deterioration of interstitial infiltration in chest X-rays and CT scans and needed a higher flow of supplemental oxygen. Considering severe oxygen desaturation and limited prognosis with epoprostenol therapy, further studies are required to determine better therapeutic strategies to treat PVOD and PCH.

Conclusions

We applied epoprostenol treatment to 8 patients with atypical clinical and radiographic findings such as IPAH. Histological findings revealed that 6 patients had PVOD and the other 2 patients had PCH. Epoprostenol was applied at a higher dose and for a longer period than previously reported cases, and worked as a bridge to lung transplantation for 4 patients. It was also applied in 4 patients who had no chance to undergo lung transplantation. All patients showed temporary amelioration in WHO functional class, exercise capacity, and cardiac index with long-term epoprostenol therapy. When patients are suspected of having PVOD or PCH by characteristic clinical and radiographic findings, careful application of epoprostenol can be considered as a bridge to lung transplantation or as the only method to improve their clinical condition because they have no other therapeutic options.

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Annual Change in Pulmonary Function and Clinical Phenotype in Chronic Obstructive Pulmonary Disease

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Rationale: **Although the rate of annual decline in FEV_1 is one of the most important outcome measures in chronic obstructive pulmonary disease (COPD), little is known about intersubject variability based on clinical phenotypes.**

Objectives: **To examine the intersubject variability in a 5-year observational cohort study, particularly focusing on emphysema severity.**

Methods: **A total of 279 eligible patients with COPD (stages I–IV: 26, 45, 24, and 5%) participated. We conducted a detailed assessment of pulmonary function and computed tomography (CT) at baseline, and performed spirometry every 6 months before and after inhalation of bronchodilator. Smoking status, exacerbation, and pharmacotherapy were carefully monitored. Emphysema severity was evaluated by CT and annual measurements of carbon monoxide transfer coefficient.**

Measurements and Main Results: **Using mixed effects model analysis, the annual decline in post-bronchodilator FEV_1 was -32 ± 24 (SD) ml/yr ($n = 261$). We classified the subjects of less than the 25th percentile as Rapid decliners, the 25th to 75th percentile as Slow decliners, and greater than the 75th percentile as Sustainers (-63 ± 2, -31 ± 1, and -2 ± 1 [SE] ml/yr). Emphysema severity, but not $\%FEV_1$, showed significant differences among the three groups. Multiple logistic regression analysis demonstrated that the Rapid decliners were independently associated with emphysema severity assessed either by CT or carbon monoxide transfer coefficient. The Sustainers displayed less emphysema and higher levels of circulating eosinophils.**

Conclusions: **Emphysema severity is independently associated with a rapid annual decline in FEV_1 in COPD. Sustainers and Rapid decliners warrant specific attention in clinical practice.**

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Although the rate of annual decline in FEV_1 is an important outcome measure in chronic obstructive pulmonary disease, little is known about intersubject variability based on clinical phenotypes.

What This Study Adds to the Field

The rate of annual change in post-bronchodilator FEV_1 is highly variable over a period of 5 years among patients with chronic obstructive pulmonary disease who receive appropriate therapy. Emphysema severity is independently associated with a rapid annual decline in FEV_1.

Keywords: annual decline in FEV_1; emphysema; diffusing capacity; rapid declines; sustainers

Chronic obstructive pulmonary disease (COPD) is a major cause of morbidity and mortality, and represents a substantial economic and social burden worldwide. COPD is characterized by progressive airflow limitation that is not fully reversible (1). FEV_1 and the rate of annual decline in FEV_1 are the most widely used outcome measures for clinical trials of pharmacotherapy or other interventions, such as smoking cessation, for COPD (2). To date, the factors that have been shown to convincingly affect annual decline in FEV_1 are smoking status (3, 4) and frequency of exacerbation (5), although some pharmacotherapies (6, 7) have only recently been shown to have the potential to alleviate this natural decline.

The airflow limitation in COPD is caused by a mixture of abnormal inflammatory responses in small airways and parenchymal destruction of the lungs (emphysema), the relative contributions of which vary from person to person (1, 8). However, there have been few studies clearly demonstrating an independent effect of emphysema severity on annual decline in FEV_1 in patients with COPD (9–11), although the emphysema phenotype is reportedly associated with poor quality of life, osteoporosis, arterial stiffness, lung cancer, and poor prognosis (12–16). In addition to emphysema severity, there are many other phenotypes based on clinical parameters, such as spirometry, nutritional status, exercise tolerance, and exacerbation, which have recently attracted attention (17, 18).

The Hokkaido COPD cohort study is a carefully designed multicenter observational cohort, which primarily aims to examine the annual decline in FEV_1 over a period of 5 years based on clinical phenotypes in patients with smoking-related COPD. At baseline, we found that emphysema severity varied widely, even in patients with the same spirometric stage of

(*Received in original form June 8, 2011; accepted in final form October 5, 2011*)

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Supported by 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. The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Author Contributions: M.N. and H.M., study concept and design, acquisition of data, interpretation of data, and drafting and finalizing of the manuscript; K.N., S.K., Y.N., M.H., K.S., T.B., S.F., T.I., Y.A., and S.O., study design, acquisition of data, and interpretation of data; and Y.M.I., statistical analysis and interpretation of data.

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This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org

Am J Respir Crit Care Med Vol 185, Iss. 1, pp 44–52, Jan 1, 2012

Originally Published in Press as DOI: 10.1164/rccm.201106-0992OC on October 20, 2011
Internet address: www.atsjournals.org

COPD, and emphysema phenotype was associated with poorer quality of life and lower body mass index (BMI) independently of pulmonary function (19). Thus, we were particularly interested in the independent effects of emphysema phenotype on the annual decline in FEV_1.

In this study, we evaluated emphysema severity in two ways: by computed tomography (CT) (20, 21) and by annual measurements of carbon monoxide diffusing capacity (D_{LCO}) (22, 23). We measured pulmonary function every 6 months before and after inhaling a short-acting bronchodilator, whereas we monitored other confounding factors, such as smoking status, exacerbation, and pharmacotherapy, during the study period. We here demonstrate that emphysema severity is independently associated with a rapid decline in FEV_1 in COPD. Some of the results in this manuscript have been previously reported in the form of an abstract (24).

METHODS

Participants

A total of 330 patients with respiratory physician-diagnosed COPD were recruited at Hokkaido University Hospital, Sapporo, Japan, and nine affiliated hospitals from May 2003 to May 2005. All were aged 40 years or older, and were either current or former smokers with a smoking history of at least 10 pack-years. Subjects with asthma diagnosed clinically, but not based on any bronchodilator reversibility, at the time of study entry were excluded. Details of enrolment of the subjects and other exclusion criteria are listed in the online supplement. Thirty patients were excluded for consent withdrawal or were ineligible for inclusion before visit 1, and a total of 300 patients were followed. During the first follow-up year (visits 1–3), the diagnosis was reconfirmed based on the spirometric criteria of the Global Initiative for Chronic Obstructive Lung Disease guidelines (1) (a ratio of post-bronchodilator FEV_1 to FVC <0.70), and included those subjects for later analysis who fulfilled the criteria even once among the three visits. As a result, a total of 279 subjects with COPD (stage I, 26%; stage II, 45%; stage III, 24%; and stage IV, 5%) were eligible for subsequent follow-up (Figure 1). The Ethics Committee of Hokkaido University School of Medicine approved the study protocol and written informed consent was obtained from all participants.

Study Protocol

Most subjects, except for those with stage I COPD, visited outpatient clinics at each hospital monthly or bimonthly for regular clinical check-ups (*see* online supplement), and all were advised to participate in the follow-up study every 6 months for the following 5 years (from visits 1–11). Each physician was allowed to manage and treat subjects in such a way that he or she considered appropriate at all times, and thus changes in smoking status or pharmacotherapy often occurred in many subjects during the study period. Particularly, the subjects had been advised to cease smoking before they were enrolled in the study and those who could not give up smoking by entry were continuously encouraged to do so during the follow-up period. On the first visit, demographic information, including sex, age, height, weight, smoking history, medical history and any medications, and information on pulmonary symptoms were collected. Every 6 months, any changes in smoking status, medical history, and pharmacotherapy were monitored. Subjects were described as continuous, intermittent, or former smokers, depending on the smoking status during the study period. Actual use of any respiratory medicine was recorded on each visit, and usage was considered to be positive when any respiratory medicine was used for more than half of the entire follow-up period. Assessment of exacerbation during the study is described in detail in the online supplement (25). Health-related quality of life assessed by St. George's Respiratory Questionnaire (26) was examined every year, and blood was sampled also every year for measurements of circulating blood cell counts, serum IgE, erythrocyte sedimentation rate, or C-reactive protein. We confirmed at baseline that there were no subjects with α-antitrypsin deficiency. If permitted, blood samples for measurements of biomarkers or genetic samples were stored for later analysis.

Pulmonary Function Tests

Spirometry before and after inhalation of a bronchodilator was conducted on every visit, and D_{LCO} was examined every 12 months (visits 1, 3, 5, 7, 9, and 11). On each visit, withdrawal of any respiratory medicine was confirmed before testing. D_{LCO} was measured by the single breath method immediately after prebronchodilator spirometry and results were corrected by hemoglobin concentration, using the equation provided by American Thoracic Society guidelines (27), but not corrected by carboxyhemoglobin level. Transfer factor coefficient of the lung for carbon monoxide (K_{CO}), which is D_{LCO} corrected by alveolar volume, is used for later analysis. D_{LCO} and K_{CO} were expressed as percentages of predicted normal values (28). The reversibility of airflow limitation was evaluated before and at 30 minutes after inhalation of salbutamol (0.4 mg) at visits 1–5, 7, 9, and 11, or at 60 minutes after inhalation of oxitropium (0.4 mg) at visits 6, 8, and 10. Further details of pulmonary function tests and the reason for use of two classes of bronchodilators (29) are described in the online supplement.

Chest CT Analyses

Chest CT scans were performed in the supine position, with breath held at full inspiration. The CT scanners used in this study and details of technical parameters are described in the online supplement. Severity of emphysema was visually assessed by three independent pulmonologists according to the modified Goddard scoring system (19, 30). Computerized three-dimensional CT analysis of the lung (31, 32) was performed using custom software (AZE Ltd., Tokyo, Japan) only in those subjects who visited Hokkaido University Hospital (n = 108). Computerized analysis was not performed for all subjects because we could not obtain the Digital Imaging and Communications in Medicine images from some hospitals and it was considered to be hard to satisfactorily standardize quantitative computerized assessment of emphysema when the study was started. A good correlation was obtained between visually assessed severity of emphysema and severity based on computerized analysis (n = 108; $r = 0.79$; $P < 0.0001$) (*see* Figure E1 in the online supplement). Details for both visual assessment and computerized assessment are provided in the online supplement.

Statistical Analyses

Summary statistics for subject characteristics were constructed using frequencies and proportions for categorical data, and mean ± SD for continuous variables, or median values with interquartile range for skewed continuous variables. Two types of analysis were performed for determination of individual annual changes in FEV_1. First, linear regression analysis was used for each individual who underwent spirometric measurements more than seven times with a follow-up period of 3 to 5 years (n = 217). Normal distribution of intersubject variation in annual changes in FEV_1 in the first analysis was confirmed by Kolmogorov-Smirnov tests. A mixed-effects model was then used for those subjects who had at least three spirometric measurements to accommodate loss-to-follow-up subjects, and the Best Linear Unbiased Prediction of the annual changes in post-bronchodilator FEV_1 (milliliter per year) was estimated using the random coefficient regression model (33). Univariate analysis used chi-square tests for categorical variables, one-way analysis of variance for quantitative continuous variables with Tukey multiple comparison tests, and Kruskal-Wallis with Mann-Whitney *U*-tests for skewed continuous variables. Logistic regression analysis was performed where necessary (34). Data are shown as means ± SEM, unless otherwise specified. *P* value less than 0.05 was considered statistically significant.

RESULTS

The flow chart in Figure 1 depicts how the subjects were followed. Of 279 subjects with spirometry-confirmed COPD by the first year, 216 (77%) completed a 4-year follow-up period, and 195 (70%) completed a 5-year follow-up period (*see* Figure E2). The reasons for dropout (n = 84) during the study period are shown in Figure E3, and among these patients, 34 died.

First, we examined the annual change in FEV_1 for 217 subjects who had more than seven spirometric measurements,

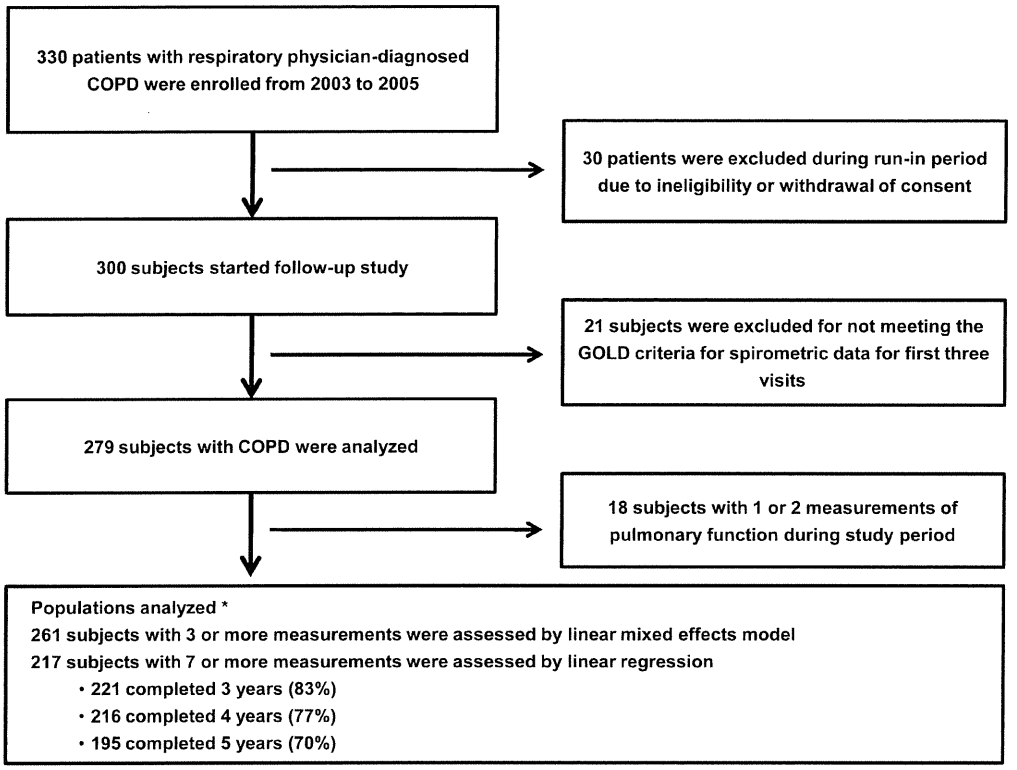

Figure 1. Flow chart for subject selection. COPD = chronic obstructive pulmonary disease; GOLD = Global Initiative for Chronic Obstructive Lung Disease. * Subjects with three or more measurements of pulmonary function for the 5-year study period (n = 261) were included in the analysis of annual changes in FEV_1 using a mixed effects model after confirming normal distribution of subjects with seven or more measurements of pulmonary function.

with a follow-up period of 3–5 years (*see* Figure E4). The annual changes in FEV_1, either for prebronchodilator values (mean ± SD: −26 ± 41 ml/yr, n = 215) or for post-bronchodilator values (−31 ± 38 ml/yr, n = 217) varied widely among subjects with normal distribution. Of particular note is that a significant proportion of subjects maintained pulmonary function over the study period.

We then used a linear mixed-effects model to assess the annual changes in FEV_1 for all subjects who had at least three spirometric measurements (n = 261), regardless of whether they dropped out during the study period. The calculated annual change in post-bronchodilator FEV_1 was −32 ± 24 SD ml/yr (Figure 2A). We next classified the subjects into three groups based on the magnitude of annual change in FEV_1 (Tables 1 and 2). We labeled those of less than the 25th percentile as Rapid decliners (−63 ± 2 SE ml/yr); the 25th to 75th percentile as Slow decliners (−31 ± 1 SE ml/yr); and greater than the 75th percentile as Sustainers (−2 ± 1 SE ml/yr). Figures 2B and 2C displays the chronologic course of the mean FEV_1 and that expressed as percent change from baseline in the three groups.

There were no significant differences in spirometric data, reversibility of airflow limitation, or quality of life measurements among the three groups at baseline (Table 1). Furthermore, smoking status, exacerbation frequency, and pharmacotherapy during the study period did not significantly differ. Rather, there were fewer continuous smokers and pharmacotherapy was more intense in the Rapid decliners, although these differences were not statistically significant (Table 2). Among the clinical features, BMI was significantly lower in the Rapid decliners compared with the Sustainers. Interestingly, circulating eosinophil count was significantly higher in the Sustainers compared with the other two groups. Most markedly, emphysema score was significantly higher in the Rapid decliners on visual assessment of all subjects compared with the other two groups ($P < 0.05$) (Figure 3A). Computerized assessment of a limited number of subjects (n = 108) revealed the same results (Figure 3B). Consistent with this, %Kco at baseline was the lowest in the Rapid decliners, followed by the Slow decliners, and then the Sustainers (Figure 3C). Furthermore, the annual decline in D_{LCO} or Kco expressed as percent change from baseline was significantly greater in the Rapid decliners compared with the other two groups ($P < 0.05$) (Figure 4A) or compared with the Slow decliners ($P < 0.05$) (Figure 4B).

To elucidate independent predictors for the Rapid decliners or the Sustainers we then used logistic regression models between the Rapid decliners and the Slow decliners, and between the Sustainers and the others (Tables 3 and 4). We included 10 items as potential confounding factors and treated emphysema score and %Kco separately, because they were mutually correlated. In addition, BMI was eliminated from this analysis because it was closely related to emphysema severity. As a result, higher emphysema score or lower %Kco was an independent predictor for the Rapid decliners compared with the Slow decliners. In addition, higher circulating neutrophil count was also a significant predictor for the Rapid decliners. However, the Sustainers were significantly associated with more chronic bronchitis symptoms, higher circulating eosinophil count, and lower emphysema score or higher %Kco.

Finally, the rate of annual decline in FEV_1 was examined in subjects who were classified based on emphysema severity at baseline (19). As expected, the annual decline was significantly larger in subjects who were diagnosed as having severe emphysema (score ≥2.5) compared with those having no or mild emphysema (score <1; $P < 0.001$) or moderate emphysema (score ≥1, and <2.5; $P < 0.05$) (Figure 5A). The same trend was found when subjects were classified into three groups based on percent low attenuation volume at baseline; subjects who showed lower percent low attenuation volume (<25th percentile) demonstrated a larger annual decline in FEV_1 compared with the other two groups (interquartile and >75th percentile) (Figure 5B).

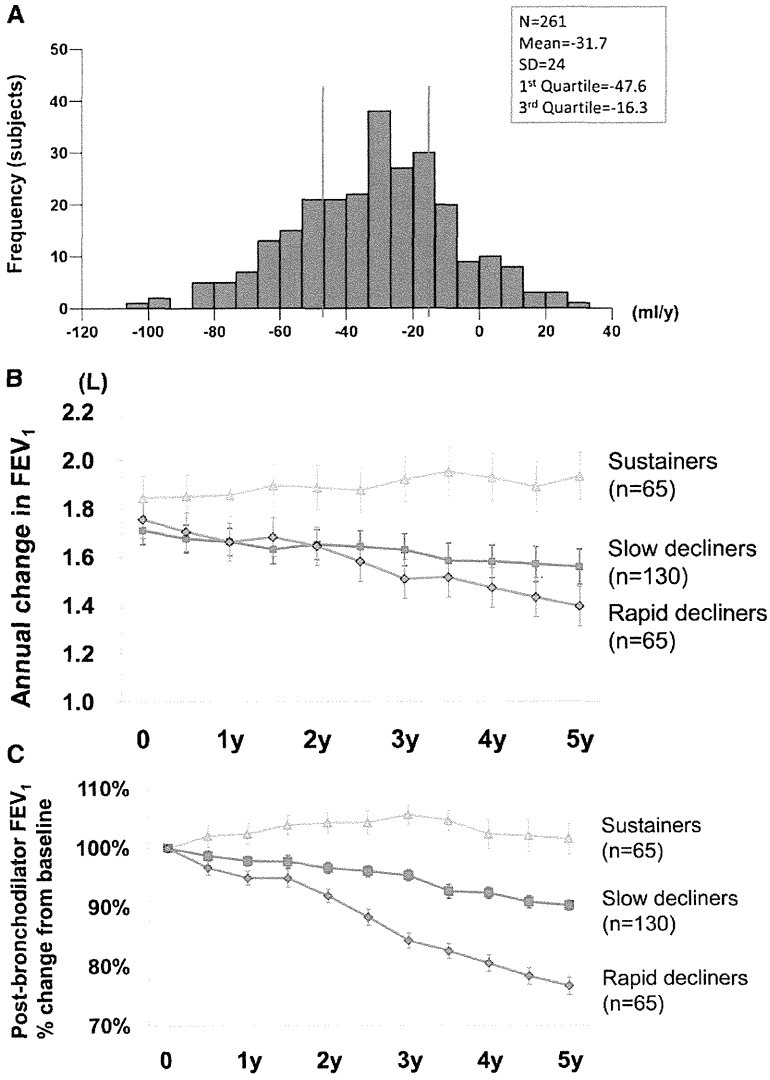

Figure 2. Annual change in post-bronchodilator FEV_1 among three groups classified by annual declines in post-bronchodilator FEV_1. (*A*) Annual changes in post-bronchodilator FEV_1 varied widely, with the mean (SD) post-bronchodilator FEV_1 being −32 (24) ml/yr (n = 261). The subjects were categorized into three groups using the 25th percentile and the 75th percentile (*lines* in *A*): less than the 25th percentile as Rapid decliners (−63 ± 2 ml/yr, mean ± SEM); the 25th to 75th percentile as Slow decliners (−31 ± 1 ml/yr); and greater than the 75th percentile as Sustainers (−2 ± 1 ml/yr). (*B*) Mean post-bronchodilator FEV_1 (with SEM) expressed as absolute values. (*C*) Mean post-bronchodilator FEV_1 (with SEM) expressed as percent changes from baseline.

DISCUSSION

In this study, we demonstrated that there is a wide variability in the rate of annual change in FEV_1, with normal distribution among the subjects with COPD, over a period of 5 years. Most importantly, we found that emphysema severity assessed by either CT or %Kco was independently associated with a rapid annual decline in FEV_1 and that the Rapid decliners displayed an accelerated decline in D_{LCO} and Kco over the 5-year follow-up period when expressed as percent change from baseline compared with the other two groups. Of additional note is that a significant proportion of subjects maintained FEV_1 over a period of 5 years and they displayed increased levels of circulating eosinophils, although within normal limits.

This study has several strengths. First, it was a carefully designed, carefully conducted, prospective cohort, and the final follow-up rate of the subjects was very high (70%) at the 5th year, and even higher (82%) if one considers the number of the subjects who died. We accurately recorded confounding factors, such as smoking status, exacerbation, and pharmacotherapy during the study period. Second, assessment of annual change in FEV_1 was based on measurements every 6 months over a period of 5 years; indeed, 217 subjects underwent more than seven measurements, and as many as 197 subjects underwent more than nine measurements. Burrows (35) noted in the early 1980s that the variability in FEV_1 makes true rates of decline difficult to measure in individual patients, unless there are numerous time points over many years of follow-up. He also noted that intersubject variation in calculated rates of annual decline in FEV_1 would be smaller with longer follow-up periods. Finally, emphysema severity was evaluated in this study using two methods: CT scans (20, 21) and annual measurements of D_{LCO} and Kco (22, 23). This is important in the assessment of emphysema severity because neither of the two modalities is a perfect predictor of emphysema severity on a pathologic basis, and should thus be considered to be mutually complementary.

In most clinical trials of pharmacotherapy for COPD, including recently reported large-scale trials, annual decline in FEV_1 is one of the most important outcome measurements (36, 37). Indeed, COPD is characterized by progressive airflow limitation, and thus an attempt to alleviate the annual decline in FEV_1 is a major target of any therapeutic intervention. Smoking cessation is the best established and most effective intervention for this goal (3, 4). Recent studies have shown that some pharmacotherapy has the capacity to alleviate the annual decline in FEV_1 by approximately 16 ml/yr (6, 7). However, no previous studies have considered clinical phenotype defined by emphysema severity in

TABLE 1. CHARACTERISTICS OF SUBJECTS WITH COPD CLASSIFIED BY ANNUAL RATES OF DECLINE IN FEV_1 AT BASELINE

	Rapid Decliners (*N = 65*)	Slow Decliners (*N = 131*)	Sustainers (*N = 65*)	*P* Value
Age, yr	69 ± 6	70 ± 8	68 ± 9	0.11
Female sex, N (%)	1 (1.5)	10 (7.6)	4 (6.2)	0.22
Body mass index, kg/m^2	21 ± 3*	22 ± 3	23 ± 4	0.017
Current smoker at entry, N (%)	13 (20)	40 (31)	20 (31)	0.26
Smoking index at entry, pack-years	67 ± 27	64 ± 33	55 ± 25	0.05
Lung function				
Prebronchodilator				
FEV_1, L	1.58 ± 0.65	1.55 ± 0.67	1.70 ± 0.73	0.36
FEV_1, % predicted	57 ± 22	58 ± 22	61 ± 24	0.59
FVC, % predicted	95 ± 19	92 ± 20	92 ± 23	0.70
Post-bronchodilator				
FEV_1, L	1.76 ± 0·62	1.71 ± 0.66	1.84 ± 0.67	0.42
FEV_1, % predicted	64 ± 21	64 ± 22	66 ± 23	0.74
FVC, % predicted	103 ± 17	100 ± 19	99 ± 22	0.47
FEV_1/FVC	0.50 ± 0·13	0.51 ± 0.12	0.53 ± 0.13	0.20
Reversibility of FEV_1, %	14 ± 14	14 ± 12	11 ± 14	0.33
Reversibility of FEV_1, ml	176 ± 152	167 ± 121	148 ± 141	0.47
D_{LCO}, mmol/min/mm Hg	11.2 ± 5.2†	12 ± 4.6*	14 ± 4	0.003
Kco, mmol/min/mm Hg/L	2.5 ± 1.2†	2.8 ± 1†	3.3 ± 1	<0.001
Patient-reported outcomes				
Chronic bronchitis, N (%)	7 (11)	11 (8)	11 (17)	0.20
MRC dyspnea score, ≥2 (%)	56 (86)	111 (85)	52 (80)	0.59
SGRQ total score	31 ± 17	32 ± 17	31 ± 19	0.84
Laboratory values				
Blood neutrophil count, cells/mm^3	3,597 (2,759–4,601)	3,342 (2,704–3,953)	3,534 (2,893–4,287)	0.13
Blood eosinophil count, cells/mm^3	120 (80–221)†	169 (94–248)*	233 (131–353)	0.001
Serum total IgE, IU/ml	62 (19–153)	73 (19–184)	86 (27–216)	0.64

Definition of abbreviations: COPD = chronic obstructive pulmonary disease; D_{LCO} = carbon monoxide diffusing capacity; Kco = carbon monoxide transfer coefficient; MRC = Medical Research Council; SGRQ = The St. George's Respiratory Questionnaire.

Subjects were classified into three groups based on the magnitude of annual decline in FEV_1, using the 25th percentile and the 75th percentile: Rapid decliners, Slow decliners, and Sustainers. Pulmonary function tests were performed after inhalation of salbutamol (0.4 mg). SGRQ scores range from 0–100, with a lower score indicating better quality of life. The total number of the subjects was 261 except for D_{LCO} and Kco data (n = 257).

Plus–minus values are means ± SD. Other values are number (%) or median (interquartile range).

P values were determined by one-way analysis of variance with *post hoc* comparisons using Tukey multiple comparison tests for continuous variables

* *P* value < 0.05 versus Sustainers.

assessment of annual decline in FEV_1. COPD is characterized by a combination of pulmonary emphysema and small airway disease (1, 8), both of which are known to vary substantially among patients even with the same spirometric COPD stages (8, 19), and emerging evidence exists to support the independent genetic influence on emphysema and airway disease (38–40). This study

TABLE 2. CHARACTERISTICS OF SUBJECTS WITH COPD CLASSIFIED BY ANNUAL RATES OF DECLINE IN FEV_1 DURING THE FOLLOW-UP

	All Patients (*N = 261*)	Rapid Decliners (*N = 65*)	Slow Decliners (*N = 131*)	Sustainers (*N = 65*)	*P* Value
Smoking status*					
Continuous smoker, N (%)	40 (15)	4 (6)	24 (18)	12 (19)	0.21
Intermittent smoker, N (%)	40 (15)	12 (19)	18 (14)	10 (15)	0.21
Former smoker, N (%)	181 (69)	49 (75)	89 (68)	43 (66)	0.21
Exacerbation (events/person/yr)†					
Symptom definition	0.22 ± 0.39	0.19 ± 0.33	0.24 ± 0.44	0.22 ± 0.33	0.64
Prescription change	0.17 ± 0.33	0.15 ± 0.32	0.18 ± 0.36	0.18 ± 0.28	0.87
Hospital admission	0.06 ± 0.20	0.07 ± 0.21	0.06 ± 0.23	0.05 ± 0.11	0.84
Medication for COPD (%)‡					
Any medication	190 (73)	51 (78)	97 (74)	42 (65)	0.19
Anticholinergics	135 (52)	36 (55)	71 (54)	28 (43)	0.27
β-Receptor agonists	92 (35)	23 (35)	47 (36)	22 (34)	0.96
Theophylline	116 (44)	32 (49)	58 (44)	26 (40)	0.57
Inhaled corticosteroids	36 (14)	10 (15)	18 (14)	8 (12)	0.88

Definition of abbreviation: COPD = chronic obstructive pulmonary disease.

Plus–minus values are means ± SD. Other values are number (%). We used one-way analysis of variance with *post hoc* comparisons using Tukey multiple comparison tests for continuous variables and chi-square testing for categoric variables.

* Subjects were described as continuous, intermittent, or former smokers, depending on the smoking status during the study period.

† Exacerbation information was collected using prepaid postcard every month with telephone interview if necessary.

‡ Numbers denote the number of subjects with more than 50% of usage during the follow-up period.

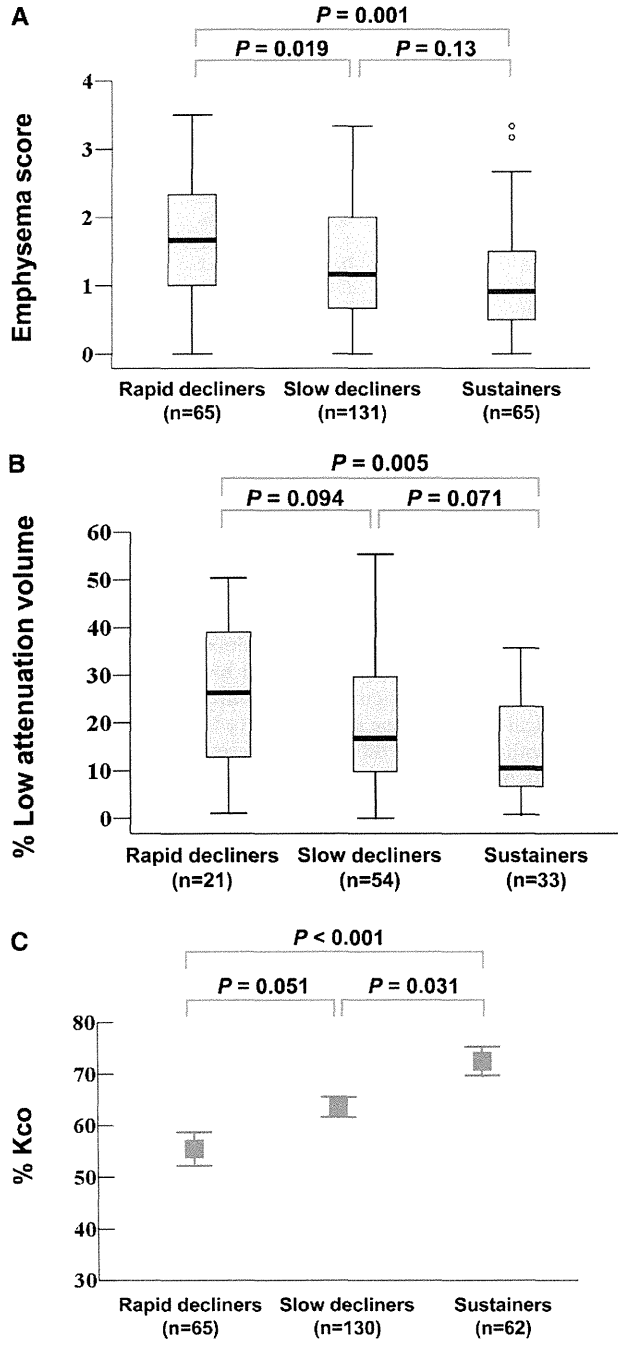

Figure 3. Emphysema severity at baseline among three groups classified by annual declines in post-bronchodilator FEV_1. Emphysema severity was assessed by (*A*) emphysema score visually assessed (n = 261), (*B*) percent low attenuation volume (n = 108), and (*C*) percent carbon monoxide transfer coefficient (%Kco, n = 257). Data show medians with interquartile range for skewed data (*A* and *B*) and means with SEM for quantitative continuous variables (*C*). *See* Figure 2 A for classification of the three groups.

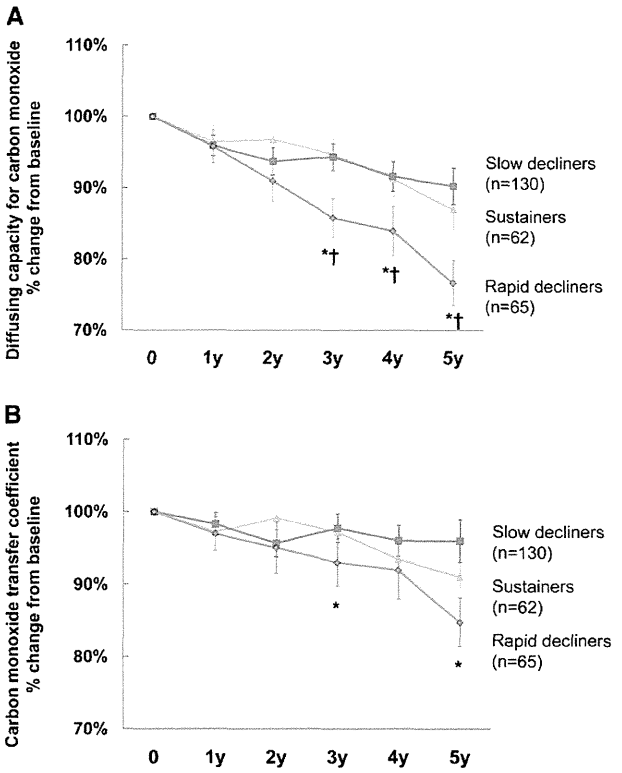

Figure 4. Annual declines in diffusing capacity of carbon monoxide and carbon monoxide transfer coefficient in three groups classified by annual declines in post-bronchodilator FEV_1. Both diffusing capacity of carbon monoxide and carbon monoxide transfer coefficient were expressed as percent changes from baseline over a period of 5 years. *See* Figure 2 A for classification of the three groups. * $P < 0.05$ versus Slow decliners; † $P < 0.05$ versus Sustainers.

provides the first evidence that the phenotype defined by emphysema severity should be considered in future clinical trials when annual decline in FEV_1 is a primary outcome measurement.

Few studies have examined the effects of emphysema on annual decline in lung function. In healthy smokers, Remy-Jardin and coworkers (9) reported that subjects with subtle morphologic abnormalities assessed by CT, including emphysema, at baseline showed a more rapid decline in lung function compared with those with normal CT findings. Another study by Yuan and coworkers (10) reported that quantitative assessment of overinflation of the lung by CT may be able to identify the "susceptible minority of smokers" who will eventually develop COPD. Only recently, Hoesein and coworkers (11) demonstrated that greater baseline severity of CT-detected emphysema is related to lower lung function and greater rates of lung function decline in a larger population. However, in these studies, the authors focused on the importance of early detection of emphysema in seemingly healthy subjects, not on emphysema phenotype in all stages of COPD. Unfortunately, they did not use post-bronchodilator values in FEV_1, and calculated the annual decline with only two-point measurements. However, in another study examining emphysema severity and annual decline in FEV_1 in patients with advanced COPD (41), only patients with moderate to severe COPD with the presence of significant emphysema were recruited because they were candidates for volume reduction surgery. Thus, it is understandable that no relationship between emphysema severity and annual decline in lung function was detected.

The magnitude of annual decline in FEV_1 observed in this study may be relatively small compared with that reported in recently conducted clinical trials worldwide (36, 37). Possible reasons include that the subjects were allowed to receive pharmacotherapy during the study, as decided by their physicians, and that the percentage of continuous or intermittent smokers was