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Author Contributions

Conceived and designed the experiments: AS HS MH TN. Performed the experiments: AS HS MH YM. Analyzed the data: AS HS MH MO YA. Contributed reagents/materials/analysis tools: AS HS MH YM MO YA. Wrote the paper: AS HS MH TN.

Comparison of Distance of 6-min Walk Test and the Incremental Shuttle Walk Test with Lung Function or Quality of Life in Patients with Chronic Obstructive Pulmonary Disease

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Background: Field walk tests such as the 6-min walk test (6MWT) and the incremental shuttle walk test (ISWT) are simple tests for assessing the degree of disability in individuals with chronic obstructive pulmonary disease (COPD). In the present study, the correlation between exercise performance and lung function or health-related quality of life (HRQoL) in both the 6MWT and the ISWT was examined in COPD patients.

Methods: A retrospective examination of 105 patients with COPD using the 6MWT or the ISWT and both lung function tests and assessment of HRQoL with the St George's Respiratory Questionnaire (SGRQ) was performed, and the correlation between walking distance and lung function parameters or SGRQ scores were assessed in each test.

Results: Walking distance was not correlated with lung function parameters, but was significantly correlated with the activity and impact domains, as well as the total score of the SGRQ in the 6MWT. In the ISWT, walking distance correlated significantly with inspiratory capacity, forced vital capacity, and forced expiratory volume in one second, but not with the total score of the SGRQ.

Conclusion: The 6MWT is reflective of health-related quality of life and the ISWT is reflective of lung function. *Shinshu Med J 61: 57—64, 2013*

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I Introduction

Exercise testing is useful for the assessment of the degree of disability, prognosis for survival, presence of exercise-induced hypoxemia, and response to treatment in patients with chronic obstructive pulmonary disease (COPD)[1]. Several modalities are available for the objective evaluation of functional exercise capacity. Of these modalities, field walk tests require less technical expertise and equipment, are simple to perform and are inexpensive[2]. Field walk tests are therefore extensively used for clinical or research purposes. A variety of walk tests exist, including time-based tests, fixed-distance tests, and controlled-pacing tests.

The 6-min walk test (6MWT) is currently the test of choice as a functional walk test for clinical or research purposes[3]. However the 6 min walking distance depends on the motivation of the patient because the 6MWT is a self-paced test.

The incremental shuttle walk test (ISWT) is a

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submaximal exercise test based on incremental increase in walking pace[4]. Consequently, maximal exercise capacity is probably better correlated with the ISWT than with the 6MWT. The ISWT is a reproducible measure of functional capacity in patients with chronic airflow limitation, and strong correlations between ISWT distance and maximal oxygen consumption have been reported, measured during treadmill testing[5].

Whether the walking distance in the 6MWT or that in the ISWT shows a better correlation with lung function or health-related quality of life (HRQoL) is still unclear, although both walking tests have been used for evaluation of exercise performance in patients with COPD. We hypothesized that the 6MWT is more reflective of HRQoL, because the 6MWT is a self-paced test and reflects activity of daily life, and the ISWT better reflects lung function because it is closer to a submaximal exercise test. In the present study, we examined the results of field walk tests (6MWT or ISWT), lung function tests, and HRQoL using St George's Respiratory Questionnaire (SGRQ), and analyzed the walking distance in each walking test in correlation with lung function parameters and SGRQ scores in patients with stable COPD.

II Subjects and Methods

A Subjects

This study was a retrospective study. One hundred and five consecutive symptomatic patients with stable COPD who had been seen at the outpatient clinic of Shinshu University Hospital from 2005 to 2008 were recruited and the results of field walk tests, lung function tests, and HRQoL using SGRQ were examined concurrently. COPD was diagnosed in accordance with the Global Initiative for Chronic Obstructive Lung Disease guidelines[6]. Patients with a history of long-term oxygen therapy, respiratory tract infection or exacerbation during the preceding 3 months, and ischemic heart disease or locomotive problems were excluded. Patients were divided into 2 groups according to the examination date : the 6MWT group (from 2005 to 2006) or the ISWT group (from 2007 to 2008). This study was approved by the institutional research ethics committee of Shinshu University School of Medicine and informed consent was obtained from each patient.

B Lung function tests

Spirometry, lung volume, and diffusion capacity for carbon monoxide (DLCO) were measured using Chestac-8800 (Chest Co. Ltd, Tokyo, Japan) in accordance with the American Thoracic Society protocol[7]. Functional residual capacity (FRC) was measured using a gas-dilution method, after which the subject immediately inspired to total lung capacity (TLC) and expired maximally to residual volume (RV), allowing calculation of lung volume and RV/TLC. DLCO was measured using the single-breath method. For the predicted values for forced expiratory volume in one second (FEV$_1$) and vital capacity (VC), Japanese local reference data[8] developed by the Japanese Respiratory Society were adopted, and the predicted values for DLCO and lung volumes (FRC, RV, and TLC) measured by body plethysmography were determined using the formulas of Nishida et al.[9] and Kory et al.[10], respectively.

C Health-related quality of life

HRQoL was assessed using a Japanese version of the SGRQ, which consists of 76 items and calculates a 3-component score (symptoms, activity, and impact) and a total score. The symptom component contains items related to symptomatology, including frequency of cough, sputum production, wheezing, and breathlessness. The activity component is concerned with physical activities that either cause or are limited by breathlessness. The impact component covers such factors as employment, being in control of health, panic, medication needs and side effects, and disturbance of daily life. Each of these scores ranges from 0 to 100, with a score of 100 indicating maximum disability[11]. The Japanese version of SGRQ is a valid and reliable measure of impaired health in COPD[12].

D Field walk test

1 The 6MWT

The 6MWT was performed according to the American Thoracic Society Guidelines[13]. In summary, the patients were instructed to walk at their fastest pace, attempting to cover the maximum possible distance within 6 min. Percutaneous oxygen saturation (SpO_2) and pulse rate (PR) were continuously monitored. An investigator timed the walk, and used the standard phrases of encouragement. The patients were allowed to stop anytime and anywhere until they had sufficiently rested to start walking again. Before and after the test, SpO_2, PR, and blood pressure were measured, and dyspnea was rated using the modified Borg breathlessness scale (BS).

2　The ISWT

The ISWT was performed according to a previously reported method[8]. In summary, the patient walked up and down a 10-m course. The speed at which the patient walked was dictated by an audio signal played on a compact disc. This compact disc emitted a single bleep at regular intervals, at which point the subject attempted to reach the opposite end of the course. Walking speed was increased every minute by a small increment, which meant that the patient was required to walk at a progressively faster pace. The end of the test was determined by either (a) the patient, when the patient was too breathless to maintain the required speed, or (b) the investigator, if the patient failed to complete a shuttle in the time allowed. SpO_2 and PR were continuously monitored. Before and after the test, SpO_2, PR, respiration rate, and blood pressure were measured, and dyspnea was rated using the modified BS.

E　Statistical analysis

All results were expressed as mean±SD. Data distributions of variables in the various groups were first assessed using the goodness-of-fit test. When data for variables showed a normal distribution, the 2 groups were compared using the unpaired t test. When data for variables did not show a normal distribution, variables between the 2 groups were compared using the Mann-Whitney U test. Gender distribution difference between the 2 groups was compared using Fisher's exact test. Simple correlations between various parameters were examined using Pearson's correlation coefficient. A Pearson correlation coefficient of 0.40 or above was considered satisfactory. Multiple stepwise linear regression analysis was performed to identify which variables were significant determinants for the walking distance or HRQoL. A value of $p \leq 0.15$ was used first to identify candidate variables, and then variables were removed from the regression model if the p value was more than 0.1. All statistical analyses were performed using a Windows-compatible software program (Stat Flex ver. 5.0, Artech Ltd., Osaka, Japan). A p value of <0.05 was considered statistically significant.

III　Results

A　Patient details

Patient characteristics and lung function values are shown in **Table 1**. Among the 105 patients, 52 patients (5 female patients) were classified into the 6MWT group, and 53 patients (3 female patients) into the ISWT group. There were no significant differences in patient characteristics between the 2 groups, except for the mean inspiratory capacity (IC)/TLC and DLCO, which were significantly lower in the 6MWT group than in the ISWT group, and the mean, which was significantly higher in the 6MWT group than in the ISWT group. The mean SGRQ symptom score, activity score, impact score, and total score in the 6MWT group were significantly higher than in the ISWT group (**Table 2**). The results of the walk tests are shown in **Table 3**. The mean post-SpO_2, delta SpO_2, and the mean pre- and post-modified BS did not significantly differ between the 2 groups. The mean pre-SpO_2, post-PR, and delta PR in the ISWT group were significantly higher than in the 6MWT group. The mean pre-PR in the 6MWT group was significantly higher than in the ISWT group.

B　The relationship between walking distance and lung function or HRQoL (Table 4)

The 6MWT distance (6MWD) showed a significant correlation with the activity and impact

Table 1 Patient characteristics and lung function parameters

	6MWT group (n=52)	ISWT group (n=53)	p value
Age (yr)	72.6±7.1	70.0±7.3	p＝0.065
Gender (male/female)	47/5	50/3	p＝0.692
BH (cm)	162.9±7.5	163.5±6.4	p＝0.662
BW (kg)	57.8±9.7	58.4±10.1	p＝0.753
BMI (kg/m²)	21.7±2.9	21.8±3.1	p＝0.929
VC, % of predicted (%)	98.0±16.9	105.0±20.4	p＝0.091
FEV_1, % of predicted (%)	63.4±24.2	69.0±22.4	p＝0.223
FEV_1/FVC (%)	52.2±14.3	56.5±13.1	p＝0.110
IC/TLC (%)	35.6±8.6	39.8±8.4	p＝0.012
FRC, % of predicted (%)	107.7±29.1	99.9±19.3	p＝0.272
RV, % of predicted (%)	156.2±48.0	141.9±42.0	p＝0.115
TLC, % of predicted (%)	114.0±18.7	112.4±18.4	p＝0.684
RV/TLC (%)	47.0±8.7	42.1±10.0	p＝0.009
DLCO, % of predicted (%)	48.2±19.3	56.1±21.1	p＝0.048

Values are mean±SD
COPD : chronic obstructive pulmonary disease ; 6MWT : 6-min walk test ; ISWT : incremental shuttle walk test ; BH : body height ; BW : body weight ; BMI : body mass index ; VC : vital capacity ; FEV_1 : forced expiratory volume in one second ; FVC : forced vital capacity ; IC : inspiratory capacity ; TLC : total lung capacity ; FRC : functional residual capacity ; RV : residual volume ; DLCO : diffusion capacity for carbon monoxide

Table 2 SGRQ scores of patients with COPD

	6MWT group (n=52)	ISWT group (n=53)	p value
SGRQ symptom score	51.106±22.138	38.200±19.016	p＝0.002
SGRQ activity score	58.492±30.273	35.688±25.735	p＜0.001
SGRQ impact score	28.010±18.055	18.589±17.497	p＝0.005
SGRQ total score	43.013±19.830	29.113±18.058	p＜0.001

Values are mean±SD
COPD : chronic obstructive pulmonary disease ; 6MWT : 6-min walk test ; ISWT : incremental shuttle walk test ; SGRQ : St George's Respiratory Questionnaire

Table 3 Summary of walk test result of patients with COPD

	6MWT group (n=52)	ISWT group (n=53)	p value
pre SpO_2 (%)	94.9±1.8	95.7±1.5	p＝0.014
post SpO_2 (%)	86.8±8.6	89.4±5.6	p＝0.262
ΔSpO_2 (%)	-8.1±8.2	-6.3±5.1	p＝0.685
pre PR (/min)	81.6±12.8	74.8±13.6	p＝0.007
post PR (/min)	108.5±12.8	119.2±19.0	p＝0.002
ΔPR (/min)	26.9±13.3	44.4±22.2	p＜0.001
pre modified Borg breathlessness scale	0.22±0.50	0.21±0.55	p＝0.422
post modified Borg breathlessness scale	3.88±2.41	3.19±2.20	p＝0.082
walk test distance (m)	387±106	365±143	

All values represent mean±SD
Pre- and post-modified Borg scales represent median and ranges.
COPD : chronic obstructive pulmonary disease ; 6MWT : 6-min walk test ; ISWT : incremental shuttle walk test ; SpO_2 : Oxygen saturation ; ΔSpO_2 : post SpO_2-pre SpO_2 ; PR : pulse rate ; ΔPR : post PR-pre PR

Table 4　Simple regression analysis using Pearson's correlation coefficient

	6MWT distance		ISWT distance	
	Coefficient	p value	Coefficient	p value
FVC	0.33	p<0.016	0.52	p<0.001
FEV$_1$	0.34	p<0.013	0.50	p<0.001
FEV$_1$/FVC	0.17	p=0.239	0.35	p=0.011
FRC	0.19	p=0.168	-0.01	p=0.922
RV	0.21	p=0.135	-0.21	p=0.124
TLC	0.28	p=0.044	0.24	p=0.888
IC	0.27	p=0.049	0.46	p<0.001
RV/TLC	0.02	p=0.860	-0.35	p=0.009
DLCO	0.61	p<0.001	0.50	p<0.001
SGRQ symptom score	-0.28	p=0.041	-0.18	p=0.190
SGRQ activity score	-0.65	p<0.001	-0.44	p=0.001
SGRQ impact score	-0.63	p<0.001	-0.28	p=0.044
SGRQ total score	-0.60	p<0.001	-0.33	p=0.015
pre SpO$_2$	0.45	p<0.001	0.28	p=0.038
delta SpO$_2$	-0.26	p=0.061	-0.12	p=0.378
post modified Borg scale	-0.21	p=0.141	-0.02	p=0.845

COPD : chronic obstructive pulmonary disease ; 6MWT : 6-min walk test ; ISWT : incremental shuttle walk test ; FVC : forced vital capacity ; FEV$_1$: forced expiratory volume in one second ; FRC : functional residual capacity ; RV : residual volume ; TLC : total lung capacity ; IC : inspiratory capacity ; DLCO : diffusion capacity for carbon monoxide ; SGRQ : St George's Respiratory Questionnaire ; delta SpO$_2$: difference post SpO$_2$ from pre SpO$_2$

$r =- 0.60$　$p < 0.001$　　$r =- 0.33$　$p = 0.015$
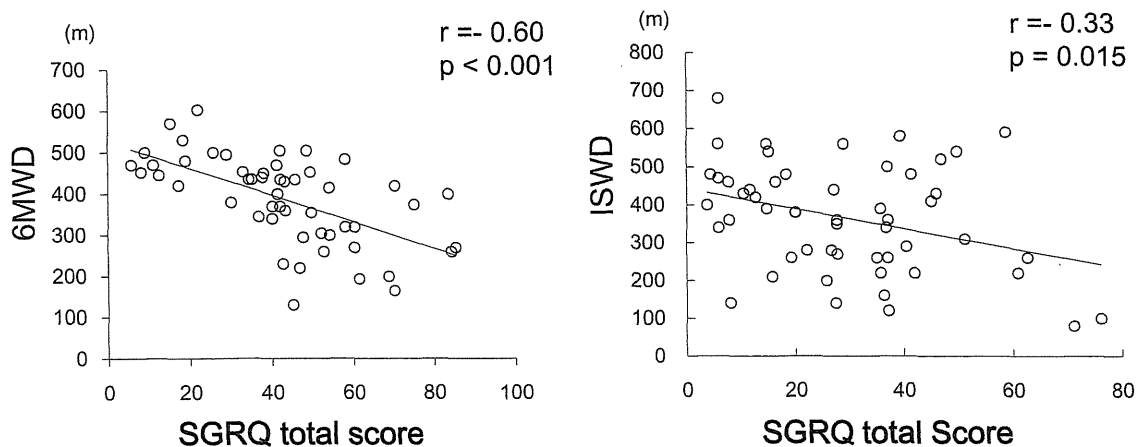
Fig. 1
The relation between St George's Respiratory Questionnaire (SGRQ) total score and the field walk tests. The correlation coefficient between the 6-min walk distance (6MWD) and SGRQ total score is -0.60. The correlation coefficient between the incremental shuttle walk test distance (ISWD) and SGRQ total score is -0.33.

domain scores, and the total score of SGRQ (**Fig. 1**) with a correlation coefficient over 0.4. With regard to lung functions, DLCO was significantly correlated with 6MWD, showing a coefficient of 0.61. The correlation coefficients between 6MWD and FVC, FEV$_1$, TLC and IC were less than 0.4. On the other hand, the ISWT distance (ISWD) was significantly

correlated with FVC, FEV$_1$ (**Fig. 2**), IC, and DLCO, showing a high correlation coefficient of over 0.4. However, with respect to HRQoL, weak correlations between ISWD and the SGRQ scores were observed compared to the correlations between the 6MWD and the SGRQ scores.
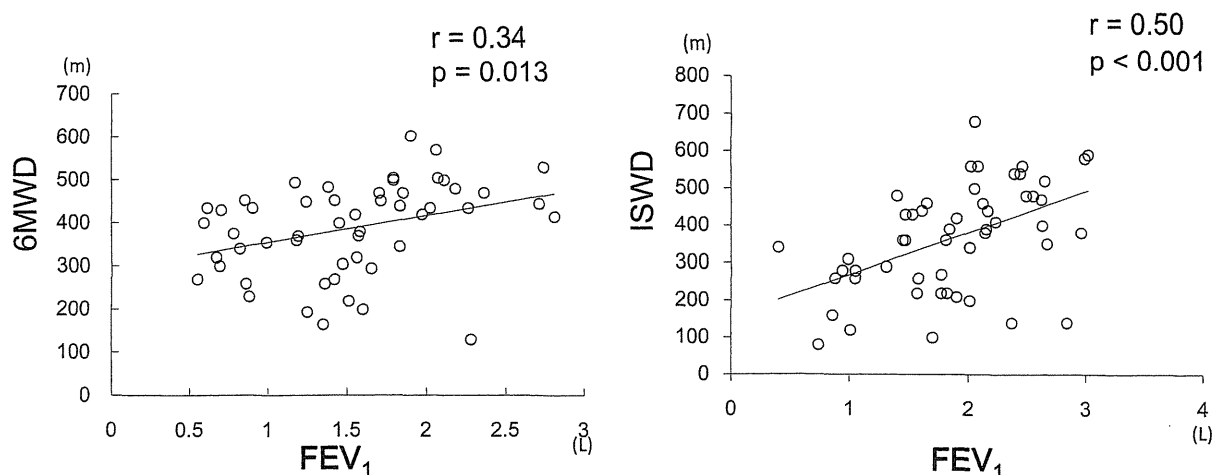
The regression model for the 6MWD was

Fig. 2

The relationship between forced expiratory volume in one second (FEV_1) and the field walk test. The correlation coefficient between the incremental shuttle walk test distance (ISWD) and FEV_1 is 0.50. The correlation coefficient between the 6-min walk distance (6MWD) and FEV_1 is 0.34.

significant (r=0.68) and comprised DLCO (R^2= 0.376, p<0.001), FEV_1 (R^2=0.117, p=0.012), and RV/ TLC (R^2<0.001, p=0.014). This model accounted for 42.8 % of the walking distance in the 6MWT, and DLCO was the most significant determinant. The regression model for the total SGRQ score was significant (r=0.70) and included 6MWD (R^2=0.359, p<0.001) and FEV_1 (R^2=0.291, p=0.001). This model accounted for 46.5 % of the SGRQ, and 6MWD was the most significant determinant. The regression model for the ISWD was significant (r= 0.64) and included DLCO (R^2=0.249, p=0.007), FVC (R^2=0.270, p=0.011), and FEV_1/FVC (R^2=0.121, p= 0.100). This model accounted for 37.1 % of the ISWD, and DLCO was the most significant determinant. The regression model for the total SGRQ score was significant (r=0.62) and included post- and pre-modified BS (R^2=0.272 and <0.001, p= 0.008 and 0.038, respectively), FVC (R^2=0.132, p= 0.036), and FRC (R^2=0.016, p=0.064). This model accounted for 33.5 % of the SGRQ, and dyspnea during ISWT was the most significant determinant.

IV Discussion

The results of the present study indicate that exercise capacity evaluated by ISWD correlated better with lung function, but was poorly correlated with HRQoL. On the other hand, exercise capacity evaluated by 6MWD correlated better with HRQoL, but did not show a good correlation with parameters of airflow limitation. Multiple, stepwise, linear regression analysis also revealed that 6MWD was the most significant contributing factor for the evaluation of HRQoL among all the lung function parameters tested. Previous reports showed that maximum heart rate and BS rating were higher at the end of the ISWT than at the end of the 6MWT[4] and ISWD was strongly correlated with the maximum oxygen uptake, whereas 6MWD was correlated poorly with maximum oxygen uptake[5]. Furthermore, our present study showed a larger delta PR in the ISWT group than in the 6MWT group. This result suggests that the ISWT is closer to a submaximal exercise test than the 6MWT is, and may be a better indicator of lung function alterations such as airflow limitation and lung hyperinflation. Previous reports also showed a correlation between ISWD and lung function, such as VC and FEV_1[14] and no correlation between 6MWD and lung function including FVC, FEV_1, and TLC[15]. However, Mak VH et al and Wijkstra PJ et al reported that 6MWD was strongly correlated with FVC and FEV_1[16][17] and Vagaggini B et al reported that SWD was not correlated with FVC, FEV_1, and TLC[15]. In these studies, the severity of COPD estimated by air flow limitation with each mean FEV_1, % of the predicted

being 40.3 %[16], 44.3 %[17], and 48 %[15], was more severe than that of our study (63.4 %). These results suggest that the correlation between lung functions and walking distance depends on the severity of COPD. In our study, when restricted to patients with severe (30 % $\leq$ FEV$_1$ < 50 % predicted) and very severe (FEV$_1$ < 30 % predicted) COPD, 6MWD was significantly correlated with TLC, IC/TLC and DLCO, and SWD was not correlated with FEV$_1$. However, because the number of patients with severe and very severe COPD was small in our study, it is difficult to discuss each severity. Further studies which recruit each severity patients of every severity are needed.

In the present study, the SGRQ score showed a good correlation with 6MWD, as previously reported[11][16]. The SGRQ is one of the most widely used questionnaires for assessing HRQoL in COPD patients and is well known for its validity, repeatability, and sensitivity[11]. On the other hand, the 6MWT also reflects daily activity for most patients with severely impaired activities, and most patients do not achieve maximal exercise capacity during the 6MWT[13]. Because the 6MWT assesses the submaximal functional exercise capacity, and most of the activities of daily living are performed at submaximal levels of exertion, the 6MWD should correlate well with the SGRQ scores, especially with the scores of the activity domain.

The present study is the first to investigate the correlation between the ISWT and SGRQ. Some investigators reported a correlation between the ISWT and QoL that did not include the SGRQ[18][19]. Mean ISWD was only about 180 m and patients in these studies had less exercise capacity than patients in our study. In our study, when restricted to patients with severe and very severe COPD, mean ISWD was 240 m, and ISWD was significantly cor-

related with SGRQ activity, impact and total score in these patients. These results suggest that ISWD is correlated with HRQoL in patients with COPD showing decreased exercise capacity.

V Limitation

The present study was a retrospective study and there was a different background in the 2 groups. The results showed that the patients in the 6MWT group showed more lung hyperinflation and disturbed gas exchange when compared with the patients in the ISWT group and reduction of HRQoL estimated by the SGRQ. This is a limitation of the present study and it is difficult to compare each parameter in the two groups. Therefore, a further prospective study is needed to compare the 6MWT with the ISWT.

VI Conclusion

It was suggested that the 6MWT may be more reflective of QoL and the ISWT more reflective of lung function in patients with COPD. However, most of the patients were of mild or moderate severity. Furthermore, the background in the 6MWT group and that in the SWT group differed. Further prospective studies which recruit each severity patients of each severity are needed.

VII Acknowledgements

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Respiration

Pulmonary Hypertension as a Prognostic Indicator at the Initial Evaluation in Idiopathic Pulmonary Fibrosis

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Abstract
Background: The impact of pulmonary hypertension (PH) on survival has been demonstrated in severe cases with idiopathic pulmonary fibrosis (IPF) who were referred for transplantation. However, whether PH is a predictor of survival remains unclear in milder cases. **Objectives:** To evaluate the survival impact of pulmonary artery pressure measured during the initial evaluation in patients with IPF. **Methods:** We retrospectively analyzed the initial evaluation data of 101 consecutive IPF patients undergoing right heart catheterization. Patients evaluated with supplemental oxygen were excluded. Predictors of 5-year survival were analyzed using the Cox proportional model. **Results:** The mean forced vital capacity (FVC) % predicted, diffusing capacity of the lung for carbon monoxide (DLCO) % predicted, and mean pulmonary artery pressure (MPAP) were 70.2 ± 20.1%, 47.9 ± 19.5%, and 19.2 ± 6.5 mm Hg, respectively. A univariate Cox proportional hazard model showed that the body mass index, %FVC, %DLCO, baseline PaO$_2$, modified Medical Research Council score, 6-min walk distance, and lowest SpO$_2$ of the 6-min walk test were significantly predictive of survival. The MPAP and pulmonary vascular resistance of right heart catheterization were also significant. With stepwise, multivariate Cox proportional analysis, MPAP (HR = 1.064; 95% CI 1.015–1.116, p = 0.010) and %FVC (HR = 0.965, 95% CI 0.949–0.982, p < 0.001) were independent determinants of survival. Analysis of the receiver operating curve revealed MPAP >20 mm Hg to be optimal for predicting the prognosis. **Conclusions:** Higher MPAP and lower %FVC at the initial evaluation were significant independent prognostic factors of IPF. The current results suggested the importance of the initial evaluation of PH for patients with IPF.

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Introduction

Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive, and devastating disease with a median survival of 3–5 years [1, 2]. Previous studies have reported several poor prognostic factors, including decreased forced vital capacity (FVC), diffusing capacity of the lung for carbon monoxide (DLCO), modified Medical Research Council

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(MMRC) scale, and degree of desaturation during the 6-min walk test (6MWT) [3–10].

Pulmonary hypertension (PH) is often observed in the clinical course of IPF patients with advanced disease [11–17]. In a retrospective study, Lettieri et al. [13] reported poor outcomes in IPF patients who were listed for lung transplantation with PH [mean pulmonary artery pressure (MPAP) >25 mm Hg] identified by right heart catheterization (RHC). In their study, a significant difference in outcomes was demonstrated with 1-year mortality.

Since PH does not always correlate with the restrictive impairment or the extent of fibrosis, the question of whether PH in less severe cases predicts mortality is interesting. Only one study, however, has reported mild or early cases. Hamada et al. [18] demonstrated the importance of PH in IPF patients at their initial workup using another cutoff point (MPAP >17 mm Hg) by RHC. However, when they performed a stepwise regression analysis, MPAP was not confirmed as an independent prognostic factor after adjusting for some parameters.

Moreover, in recent studies [11, 13, 15, 16, 18, 19] PH was evaluated in patients including those treated with supplemental oxygen, which could improve hypoxemic vasoconstriction and influence MPAP. No study has targeted IPF patients without supplemental oxygen at the initial evaluation by RHC.

In the current definition [20], the class of patients with MPAP 21–24 mm Hg remains undetermined. For example, in patients with chronic obstructive pulmonary disease (COPD), the cutoff point of PH has been defined as MPAP >20 mm Hg or >25 mm Hg [21]. However, in IPF, the optimal cutoff point has not been sufficiently discussed.

The aim of this study was to evaluate whether MPAP predicts survival in IPF patients who could be evaluated based on the background, pulmonary function test, 6MWT, and RHC at the initial evaluation in milder cases, and to evaluate the optimal cutoff point of MPAP.

Methods

Subjects

Patients who underwent systematic evaluations were registered in our database, which we retrospectively analyzed. Four hundred eighty-nine patients with interstitial pneumonia were enrolled at Tosei General Hospital between April 2001 and February 2009. One hundred seventy-seven patients were diagnosed with IPF and 76 patients were excluded for the following reasons: (1) they did not consent to RHC, (2) RHC was not performed

within 3 months, (3) they suffered from unstable disease, such as acute exacerbation, infection, or heart failure, (4) there were other obvious causes of PH, for example chronic thromboembolic PH, (5) evaluation was done with supplemental oxygen, and (6) RHC was performed, but the pulmonary capillary wedge pressure (PCWP) was over 15 mm Hg. Finally, we reviewed 101 stable IPF patients who underwent RHC for the initial evaluation in this period (fig. 1).

This study was approved by the Tosei General Hospital Institutional Review Board (IRB No. 219). The IRB did not require the patients' approval or informed consent for the retrospective review of their records and images.

The diagnosis of IPF was made in accordance with the American Thoracic Society (ATS)/European Respiratory Society (ERS) statement [1], using the following major criteria: (1) exclusion of other known causes of interstitial lung disease, (2) abnormal pulmonary function with restriction and impaired gas exchange, (3) bibasilar reticular abnormalities on high-resolution computed tomography (HRCT), and (4) transbronchial lung biopsy or bronchoalveolar lavage showing no features to support an alternative diagnosis. Minor criteria included: (1) age >50 years, (2) insidious onset of otherwise unexplained dyspnea, (3) duration of illness >3 months, and (4) bibasilar inspiratory crackles. All of the major criteria and at least 3 of the 4 minor criteria had to be satisfied. For those with a surgical lung biopsy specimen showing usual interstitial pneumonia, only the major criteria were considered relevant.
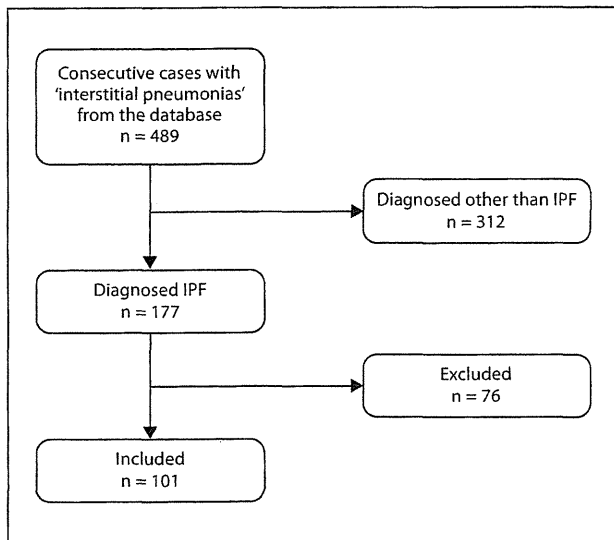
Measurements

We recorded patients' characteristics, pulmonary function tests, PaO$_2$, 6MWT, and hemodynamics, retrospectively. All patients underwent spirometry (CHESTAC-55V; Chest, Tokyo, Japan), according to the method described in the ATS 1994 update [22]. Single-breath DLCO was also measured (CHESTAC-55V). The values for FVC and DLCO were related to % predicted values [23]. 6MWT was conducted in all patients who participated in the study, according to the ATS statement [24]. Briefly, all patients were tested under standardized conditions by trained technicians. Baseline blood pressure, heart rate, and oxygen saturation were measured. Patients were instructed to walk as far as possible in 6 min. The distance that patients could walk was recorded. Oxygen saturation was also measured by pulse oximetry at rest for 5 min prior to and immediately after the test. All patients underwent the tests twice to minimize the training effects. The MMRC scale includes 5 grades (0–4) of various physiological activities that provoke dyspnea [10, 25]. After the patients had read the descriptive phrases, they selected the number that best corresponded to their level of dyspnea in daily living.

RHC was performed using a Swan-Ganz catheter percutaneously via either the cubital vein or the femoral vein.

Statistical Analysis

All data were based in February 2011. Continuous variables were expressed as means ± SD. Categorical variables were summarized by frequency. The MMRC score was analyzed as a continuous variable. Distribution of continuous variables was evaluated using the Shapiro-Wilk test. If both variables had a normal distribution, correlations were calculated using Pearson's correlation test. If either variable had a nonnormal distribution, correlations were calculated using Spearman's correlation test.

Fig. 1. Screening and inclusion process for patients in the study.

Table 1. Baseline characteristics and physiology of patients

Variables	Mean	Range
Sex (M/F)	85/16	
Age, years	65.4 ± 7.6	41–82
BMI	23.4 ± 4.1	13.9–36.1
Smoking status		
current/former/never	8/71/22	
FVC, % predicted	70.2 ± 20.1	28.3–112.6
DLCO, % predicted	47.9 ± 19.5	7.7–99.7
PaO_2, mm Hg	79.8 ± 12.0	48.8–103.0
MMRC	1.5 ± 1.0	0–4
6MWD, m	526.6 ± 154.0	68–1103
Lowest SpO_2, %	80.8 ± 10.4	46–96
MPAP, mm Hg	19.2 ± 6.5	9–39
PVRI, $dyn\cdot s\cdot cm^{-5}\cdot m^2$	285.3 ± 151.0	85.6–922.2
Cardiac index, $l\cdot min^{-1}\cdot m^{-2}$	3.11 ± 0.60	1.51–5.38
PCWP, mm Hg	8.0 ± 3.6	0–15

Data are presented as means ± SD or numbers. n = 101 except for DLCO (n = 96).

When two continuous variables were compared, the t test was used for normal distributions and the Mann-Whitney test was used for nonnormal distributions. When categorical variables were compared, the χ^2 test was used. Univariate Cox's proportional hazard models were used to examine the association of selected variables with survival. Variables that were significant ($p < 0.05$) in the univariate analysis were included in the multivariate model. To avoid multicolinearity, only one of the highly correlated variables (coefficient of correlation ≥ 0.9) was entered in the multivariate model, if present. The stepwise multivariate Cox's proportional hazards model was then used for variables that were revealed to be significant with the univariate model, in order to select more significant variables. To obtain an appropriate cutoff value of MPAP, a receiver operating curve (ROC) analysis was performed. The survival analysis was completed according to the methods of Kaplan-Meier, and the log-rank test was used to compare survival curves. All tests were performed at a significance level of $p < 0.05$. Analyses were completed using IBM SPSS statistics version 19.

Results

The baseline characteristics of 101 patients are summarized in table 1. The mean observation period was 25.1 ± 14.8 months. Twenty-three patients (22.8%) developed acute exacerbation of IPF. In this observation period 60 (59.4%) patients died. Thirty patients (29.7%) died due to respiratory failure, 18 (17.8%) due to acute exacerbation of IPF, 6 (5.9%) due to infection, 2 (2.0%) due to lung cancer, 1 (1.0%) due to acute leukemia, and 3

Table 2. Results of the univariate Cox proportional hazard model

Variables	HR	95% CI	p value
Sex			
Male	1		
Female	1.076	0.555–2.288	0.829
Age, years	0.998	0.965–1.032	0.911
BMI	0.926	0.863–0.993	0.032
Smoking status			
Never	1		
Former	1.205	0.641–2.266	0.562
Current	1.454	0.514–4.111	0.641
FVC, % predicted	0.960	0.944–0.976	<0.001
DLCO, % predicted	0.980	0.965–0.994	0.005
PaO_2, mm Hg	0.963	0.941–0.985	0.001
MMRC	2.014	1.453–2.790	<0.001
6MWD, m	0.995	0.993–0.997	<0.001
Lowest SpO_2, %	0.965	0.945–0.986	0.001
MPAP, mm Hg	1.082	1.035–1.131	0.001
PVRI, $dyn\cdot s\cdot cm^{-5}\cdot m^2$	1.003	1.001–1.004	<0.001
Cardiac index, $l\cdot min^{-1}\cdot m^{-2}$	0.841	0.534–1.322	0.452
PCWP, mm Hg	0.998	0.926–1.077	0.967

(3.0%) due to unknown causes. Fourteen patients received therapy for IPF at the initial evaluation. All of them were treated with oral corticosteroids. Ten patients were treated with an immunosuppressive agent. No patients were treated with antithrombotic agents for PH
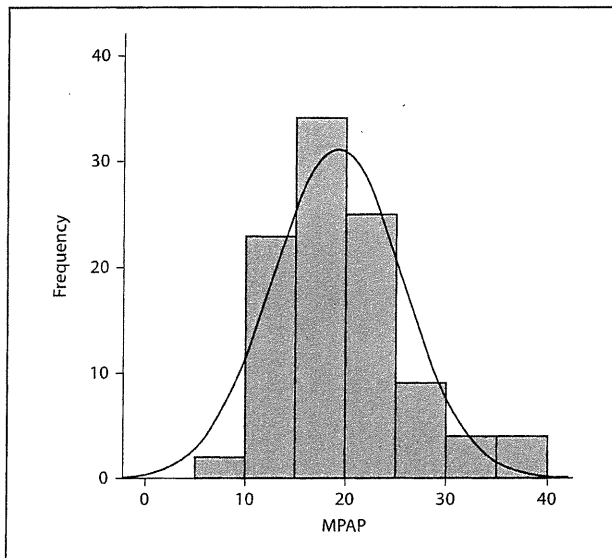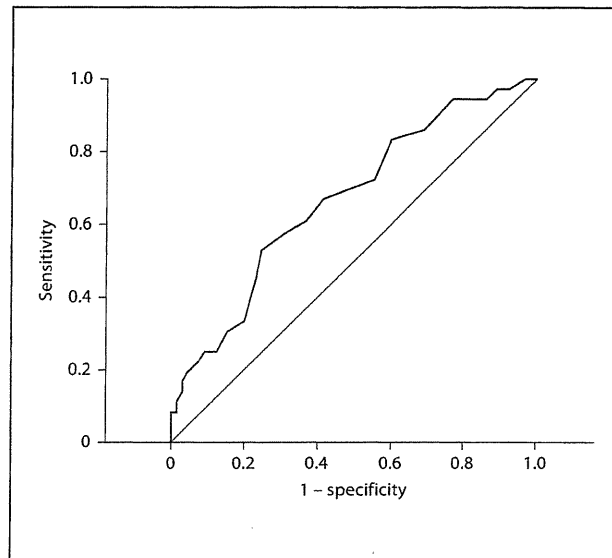
Fig. 2. Histogram of MPAP.

Fig. 3. ROC of pulmonary artery pressure for the prognosis.

Table 3. Results of stepwise multivariate Cox proportional hazards model

Variables	HR	95% CI	p value
FVC, % predicted	0.965	0.949–0.982	<0.001
MPAP, mm Hg	1.064	1.015–1.116	0.010

Adjusted for variables that were significant in univariate analysis (table 2), %FVC and MPAP were independent predictors of 5-year survival. n = 96; DLCO could not be obtained in 5 cases.

and only 6 patients were treated with PH targeted therapy (sildenafil only). The mean MPAP, pulmonary vascular resistance index (PVRI), cardiac index, and PCWP were 19.2 ± 6.5 mm Hg, 285.3 ± 151.0 dyn·s·cm^{-5}·m^2, 3.11 ± 0.60 l·min^{-1}·m^{-2}, and 8.0 ± 3.6 mm Hg, respectively.

A histogram of MPAP is shown in figure 2. Fifteen patients (14.9%) had MPAP >25 mm Hg and only 4 cases were over 35 mm Hg.

The univariate Cox regression model (table 2) demonstrated that MPAP (HR = 1.082; 95% CI 1.035–1.131; p = 0.001) and several variables have a statistically significant impact on survival.

The stepwise multivariate Cox regression model (table 3) demonstrated that MPAP (HR = 1.064; 95% CI 1.015–1.116, p = 0.010) and %FVC (HR = 0.965; 95% CI 0.949–0.982, p < 0.001) have statistically significant impacts on survival.

ROC analysis was performed to obtain an appropriate cutoff value of MPAP. As a result, a value of 20 mm Hg was revealed to be optimal (AUC 0.679, sensitivity 55.0%, specificity 75.4%) (fig. 3).

Table 4 shows the baseline characteristics and physiology of patients using the cutoff point of 20 mm Hg. Thirty-five patients (34.7%) had MPAP >20 mm Hg. Age, %DLCO, PaO$_2$, 6-min walk distance (6MWD), and lowest SpO$_2$ were significantly lower in those with over 20 mm Hg. The rate of smoking history, MMRC, PVRI, and PCWP were significantly higher in those with over 20 mm Hg.

Figure 4 shows a Kaplan-Meier curve that reveals significantly worse survival among patients whose MPAP was >20 mm Hg than among those whose MPAP was ≤20 mm Hg (log-rank test p = 0.001). The median survival estimates were 20.8 and 37.5 months, respectively. In addition, a Kaplan-Meier curve revealed a significant difference in survival between patients whose MPAP was ≤20 mm Hg, 21–25 mm Hg, and >25 mm Hg (log-rank test p = 0.003) (fig. 5).
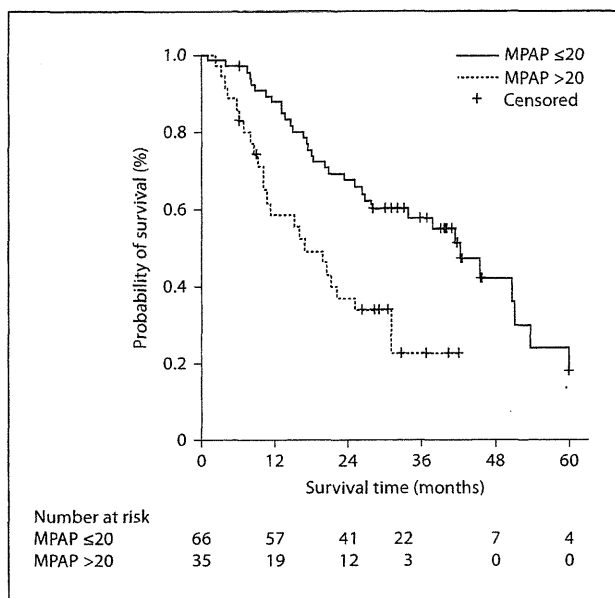
Fig. 4. Kaplan-Meier curves for 5-year survival according to MPAP (p = 0.001). Survival curves were compared with log-rank statistics.
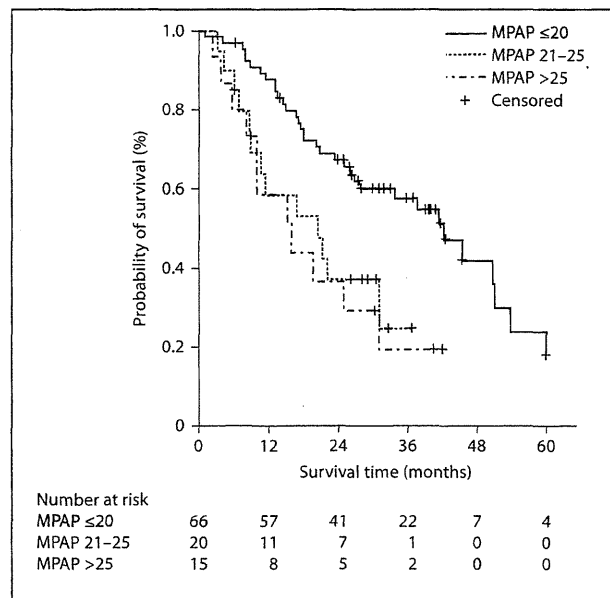
Fig. 5. Kaplan-Meier curves for 5-year survival according to MPAP (p = 0.003). Survival curves were compared with log-rank statistics.

Table 4. Baseline characteristics and physiology of patients with and without high MPAP

Variables	MPAP ≤20 mm Hg (n = 66)	MPAP >20 mm Hg (n = 35)	p value
Sex (M/F)	53/13	32/3	0.145
Age, years	66.6 ± 7.0	63.2 ± 8.3	0.027
BMI	23.1 ± 3.8	24.1 ± 4.6	0.24
Smoking status current/former/never	7/40/19	1/31/3	0.014
FVC, % predicted	71.5 ± 19.7	67.7 ± 20.9	0.373
DLCO, % predicted	52.5 ± 20.5	38.4 ± 13.1	<0.001
PaO₂, mm Hg	83.5 ± 10.0	72.8 ± 12.6	<0.001
MMRC	1.3 ± 0.9	1.9 ± 0.9	0.004
6MWD, m	561.2 ± 150.0	461.2 ± 141.8	0.002
Lowest SpO₂, %	83.8 ± 9.1	75.1 ± 10.6	<0.001
PVRI, dyn·s·cm⁻⁵·m²	225.9 ± 90.7	397.4 ± 177.5	<0.001
Cardiac index, l·min⁻¹·m⁻²	3.14 ± 0.54	3.06 ± 0.7	0.518
PCWP, mm Hg	6.8 ± 3.3	10.2 ± 3.2	<0.001

Data are presented as means ± SD or numbers. n = 101 except for DLCO (n = 96).

Discussion

This is the first study to confirm by multivariate analysis that a high MPAP at the initial evaluation is an independent predictor of survival in patients with IPF who undergo RHC. In this study, a higher MPAP was an independent prognostic predictor comparable to %FVC, a well-known prognostic factor. The study included patients with milder pulmonary function impairment (mean FVC 70.2%, mean DLCO 47.9%) than subjects of

many previous studies [13, 17, 19], and it excluded patients with left heart failure and those with supplemental oxygen, which may influence hypoxemic vasoconstriction. Therefore, the results are thought to be robust and to demonstrate the importance of PH in IPF at the initial evaluation. The results also support previous reports indicating that PH is not just a result of restrictive impairment in patients with IPF [13, 14, 26].

In advanced patients with IPF who were referred for lung transplantation, PH is reported to be a survival predictor. Lettieri et al. [13] reported that only PH diagnosed by RHC correlated with mortality, and that spirometric measurements did not predict mortality. They included 79 patients with IPF who were listed for lung transplantation. Twenty-five patients (31.6%) met the criteria for PH (MPAP >25 mm Hg), and the mean MPAP was 23.4 mm Hg. Patel et al. [16] found that PH (MPAP >25 mm Hg) was an independent predictor (HR 3.6) in 376 patients with IPF who were referred for lung transplantation. These studies did not include mild cases, so the meaning is different from our cases. However, it is notable that PH is the only prognostic factor in advanced patients with IPF.

On the other hand, Hamada et al. [18] reported the influence of elevated MPAP on the prognosis of 76 IPF patients who were evaluated with RHC in the initial workup. Although they reported that PH defined as MPAP >17 mm Hg was a prognostic factor, $D_{L}CO$ was only one significant parameter when adjusted for certain parameters. The reason for the difference between their study and ours is not apparent. One possibility is a difference in candidates. In their study patients had higher %FVC (76 vs. 70.2%) and there was a lower prevalence of patients whose MPAP was >25 mm Hg (8.1 vs. 14.9%) and a higher rate of patients proved by biopsy (77.4 vs. 41.6%) than in our study. Their cohort may have included milder cases, which might have contributed to the difference in the results.

Recently, Corte et al. [27] reported retrospectively on the prognostic significance of invasive and noninvasive parameters in patients with diffuse fibrotic lung disease and suspected PH. In their study, a raised PVR of >6.23 Wood units was strongly associated with early mortality (OR 1.30; 95% CI 1.11–1.52, p = 0.001) after adjustment for some parameters. Early mortality was not linked to MPAP. In our cases, prognostic early mortality was associated with %FVC and MPAP (data not shown). Their clinical criteria for RHC included echocardiographic right ventricular systolic pressure >40 mm Hg or right ventricular dilation and dyspnea or hypoxemia not ex-

plained by the underlying fibrosis. These criteria may have contributed to the difference in results. In fact, our cohort (mean MPAP 19.2 mm Hg, PVRI 285.3 dyn·s·cm^{-5}·m^2, PVR 2.14 Wood units, %FVC 70.2%, and %$D_{L}CO$ 47.9%) was milder than their cohort (mean MPAP 33.5 mm Hg, PVR 5.9 Wood units, %FVC 67.9%, %$D_{L}CO$ 29.6%).

In our initial evaluation for IPF, MPAP >20 mm Hg was revealed to be the optimal cutoff point for predicting the prognosis based on ROC analysis. In the case of PH owing to lung diseases, the optimal cutoff point has not been determined [20]; however, the cutoff point of MPAP >20 mm Hg has been used in COPD. In figure 4, patients with MPAP >20 mm Hg have higher mortality. Additionally, the prognosis seems to be almost the same in patients whose MPAP was 21–25 mm Hg and those whose MPAP was >25 mm Hg (fig. 5). This may suggest that it is a better cutoff point for detecting more patients at risk, who would otherwise not be diagnosed with PH in the present guidelines.

Because no treatment for PH in IPF has been established, a better understanding of the pathogenesis would be meaningful. Previous studies [14, 17, 28–31] have shown the heterogeneity of vascular proliferation in IPF. For example, Judge et al. [30] reported that neovascularization was increased in less fibrotic lesions and decreased in honeycomb lesions in patients with advanced IPF. In addition, it was suggested from another animal model [32] that endothelial apoptosis may be important during early fibrogenesis.

As we showed in table 4, patients with MPAP >20 mm Hg were found to have a higher smoking rate, a lower PaO$_2$, and a lower SpO$_2$ during the 6MWT. Recent studies [33, 34] have described the relation between smoking and pulmonary vascular remodeling. Smoking may influence not only parenchymal destruction but also vascular remodeling through various pathways. In addition, it may be speculated that hypoxia induces vascular remodeling through various factors, such as vascular endothelial growth factor and hypoxia-inducible factor 1 alpha [31, 35]. Our results indicate that smoking and a low PaO$_2$ may play a crucial role in vascular remodeling in mild IPF. Further investigation will be required to determine whether this is the case.

The limitations of this study are as follows. First, the percentage of patients evaluated with biopsy-proven IPF in the previous studies of Lettieri et al. [13] and Hamada et al. [18] was 100 and 74.7%, respectively; however, only 44 (43.6%) patients were diagnosed by surgical lung biopsy in our cases. We suppose our population is closer to

reality because the majority of patients with IPF are diagnosed by clinical criteria in general practice [1]. Secondly, we did not evaluate HRCT findings sufficiently, especially fibrosis and emphysema. Flaherty et al. [4] and Sumikawa et al. [36] reported that the CT fibrotic score was predictive of subsequent mortality. Cottin et al. [37] and Mejia et al. [38] reported the importance of evaluating emphysema. In this study, although we checked the HRCT to diagnose IPF, we did not analyze the relationship between the proportion of fibrosis and emphysema. Further studies are needed to examine this interesting issue. Finally, this is a retrospective study. Collection of additional prospective data is warranted to confirm our findings.

In summary, we demonstrated that higher MPAP and lower %FVC are independent prognostic predictors of IPF. The current results emphasized the importance of evaluating PH for patients with IPF at the initial evaluation. MPAP by RHC is warranted not only for severe IPF patients but also for mild-to-moderate patients with IPF. MPAP >20 mm Hg may be a better cutoff point for detecting more patients at risk among patients with mild IPF.

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Increased periostin associates with greater airflow limitation in patients receiving inhaled corticosteroids

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Background: Periostin, an extracellular matrix protein, contributes to subepithelial thickening in asthmatic airways, and its serum levels reflect airway eosinophilic inflammation. However, the relationship between periostin and the development of airflow limitation, a functional consequence of airway remodeling, remains unknown.

Objective: We aimed to determine the relationship between serum periostin levels and pulmonary function decline in asthmatic patients on inhaled corticosteroid (ICS) treatment.

Methods: Two hundred twenty-four asthmatic patients (average age, 62.3 years) treated with ICS for at least 4 years were enrolled. Annual changes in FEV_1, from at least 1 year after the initiation of ICS treatment to the time of enrollment or later (average, 16.2 measurements over 8 years per individual), were assessed. At enrollment, clinical indices, biomarkers that included serum periostin, and periostin gene polymorphisms were examined. Associations between clinical indices or biomarkers and a decline in FEV_1 of 30 mL or greater per year were analyzed.

Results: High serum periostin levels ($\geq$95 ng/mL) at enrollment, the highest treatment step, higher ICS daily doses, a history of admission due to asthma exacerbation, comorbid or a history of sinusitis, and ex-smoking were associated with a decline in FEV_1 of 30 mL or greater per year. Multivariate analysis showed that high serum periostin, the highest treatment step, and ex-smoking were independent risk factors for the decline. Polymorphisms of periostin gene were related to higher serum periostin levels (rs3829365) and a decline in FEV_1 of 30 mL or greater per year (rs9603226).

Conclusions: Serum periostin appears to be a useful biomarker for the development of airflow limitation in asthmatic patients on ICS. (J Allergy Clin Immunol 2013;132:305-12.)

Key words: Asthma, inhaled corticosteroids, lung function decline, periostin, POSTN gene polymorphism, sinusitis, treatment step

Airway inflammation and remodeling are key features of asthma that have been demonstrated by pathologic[1] and radiologic[2,3] findings. Physiologically, patients with asthma show a greater decline in pulmonary function than subjects without asthma.[4] Studies that were mostly conducted in the era before inhaled corticosteroids (ICS) reported that more severe symptoms or severe exacerbations,[5-7] long-standing asthma,[8] and smoking history[4,8] were moderate-to-strong risk factors for greater decline in pulmonary function.[5] Blood and sputum eosinophilia[9,10] and genetic predisposition[11-13] were also potential risk factors. Because of early intervention with ICS, however, airway inflammation and the degree of annual decline in pulmonary function have been attenuated in most asthmatic patients.[14-16] Meanwhile, a subset of patients still show accelerated decline in FEV_1 and develop irreversible airway obstruction despite adequate treatment.[17,18] van Veen et al[18] found that exhaled nitric oxide of 20

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ppb or higher is a predictor of accelerated decline in pulmonary function in patients with difficult-to-treat asthma. However, other biomarkers for greater decline in FEV_1 despite treatment with ICS remain unknown.

The airway inflammation of asthma is classically characterized by infiltration and activation of eosinophils, mast cells, and T_H2 cells with several mediators and T_H2 cytokines, such as IL-4, IL-5, and IL-13.[19,20] Periostin, a secreted, 90-kDa, extracellular matrix protein that is induced by IL-4 and IL-13, was originally isolated as an osteoblast-specific factor; it shares structural homology to the insect cell adhesion molecule fasciclin I (FAS I) and binds to fibronectin, tenascin-C, and collagen.[21,22] In airway epithelial cells collected from patients with asthma, periostin is one of the upregulated genes,[23] and its expression is correlated with thickness of the airway basement membrane.[24] Takayama et al[21] clearly demonstrated that periostin is deposited in the airway subepithelial layer in asthmatic patients. Moreover, serum periostin is identified as the single best predictor of airway eosinophilia in patients with severe asthma who remain symptomatic despite maximal ICS treatment.[25] Therefore, we hypothesized that periostin would be a novel biomarker of T_H2/eosinophil-driven airway inflammation and greater decline in pulmonary function, a functional consequence of airway remodeling in patients with asthma.

In this study, the effects of biomarkers and clinical indices on greater annual decline in pulmonary function in asthmatic patients on ICS treatment were examined, with the specific aim of determining the association between serum periostin levels and pulmonary function decline. Polymorphisms of the *POSTN* gene, which encodes periostin, were also examined on the hypothesis that *POSTN* gene polymorphisms may affect serum periostin levels.

METHODS
Patients
Patients with asthma were recruited from 9 institutions belonging to the Kinki Hokuriku Airway disease Conference where asthma specialists manage patients. Asthma was diagnosed according to the American Thoracic Society criteria (see the Methods section in this article's Online Repository at www.jacionline.org).[26] From September 2009 to December 2011, patients were enrolled if they had received ICS treatment for 4 years or more, undergone 3 or more pulmonary function tests when they were stable, and were free from exacerbations for at least 1 month. The first pulmonary function test was performed at least 1 year after the commencement of ICS treatment and at 25 years of age or older. Patients who had smoked >10 pack years, smoked in the past 1 year, or had other pulmonary diseases were excluded.

This study was approved by the ethics committee of each participant institution and was registered in the UMIN Clinical Trials Registry (Registry ID UMIN000002414). Written informed consent was obtained from all participants.

Measurements
At enrollment, patients underwent a workup that included answering a self-completed questionnaire, spirometry, and blood tests. After enrollment, spirometry was repeated at least 6 months later for up to 12 months.

Self-completed questionnaire and clinical indices
The self-completed questionnaire was composed of 4 major items, as presented in Table I. (More detail is included in the Methods section in this article's Online Repository.) The Asthma Control Test (ACT) was also scored. The treatment step at enrollment was determined according to the Global Initiative for Asthma 2010 guideline.[27]

Pulmonary function
Spirometry was performed with an electrical spirometer, which was calibrated once a week, at each institution. Spirometry data were obtained only when patients were stable. To determine pulmonary function on daily medications, ICS, and other controllers, including long-acting β_2 agonists, leukotriene receptor antagonists, or slow-release theophylline, were not withdrawn before spirometry.

Measurement of systemic biomarkers
Blood eosinophil and neutrophil counts, and serum levels of total IgE, specific IgE against common inhaled allergens, eosinophil cationic protein (ECP), high-sensitivity C-reactive protein (hsCRP), and periostin were determined.

Serum periostin levels were measured with an enzyme-linked immunosorbent assay at Shino-test (Kanagawa, Japan), as described previously (for additional information, see the Methods section in this article's Online Repository).[28] Pooled serum periostin level data from 66 healthy subjects (age, mean ± SD, 60.7 ± 16.7 years; 40 males)[28,29] were used for comparison with those of asthmatic patients.

Haplotype analysis, DNA extraction, and genotyping of the *POSTN* gene
A total of 47 single-nucleotide polymorphisms (SNPs) in the region of the *POSTN* gene and its upstream, total 39 kb, was captured in the HapMap Japanese data set. Haplotype analysis identified 4 major haplotypes and 2 minor haplotypes. Two minor haplotypes were grouped into the closest major haplotype, and 3 tag SNPs that determined the 4 haplotypes were identified (Fig 1). (More detail is included in the Methods section in this article's Online Repository.)

Genomic DNA was isolated from blood cells with the use of a QIAamp DNA Blood Mini Kit (Qiagen, Tokyo, Japan). SNPs were genotyped with a Taqman genotyping assay according to the manufacturer's instructions (Applied Biosystems, Tokyo, Japan) and analyzed with an Applied Biosystems 7300 Real-Time PCR System (Applied Biosystems).

Statistical analysis
Statistical analyses were performed with JMP version 9.0 (SAS Institute Inc, Tokyo, Japan). Annual changes in FEV_1 (ΔFEV_1) were estimated for each subject by fitting a least-square regression line to all of the subject's available data points. Receiver operating characteristic (ROC) curve analysis was performed to determine a serum periostin cutoff value for asthmatic patients. The effects of serum biomarkers or other indices on ΔFEV_1 were estimated with a generalized linear mixed model with adjustment for sex, height, age at enrollment, and FEV_1 at the first measurement. The institutions were included as random effects in this model. On univariate analysis of ΔFEV_1, the adjusted P value, that is, q value, which was a measure of significance in terms of the false discovery rate, was obtained with R and QVALUE software[30] to determine spurious significance in multiple testing. The effects on the dichotomous data for a decline in FEV_1 of 30 mL or greater per year[31] were similarly estimated with a generalized linear mixed model by IBM

TABLE I. Contents of the self-completed questionnaire

Asthma-related history
  Family history of asthma
  Age of asthma onset
  History of pediatric asthma
  History of admission due to asthma worsening or exacerbation
  Aspirin hypersensitivity
  Asthma deterioration at the working place
Comorbidity or a history of the following diseases:
  Allergic dermatitis
  Allergic rhinitis
  Seasonal rhinitis
  Allergic conjunctivitis
  Chronic sinusitis
  Cardiovascular diseases, including ischemic heart disease
  Gastrointestinal diseases, including GERD
  Collagen vascular diseases, including rheumatoid arthritis
  Diabetes mellitus
  Pulmonary diseases other than asthma
  Other diseases including malignancy
Lifestyle and environment
  Smoking history
  Pet breeding
  Type of occupation
  A highway near the home
  Age at menopause
Adherence to medication, sputum production, and exacerbations
  How often do you forget to take inhaled corticosteroids or other
    medications?
  0, never; 1, seldom; 2, sometimes; 3, often; 4, always
  How often do you produce sputum?
  0, never; 1, once in a few days: 2, every morning; 3, every morning and
    daytime
  How often did you receive systemic steroids due to asthma exacerbations
   during the recent 6 months?
  0, never; 1, once; 2, twice or more

GERD, Gastroesophageal reflux disease.

SPSS Advanced Statistics 19 (SPSS Inc, Tokyo, Japan). Multivariate analysis was performed with variables with $P < .10$ on univariate analysis, except for ICS daily maintenance dose because of its strong correlation with treatment step. On multivariate analysis, the periostin level was considered as a dichotomous variable (high or low) instead of a continuous variable. Correlation coefficients between serum periostin levels and clinical indices were estimated by fitting least-square regression lines to data, in which institutions were included as random effects. Unpaired t and χ^2 tests were performed for comparisons of continuous and dichotomous variables, respectively. When data were not normally distributed, they were log-transformed. Data are presented as means ± SDs. P values ≤ .05 were considered significant.

RESULTS

Patients' characteristics

Initially, 233 patients were enrolled in this study, but 9 patients were excluded: 5 with a smoking history of >10 pack years and 4 who did not have enough pulmonary function data available. The demographic data of the remaining 224 patients are presented in Table II. The mean age at enrollment was 62.3 ± 13.7 years. Overall, 130 (58%) had onset of asthma at 40 years of age or older. The average number of measurements of FEV_1, follow-up period, and ΔFEV_1 of 224 patients were 16.2 ± 13.9 times, 8.0 ± 4.5 years, and −7.8 ± 34.6 mL per year, respectively. The distribution of ΔFEV_1 in this population is shown in Fig E1 (in the Online Repository available at www.jacionline.org). Within 2 years after
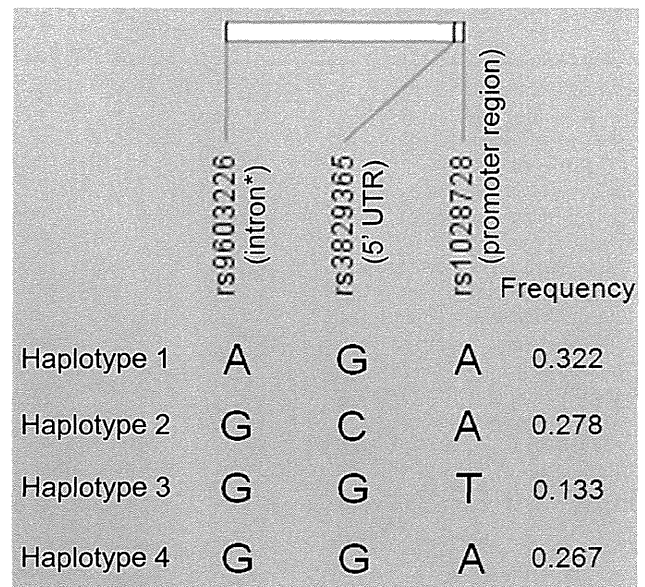
FIG 1. Three tag SNPs that determine 4 major haplotypes of the *POSTN* gene and haplotype frequencies in the Japanese population are presented. *At intron 66 bp upstream of exon 21.

diagnosis, 46% of patients started ICS treatment. At enrollment, 82% of patients took controllers such as long-acting β_2 agonists, leukotriene receptor antagonists, or sustained release theophylline to achieve adequate asthma control. On the basis of a questionnaire, adherence to medication was satisfactory; 49% of the participants never and 38% seldom forgot to take ICS or other medications. On the basis of ACT scores, 50% was totally controlled, and 38% scored from 20 to 24, indicating that they were well controlled at enrollment.

Serum periostin levels of asthmatic patients (92.8 ± 38.4 ng/mL) were significantly higher than those of healthy subjects (39.1 ± 24.5 ng/mL; $P < .001$). The ROC curve analysis was performed to discriminate patients with asthma who were thought to have refractory T_H2 inflammation despite long-term ICS treatment from healthy subjects. The highest specificity among the 4 cutoff values tested was achieved at 95 ng/mL (0.985) in the comparison study of 224 asthmatic patients and 66 healthy subjects. Therefore, a cutoff value of 95 ng/mL was used to define a high serum periostin group, although it had relatively lesser sensitivity (0.379) (see Fig E2 in this article's Online Repository at www.jacionline.org). In asthmatic patients, 85 patients (38%) had high serum periostin levels (≥95 ng/mL). Of the 85 patients, 40 patients (47%) were on treatment step 4, according to the treatment step classification,[27] and 9 patients (11%) were on treatment step 5.

Associations between serum periostin levels and greater annual decline in FEV_1 and a decline in FEV_1 of 30 mL or greater per year

In an analysis of continuous values of ΔFEV_1, greater decline in FEV_1 was associated with higher serum periostin levels at enrollment, treatment step 5, lower ACT scores, incomplete adherence to medications, comorbid or a history of sinusitis, and comorbid diabetes mellitus (Table III). When patients were stratified into 2 groups according to their serum periostin levels, high serum periostin (≥95 ng/mL) was also associated with greater