number of A alleles of −123 C/A SNP, T alleles of −88 G/T SNP, and A alleles of +20 C/A SNP carried by HBE cells according to a simple regression analysis (p=0.013, p=0.0035, and p<0.0001, respectively) (Fig. 4b). Multiple regression analysis showed that the association of +20 C/A SNP remained significant (p=0.016), whereas the association of −123 C/A and −88 G/T SNPs was insignificant (p=0.67 and p=0.78, respectively). In contrast, baseline expression of the T0 transcript was slightly higher in proportion to the number of C alleles of −77 C/G SNP; however, this increase was not statistically significant (p=0.086) (Fig. 4a).

When the relative expression of the total MxA transcripts was compared with expression of the T1 and T0 transcripts under the unstimulated condition, the overall expression of MxA strongly correlated with expression of the T1 transcript (Spearman's rank correlation coefficient; rs=0.759), whereas it weakly correlated with expression of the T0 transcript (rs=0.388). The total expression of MxA was significantly higher in proportion to the number of −123 A, −88 T, and +20 A alleles (p=0.0004, p<0.0001, and p< 0.0001, respectively), which was similar to the linear relationship between the T1 transcript and number of alleles shown above.

When immortalized HBE cell line BEAS-2B was stimulated with poly(I:C), time-course analysis revealed that expression of the T0 transcript was the highest after 24 h, whereas that of the T1 transcript was the highest after 6–12 h of incubation (Online Resource 3). We therefore investigated the expression of T0 and T1 transcripts in HBE cells (n=29) stimulated with poly(I:C) for 24 h. The expression of the T0 transcript increased eightfold, whereas that of the T1 transcript increased 870-fold. Poly(I:C)-induced expression of both transcripts was not associated with either allele of the four SNPs (Fig. 5a, b). When HBE cells (n=9) were stimulated with IFN-β for 12 h, the T0 transcript was induced eightfold, and the T1 transcript was induced 640-fold. IFN-β-induced expression of the transcripts did not vary among genotypes (data not shown).

Discussion

In this study, we investigated the expression profile of MxA by analyzing expression of the original transcript T1 and the transcript variant T0 in primary cultured HBE cells. According to our absolute quantification method using real-time RT-PCR, the amount of the T0 transcript was approximately half of that of the T1 transcript at the baseline level. Although expression of the T0 transcript was also induced by type I IFNs and poly(I:C) and its 5′ proximal region has a potential ISRE motif, IFN-β and poly(I:C) inducibility of the T1 transcript was at least 100-fold higher than that of the

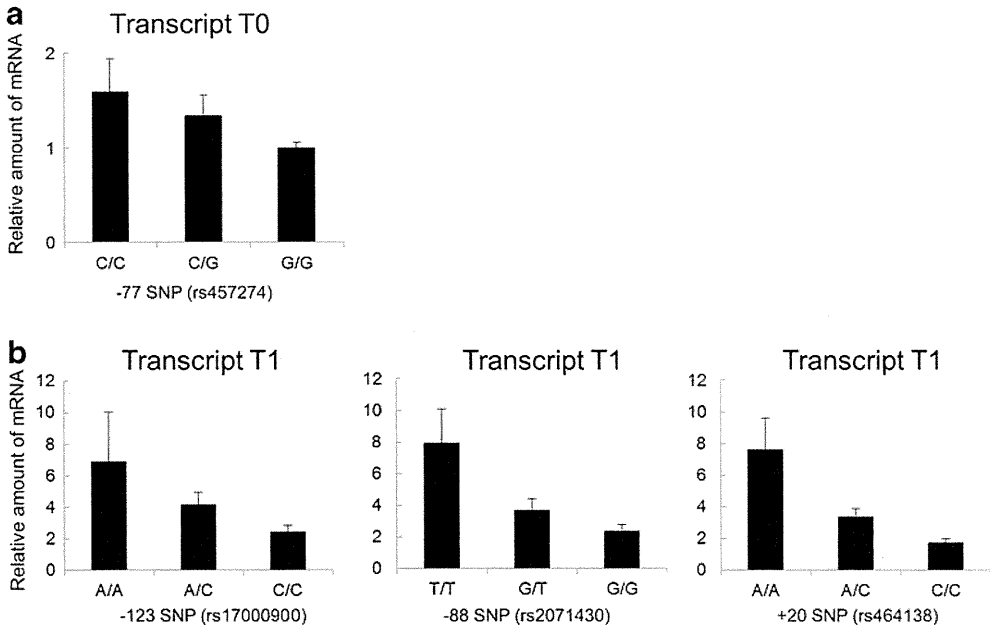

Fig. 4 Differences in the baseline expression of transcript variants among the genotypes of the promoter and exon 1 SNPs in HBE cells. Expression of **a** T0 and **b** T1 transcripts under the unstimulated condition in HBE cells with each genotype of −77 C/G SNP for the T0 transcript (C/C, n=8; C/G, n=18; G/G, n=12), of −123 C/A SNP (A/A, n=2; A/C, n=18; C/C, n=18), of −88 G/T SNP (T/T, n=3; G/T, n=19; G/G, n=16), and of +20 C/A SNP (A/A, n=5; A/C, n=22; C/C, n=11) for the T1 transcript is shown. The relative amounts of mRNA of each transcript compared with that of the T0 transcript in GG cells without poly(I:C) stimulation are shown as mean±SEM. Possible associations between the number of alleles and the amount of the corresponding transcripts were assessed by a simple regression model respectively (p=0.086 for the number of −77 C alleles, p=0.013 for −123 A alleles, p=0.0035 for −88 T alleles, and p<0.0001 for +20 A alleles)

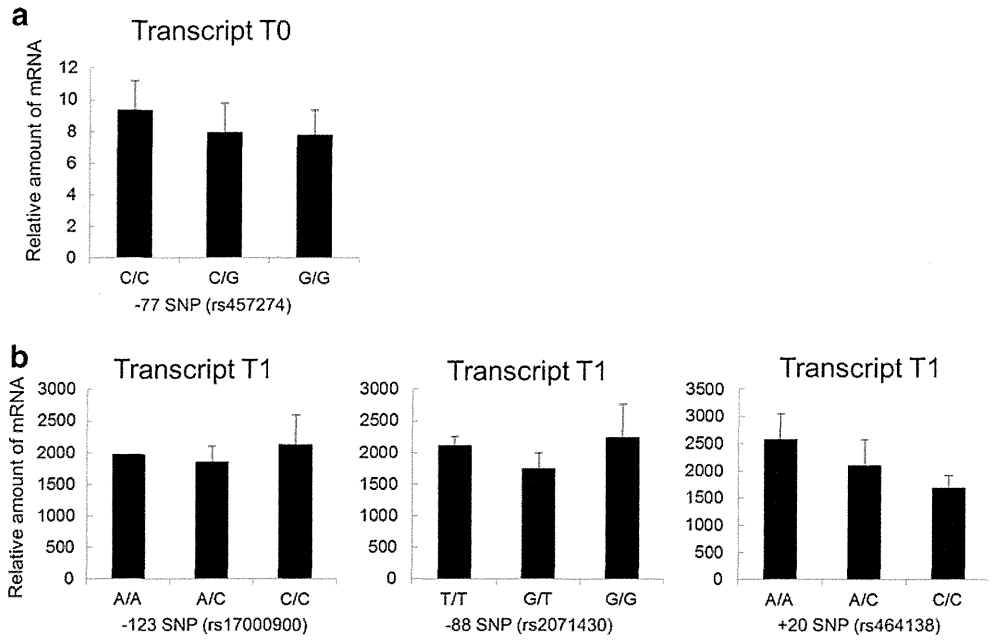

Fig. 5 Differences in the poly(I:C)-induced expression of transcript variants among the genotypes of the promoter and exon 1 SNPs in HBE cells. Expression of **a** T0 and **b** T1 transcripts in the presence of poly(I:C) in HBE cells with each genotype of −77 C/G SNP for the T0 transcript (C/C, n=5; C/G, n=13; G/G, n=11), of −123 C/A SNP (A/A, n=1; A/C, n=12; C/C, n=16), of −88 G/T SNP (T/T, n=2; G/T, n=13; G/G, n=14), and of +20 C/A SNP (A/A, n=3; A/C, n=16; C/C, n=10) for the T1 transcript is shown. The relative amounts of mRNA of each transcript compared with that of the T0 transcript in GG cells without poly(I:C) stimulation are shown as mean±SEM. Possible associations were assessed by a simple regression model (p=0.647 for the number of −77 C alleles, p=0.662 for −123 A alleles, p=0.539 for −88 T alleles, and p=0.331 for +20 A alleles)

T0 transcript. This observation implies that MxA response to viral infection of respiratory cells is mostly controlled by the expression of the T1 transcript.

Remarkably increased levels of the T1 transcript after stimulation with type I IFNs or an IFN-inducing agent, poly(I:C), were consistent with the general consensus that the induction of MxA requires type I or type III IFN signaling (Haller and Kochs 2011). However, levels of the T1 transcript were also elevated in HBE cells by other physiological stimuli. Moderate elevation of levels of the T1 transcript in the presence of IFN-γ and TNF-α may be mediated by the secondary induction of type I or type III IFNs. Although Mahanonda et al. (2012) demonstrated the induction of MxA by α-defensin in primary human gingival epithelial cells, the mechanism by which the T1 transcript was upregulated by α-defensin remains unknown. These observations support the idea that the T1 transcript plays a major role in airway diseases.

We also analyzed the expression of T0 transcript with alternative 5′ untranslated exons. Alternative promoter usage is now recognized as a common mechanism in the transcriptional regulation of mammalian genes (Davuluri et al. 2008). Among IFN-stimulated genes, ADAR1 was shown to have alternative promoters (George and Samuel 1999): one promoter contributes to constitutive expression and the other to inducible expression. To our knowledge, no IFN-stimulated genes other than MxA, whose antiviral function is well known (Sadler and Williams 2008), have been reported to possess two IFN-inducible transcripts with distinct first exons. The molecular mechanism for tissue specificity of these transcripts is unknown; however, of note, the promoter of the T0 transcript contains the putative steroidogenic factor-1 binding site (position −636 to −644) thought to be important to testis- and adrenal gland-specific gene expression (Schimmer and White 2010). A study of the expression profiles of MxA in various organs would be interesting.

Although IFN-mediated upregulation of the T0 transcript was moderate in contrast to that of the T1 transcript, baseline levels of the T0 transcript were not negligible in HBE cells. It is thus conceivable that the T0 transcript plays a minor but independent role in the human airway. Furthermore, considering the difference between the time course of mRNA expression of the T0 and T1 transcripts after poly(I:C) stimulation, it is likely that other factors further modulate their induction levels. Because some reports (Aebi et al. 1989; Goetschy et al. 1989; Prescott et al. 2005) indicate the presence of IFN-independent induction of MxA in contrast to the results of Holzinger et al. (2007), it would be worth investigating whether the T0 transcript can be induced through an IFN-independent signaling system in viral infection.

We observed the regulatory effects of −123, −88, and +20 SNPs on mRNA levels of the T1 transcript in HBE cells

under the unstimulated condition. When we evaluated the overall expression of the MxA transcripts by real-time RT-PCR, it was found to be closely correlated with levels of the T1 transcript, suggesting that individual variation of the total expression of MxA is mainly explained by the T1 transcript. Indeed overall levels of MxA as well as expression of the T1 transcript were strongly associated with these three SNPs at baseline levels. It has been repeatedly reported that the minor A allele of −123 SNP and the minor T allele of −88 SNP, which are in strong LD, were associated with the overall transcriptional activity of the gene (Hijikata et al. 2001; Torisu et al. 2004). Expression of MxA was associated with −88 G/T SNP when PBMC cells were stimulated with IFN-α2 for 12 h (Fernandez-Arcas et al. 2004). In one study (Furuyama et al. 2006), the results of a luciferase reporter assay suggested that −123 SNP contributed to basal expression levels of MxA, whereas −88 SNP contributed to the induction of expression by IFNs. Ching et al. (2010) further showed that the −123A allele had a stronger binding affinity to nuclear proteins from unstimulated cells and that the −88 T allele preferentially bound to the protein after IFN-β stimulation. In our study using HBE cells, −123, −88, and +20 SNPs were all associated with baseline expression of the T1 transcript, and according to a multiple regression analysis, among the three SNPs, +20 C/A SNP was still associated with baseline expression of the T1 transcript. This finding may be attributed to the difference in cell type; however, extensive investigation is required to determine the possible effect of +20 SNP or other unknown functional polymorphisms in strong LD. Recently, Tran Thi Duc et al. (2012) reported that three SNPs (−309 C/G, −101 G/A, and +20 C/A) also contributed to the promoter activity in combination with well-known effects of −123 and −88 SNPs. We could not examine −309 and −101 SNPs in our samples because −309 C/G and −101 G/A SNPs were detected only in the African population and their minor allele frequencies were relatively low (Duc et al. 2012).

Under the poly(I:C)-stimulated condition, the +20 SNP also tended to be associated with expression of the T1 transcript in our study; however, this tendency was not statistically significant. When HBE cells were stimulated with IFN-β for 12 h, the same three SNPs were not associated with the expression level. These findings may conflict with the in vitro effects of these 5′ SNPs on the IFN-inducible promoter activity previously assessed by a luciferase reporter system (Hijikata et al. 2001; Torisu et al. 2004; Tran Thi Duc et al. 2012). In our study, however, the mRNA induced by poly (I:C), the dsRNA analog to mimic viral infection, was directly assessed in primary cultured HBE cells, which implies that individual variance of relevant factors such as toll-like receptor 3 and subsequent IFN signaling pathways might have affected the mRNA levels in the IFN-stimulated condition and have masked the independent effects of these promoter SNPs of the MxA gene.

We previously reported that the promoter −88 SNP was associated with severity of SARS in the Vietnamese population (Hamano et al. 2005), and the promoter −123 SNP was associated with SARS in the Chinese population (Ching et al. 2010). According to Chen and Subbarao (2007), IFN induction is completely suppressed in SARS coronavirus-infected cells. Our ex vivo findings that these regulatory SNPs were mainly involved in baseline expression of the T1 transcript support the results of these disease association studies. However, we could not show significant difference in the regulatory effects between −88 and −123 SNPs, possibly because of strong LD between these two SNPs in the Japanese population (r^2=0.83) compared with moderate LD in the Chinese population (r^2=0.39) (Ching et al. 2010).

In conclusion, we characterized the expression profile of the previously known transcript and the transcript variant of MxA and demonstrated a significant effect of its 5′ SNPs on basal expression of the overall transcripts in HBE cells. Our findings may lead to an improved understanding of the association of MxA SNPs with susceptibility to respiratory viral infections.

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Conflict of interest All authors have no conflict of interest on this work.

References

Abe H, Hayes CN, Ochi H, Maekawa T, Tsuge M, Miki D, Mitsui F, Hiraga N, Imamura M, Takahashi S, Kubo M, Nakamura Y, Chayama K (2011) IL28 variation affects expression of interferon stimulated genes and peg-interferon and ribavirin therapy. J Hepatol 54:1094–1101

Aebi M, Fah J, Hurt N, Samuel CE, Thomis D, Bazzigher L, Pavlovic J, Haller O, Staeheli P (1989) cDNA structures and regulation of two interferon-induced human Mx proteins. Mol Cell Biol 9:5062–5072

Barrett JC, Fry B, Maller J, Daly MJ (2005) Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 21:263–265

Chen J, Subbarao K (2007) The immunobiology of SARS. Annu Rev Immunol 25:443–472

Ching JC, Chan KY, Lee EH, Xu MS, Ting CK, So TM, Sham PC, Leung GM, Peiris JS, Khoo US (2010) Significance of the myxovirus resistance A (MxA) gene −123C>a single-nucleotide polymorphism in suppressed interferon beta induction of severe acute respiratory syndrome coronavirus infection. J Infect Dis 201:1899–1908

Davuluri RV, Suzuki Y, Sugano S, Plass C, Huang TH (2008) The functional consequences of alternative promoter use in mammalian genomes. Trends Genet 24:167–177

Duc TT, Farnir F, Michaux C, Desmecht D, Cornet A (2012) Detection of new biallelic polymorphisms in the human MxA gene. Mol Biol Rep 39:8533–8538

Fernandez-Arcas N, Blanco A, Gaitan MJ, Nyqvist M, Alonso A, Reyes-Engel A (2004) Differential transcriptional expresion of

the polymorphic myxovirus resistance protein A in response to interferon-alpha treatment. Pharmacogenetics 14:189–193

Furuyama H, Chiba S, Okabayashi T, Yokota S, Nonaka M, Imai T, Fujii N, Matsumoto H (2006) Single nucleotide polymorphisms and functional analysis of MxA promoter region in multiple sclerosis. J Neurol Sci 249:153–157

George CX, Samuel CE (1999) Human RNA-specific adenosine deaminase ADAR1 transcripts possess alternative exon 1 structures that initiate from different promoters, one constitutively active and the other interferon inducible. Proc Natl Acad Sci U S A 96:4621–4626

Goetschy JF, Zeller H, Content J, Horisberger MA (1989) Regulation of the interferon-inducible IFI-78K gene, the human equivalent of the murine Mx gene, by interferons, double-stranded RNA, certain cytokines, and viruses. J Virol 63:2616–2622

Gray TE, Guzman K, Davis CW, Abdullah LH, Nettesheim P (1996) Mucociliary differentiation of serially passaged normal human tracheobronchial epithelial cells. Am J Respir Cell Mol Biol 14:104–112

Haller O, Kochs G (2011) Human MxA protein: an interferon-induced dynamin-like GTPase with broad antiviral activity. J Interferon Cytokine Res 31:79–87

Hamano E, Hijikata M, Itoyama S, Quy T, Phi NC, Long HT, Ha LD, Ban VV, Matsushita I, Yanai H, Kirikae F, Kirikae T, Kuratsuji T, Sasazuki T, Keicho N (2005) Polymorphisms of interferon-inducible genes OAS-1 and MxA associated with SARS in the Vietnamese population. Biochem Biophys Res Commun 329:1234–1239

Hijikata M, Mishiro S, Miyamoto C, Furuichi Y, Hashimoto M, Ohta Y (2001) Genetic polymorphism of the MxA gene promoter and interferon responsiveness of hepatitis C patients: revisited by analyzing two SNP sites (−123 and −88) in vivo and in vitro. Intervirology 44:379–382

Hijikata M, Ohta Y, Mishiro S (2000) Identification of a single nucleotide polymorphism in the MxA gene promoter (G/T at nt −88) correlated with the response of hepatitis C patients to interferon. Intervirology 43:124–127

Holzinger D, Jorns C, Stertz S, Boisson-Dupuis S, Thimme R, Weidmann M, Casanova JL, Haller O, Kochs G (2007) Induction of MxA gene expression by influenza A virus requires type I or type III interferon signaling. J Virol 81:7776–7785

Horisberger MA, McMaster GK, Zeller H, Wathelet MG, Dellis J, Content J (1990) Cloning and sequence analyses of cDNAs for interferon- and virus-induced human Mx proteins reveal that they contain putative guanine nucleotide-binding sites: functional study of the corresponding gene promoter. J Virol 64:1171–1181

Kong XF, Zhang XX, Gong QM, Gao J, Zhang SY, Wang L, Xu J, Han Y, Jin GD, Jiang JH, Zhang DH, Lu ZM (2007) MxA induction may predict sustained virologic responses of chronic hepatitis B patients with IFN-alpha treatment. J Interferon Cytokine Res 27:809–818

Leong DT, Gupta A, Bai HF, Wan G, Yoong LF, Too HP, Chew FT, Hutmacher DW (2007) Absolute quantification of gene expression in biomaterials research using real-time PCR. Biomaterials 28:203–210

Mahanonda R, Sa-Ard-Iam N, Rerkyen P, Thitithanyanont A, Subbalekha K, Pichyangkul S (2012) MxA expression induced by alpha-defensin in healthy human periodontal tissue. Eur J Immunol 42:946–956

McGilvray I, Feld JJ, Chen L, Pattullo V, Guindi M, Fischer S, Borozan I, Xie G, Selzner N, Heathcote EJ, Siminovitch K (2012) Hepatic cell-type specific gene expression better predicts HCV treatment outcome than IL28B genotype. Gastroenterology 142:1122–1131

Peng XM, Lei RX, Gu L, Ma HH, Xie QF, Gao ZL (2007) Influences of MxA gene−88 G/T and IFN-gamma +874 A/T on the natural history of hepatitis B virus infection in an endemic area. Int J Immunogenet 34:341–346

Prescott J, Ye C, Sen G, Hjelle B (2005) Induction of innate immune response genes by Sin Nombre hantavirus does not require viral replication. J Virol 79:15007–15015

Randall RE, Goodbourn S (2008) Interferons and viruses: an interplay between induction, signalling, antiviral responses and virus countermeasures. J Gen Virol 89:1–47

Ronni T, Matikainen S, Lehtonen A, Palvimo J, Dellis J, Van Eylen F, Goetschy JF, Horisberger M, Content J, Julkunen I (1998) The proximal interferon-stimulated response elements are essential for interferon responsiveness: a promoter analysis of the antiviral MxA gene. J Interferon Cytokine Res 18:773–781

Sadler AJ, Williams BR (2008) Interferon-inducible antiviral effectors. Nat Rev Immunol 8:559–568

Schimmer BP, White PC (2010) Minireview: steroidogenic factor 1: its roles in differentiation, development, and disease. Mol Endocrinol 24:1322–1337

Torisu H, Kusuhara K, Kira R, Bassuny WM, Sakai Y, Sanefuji M, Takemoto M, Hara T (2004) Functional MxA promoter polymorphism associated with subacute sclerosing panencephalitis. Neurology 62:457–460

Tran Thi Duc T, Desmecht D, Cornet A (2012) Functional characterization of new allelic polymorphisms identified in the promoter region of the human MxA gene. Int J Immunogenet. doi:10.1111/j.1744-313X.2012.01153.x

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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Key words: chronic obstructive pulmonary disease, 6-min walk test, incremental shuttle walk test, St George's Respiratory Questionnaire

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 $p \leqq 0.15$ 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

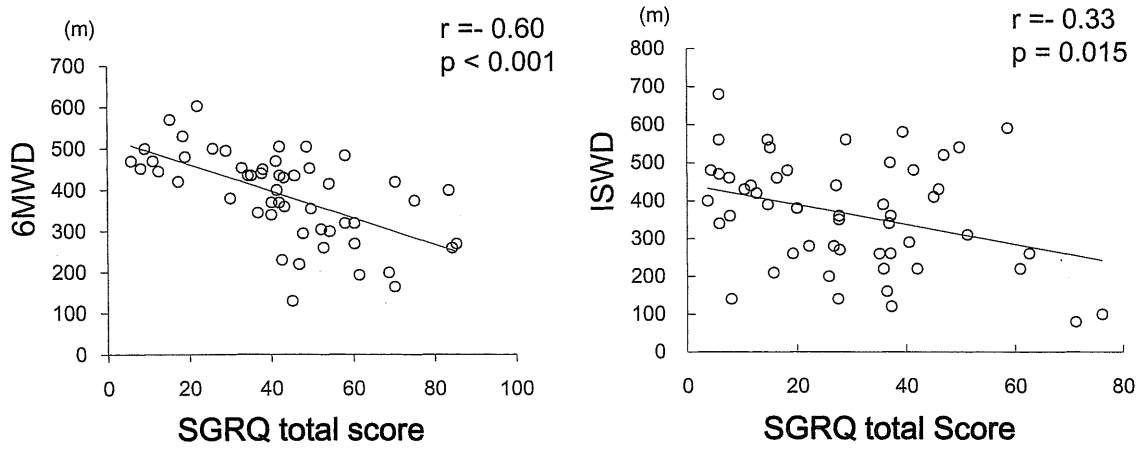

Fig. 1

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

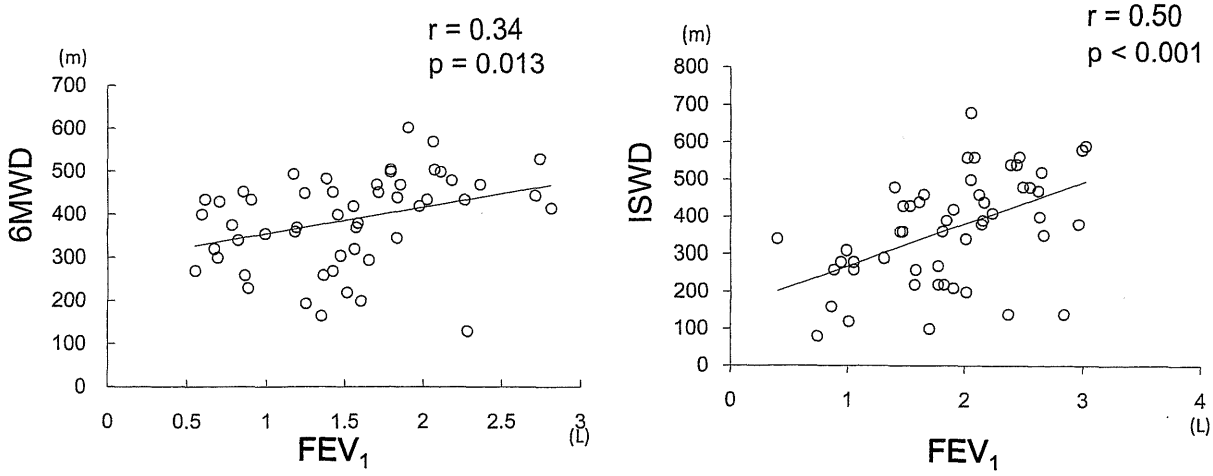

Fig. 2

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 %≤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 correlated 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 HROoL 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.

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References

1) Brown CD, Wise RA : Field tests of exercise in COPD : the six-minute walk test and the shuttle walk test. COPD 4 : 217-223, 2007

2) Mungall IP, Hainsworth R : Assessment of respiratory function in patients with chronic obstructive airways disease. Thorax 34 : 254-258, 1979

3) Solway S, Brooks D, Lacasse Y, Thomas S : A qualitative systematic overview of the measurement properties of functional walk tests used in the cardiorespiratory domain. Chest 119 : 256-270, 2001
4) Singh SJ, Morgan MD, Scott S, Walters D, Hardman AE : Development of a shuttle walking test of disability in patients with chronic airways obstruction. Thorax 47 : 1019-1024, 1992
5) Singh SJ, Morgan MD, Hardman AE, Rowe C, Bardsley PA : Comparison of oxygen uptake during a conventional treadmill test and the shuttle walking test in chronic airflow limitation. Eur Respir J 7 : 2016-2020, 1994
6) National Institute of Health, National Heart, Lung, and Blood Institute Global Initiative for Chronic Obstructive Lung Disease : Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease. Publication number 2701, 2001
7) American Thoracic Society : Standardization of Spirometry, 1994 Update. Am J Respir Crit Care Med 152 : 1107-1136, 1995
8) Standards of pulmonary function tests for Japanese. Jpn J Respir Society 31 : 421-427, 1993 (in Japanese)
9) Nishida S, Kambe M, Sewake N, Takano M, Kawane H : Pulmonary function in healthy subjects and its prediction : 5. Pulmonary diffusing capacity in adults. Jpn J Clin Pathol 24 : 941-947, 1976 (in Japanese)
10) Kory RC, Callahan R, Boren HG, Syner JC : The Veterans Administration-Army cooperative study of pulmonary function. I. Clinical spirometry in normal men. Am J Med 41 : 96-101, 1966
11) Jones PW, Quirk FH, Baveystock CM, Littlejohns P : A self-complete measure of health status for chronic airflow limitation. The St. George's Respiratory Questionnaire. Am Rev Respir Dis 145 : 1321-1327, 1992
12) Hajiro T, Nishimura K, Tsukino M, Ikeda A, Koyama H, Izumi T : Comparison of discriminative properties among disease-specific questionnaires for measuring health-related quality of life in patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 157 (3 Pt 1) : 785-790, 1998
13) ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories. ATS Statement : Guidelines for the six-minute walk test. Am J Respir Crit Care Med 166 : 111-117, 2002
14) Dyer CA, Singh SJ, Stockley RA, Sinclair AJ, Hill SL : The incremental shuttle walking test in elderly people with chronic airflow limitation. Thorax 57 : 34-38, 2002
15) Vagaggini B, Taccola M, Severino S, Marcello M, Antonelli S, Brogi S, De Simone C, Giardina A, Paggiaro PL : Shuttle walking test and 6-minute walking test induce a similar cardiorespiratory performance in patients recovering from an acute exacerbation of chronic obstructive pulmonary disease. Respiration 70 : 579-584, 2003
16) Mak VH, Bugler JR, Roberts CM, Spiro SG : Effect of arterial oxygen desaturation on six minute walk distance, perceived effort, and perceived breathlessness in patients with airflow limitation. Thorax 48 : 33-38, 1993
17) Wijkstra PJ, TenVergert EM, van der Mark TW, Postma DS, Van Altena R, Kraan J, Koëter GH : Relation of lung function, maximal inspiratory pressure, dyspnoea, and quality of life with exercise capacity in patients with chronic obstructive pulmonary disease. Thorax 49 : 468-472, 1994
18) Sanchez FF, Faganello MM, Tanni SE, Lucheta PA, Padovani CR, Godoy I : Relationship between disease severity and quality of life in patients with chronic obstructive pulmonary disease. Braz J Med Biol Res 41 : 860-865, 2008
19) Garrod R, Bestall JC, Paul EA, Wedzicha JA, Jones PW : Development and validation of a standardized measure of activity of daily living in patients with severe COPD : the London Chest Activity of Daily Living scale (LCADL). Respir Med 94 : 589-596, 2000

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

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Key Words
Idiopathic pulmonary fibrosis · Mortality · Pulmonary hypertension

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 Pa_{O_2}, modified Medical Research Council score, 6-min walk distance, and lowest Sp_{O_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.

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.

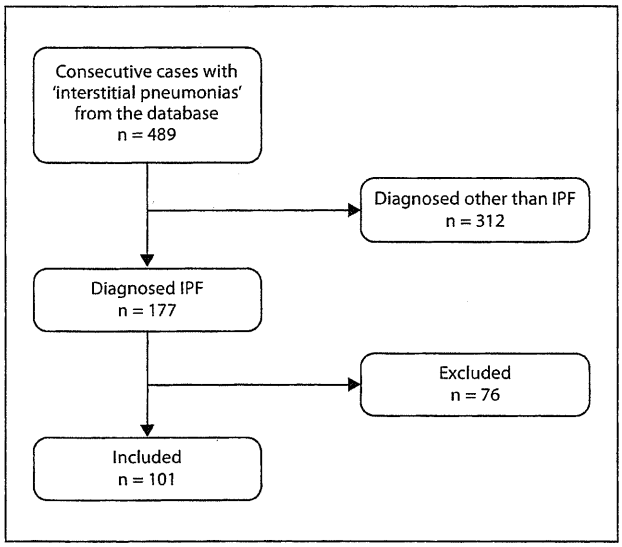

Fig. 1. Screening and inclusion process for patients in the study.

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.

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

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 (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

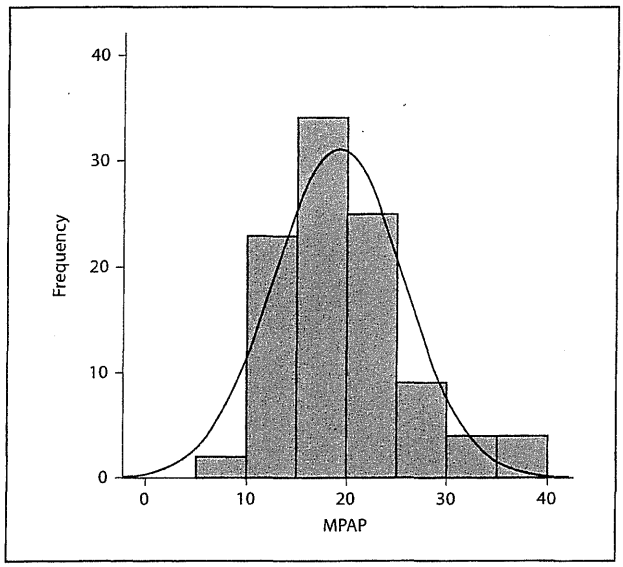

Fig. 2. Histogram of MPAP.

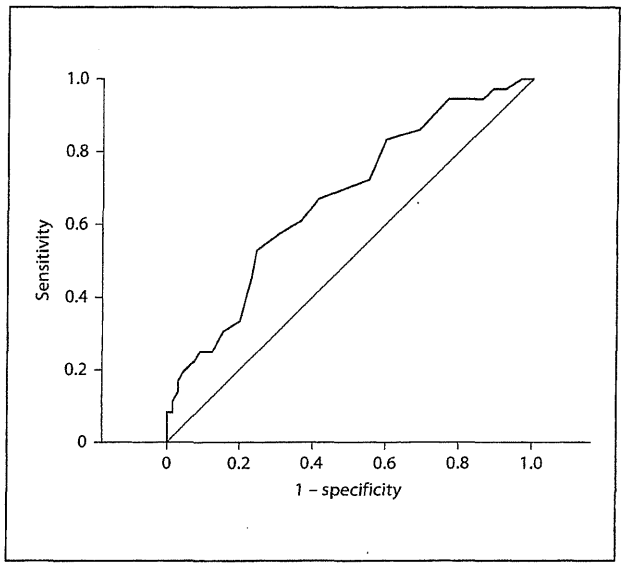

Fig. 3. ROC of pulmonary artery pressure for the prognosis.

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).

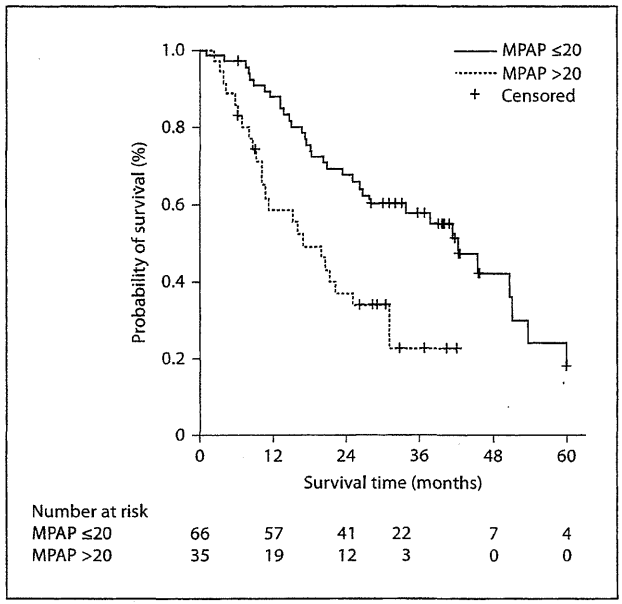

Fig. 4. Kaplan-Meier curves for 5-year survival according to MPAP (p = 0.001). Survival curves were compared with log-rank statistics.

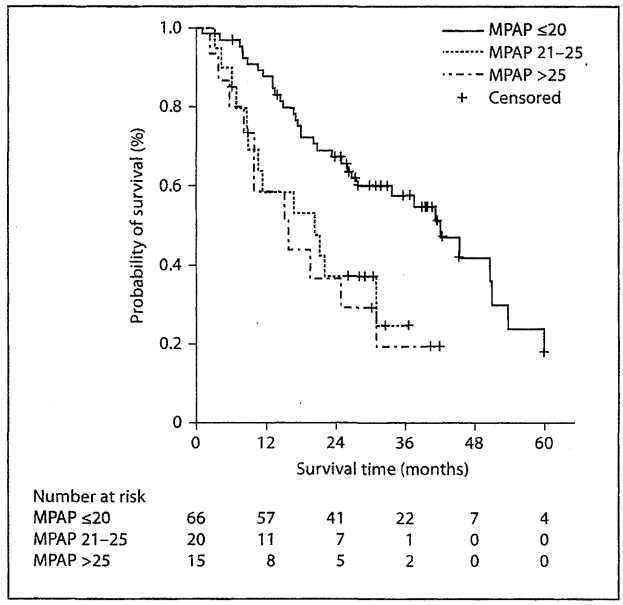

Fig. 5. Kaplan-Meier curves for 5-year survival according to MPAP (p = 0.003). Survival curves were compared with log-rank statistics.

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
Pa_{O_2}, 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 Spo_2, %	83.8 ± 9.1	75.1 ± 10.6	<0.001
PVRI, $dyn \cdot s \cdot cm^{-5} \cdot m^2$	225.9 ± 90.7	397.4 ± 177.5	<0.001
Cardiac index, $l \cdot min^{-1} \cdot m^{-2}$	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, DLCO 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 explained 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 %DLCO 47.9%) was milder than their cohort (mean MPAP 33.5 mm Hg, PVR 5.9 Wood units, %FVC 67.9%, %DLCO 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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Financial Disclosure and Conflicts of Interest

All of the authors confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

References

1 American Thoracic Society: Idiopathic pulmonary fibrosis: diagnosis and treatment. International consensus statement – American Thoracic Society (ATS), and the European Respiratory Society (ERS). Am J Respir Crit Care Med 2000;161:646–664.

2 King TE Jr, Tooze JA, Schwarz MI, Brown KR, Cherniack RM: Predicting survival in idiopathic pulmonary fibrosis: scoring system and survival model. Am J Respir Crit Care Med 2001;164:1171–1181.

3 King TE Jr, Schwarz MI, Brown K, Tooze JA, Colby TV, Waldron JA Jr, Flint A, Thurlbeck W, Cherniack RM: Idiopathic pulmonary fibrosis: relationship between histopathologic features and mortality. Am J Respir Crit Care Med 2001;164:1025–1032.

4 Flaherty KR, Toews GB, Travis WD, Colby TV, Kazerooni EA, Gross BH, Jain A, Strawderman RL 3rd, Paine R, Flint A, Lynch JP 3rd, Martinez FJ: Clinical significance of histological classification of idiopathic interstitial pneumonia. Eur Respir J 2002;19:275–283.

5 Collard HR, King TE Jr, Bartelson BB, Vourlekis JS, Schwarz MI, Brown KK: Changes in clinical and physiologic variables predict survival in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 2003;168:538–542.

6 Lama VN, Flaherty KR, Toews GB, Colby TV, Travis WD, Long Q, Murray S, Kazerooni EA, Gross BH, Lynch JP 3rd, Martinez FJ: Prognostic value of desaturation during a 6-min walk test in idiopathic interstitial pneumonia. Am J Respir Crit Care Med 2003;168:1084–1090.

7 Flaherty KR, Mumford JA, Murray S, Kazerooni EA, Gross BH, Colby TV, Travis WD, Flint A, Toews GB, Lynch JP 3rd, Martinez FJ: Prognostic implications of physiologic and radiographic changes in idiopathic interstitial pneumonia. Am J Respir Crit Care Med 2003;168:543–548.

8 Jegal Y, Kim DS, Shim TS, Lim CM, Do Lee S, Koh Y, Kim WS, Kim WD, Lee JS, Travis WD, Kitaichi M, Colby TV: Physiology is a stronger predictor of survival than pathology in fibrotic interstitial pneumonia. Am J Respir Crit Care Med 2005;171:639–644.

9 Alakhras M, Decker PA, Nadrous HF, Collazo-Clavell M, Ryu JH: Body mass index and mortality in patients with idiopathic pulmonary fibrosis. Chest 2007;131:1448–1453.

10 Nishiyama O, Taniguchi H, Kondoh Y, Kimura T, Kato K, Kataoka K, Ogawa T, Watanabe F, Arizono S: A simple assessment of dyspnoea as a prognostic indicator in idiopathic pulmonary fibrosis. Eur Respir J 2010;36:1067–1072.

11 Nadrous HF, Pellikka PA, Krowka MJ, Swanson KL, Chaowalit N, Decker PA, Ryu JH: Pulmonary hypertension in patients with idiopathic pulmonary fibrosis. Chest 2005; 128:2393–2399.

12 Leuchte HH, Baumgartner RA, Nounou ME, Vogeser M, Neurohr C, Trautnitz M, Behr J: Brain natriuretic peptide is a prognostic parameter in chronic lung disease. Am J Respir Crit Care Med 2006;173:744–750.

13 Lettieri CJ, Nathan SD, Barnett SD, Ahmad S, Shorr AF: Prevalence and outcomes of pulmonary arterial hypertension in advanced idiopathic pulmonary fibrosis. Chest 2006; 129:746–752.

14 Nathan SD, Noble PW, Tuder RM: Idiopathic pulmonary fibrosis and pulmonary hypertension: connecting the dots. Am J Respir Crit Care Med 2007;175:875–880.

15 Shorr AF, Wainright JL, Cors CS, Lettieri CJ, Nathan SD: Pulmonary hypertension in patients with pulmonary fibrosis awaiting lung transplant. Eur Respir J 2007;30:715–721.

16 Patel NM, Lederer DJ, Borczuk AC, Kawut SM: Pulmonary hypertension in idiopathic pulmonary fibrosis. Chest 2007;132:998–1006.

17 Pitsiou G, Papakosta D, Bouros D: Pulmonary hypertension in idiopathic pulmonary fibrosis: a review. Respiration 2011;82:294–304.

18 Hamada K, Nagai S, Tanaka S, Handa T, Shigematsu M, Nagao T, Mishima M, Kitaichi M, Izumi T: Significance of pulmonary arterial pressure and diffusion capacity of the lung as prognosticator in patients with idiopathic pulmonary fibrosis. Chest 2007;131: 650–656.

19 Nathan SD, Shlobin OA, Ahmad S, Urbanek S, Barnett SD: Pulmonary hypertension and pulmonary function testing in idiopathic pulmonary fibrosis. Chest 2007;131:657–663.

20 Badesch DB, Champion HC, Sanchez MA, Hoeper MM, Loyd JE, Manes A, McGoon M, Naeije R, Olschewski H, Oudiz RJ, Torbicki A: Diagnosis and assessment of pulmonary arterial hypertension. J Am Coll Cardiol 2009;54:S55–S66.

21 Chaouat A, Naeije R, Weitzenblum E: Pulmonary hypertension in COPD. Eur Respir J 2008;32:1371–1385.
22 Standardization of Spirometry, 1994 update: American Thoracic Society. Am J Respir Crit Care Med 1995;152:1107–1136.
23 Guideline of respiratory function tests-spirometry, flow-volume curve, diffusion capacity of the lung. Nihon Kokyuki Gakkai Zasshi 2004;suppl:1–56.
24 ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories: ATS statement: guidelines for the six-minute walk test. Am J Respir Crit Care Med 2002;166:111–117.
25 Mahler DA, Wells CK: Evaluation of clinical methods for rating dyspnea. Chest 1988;93: 580–586.
26 Kawut SM, O'Shea MK, Bartels MN, Wilt JS, Sonett JR, Arcasoy SM: Exercise testing determines survival in patients with diffuse parenchymal lung disease evaluated for lung transplantation. Respir Med 2005;99:1431–1439.
27 Corte TJ, Wort SJ, Gatzoulis MA, Macdonald P, Hansell DM, Wells AU: Pulmonary vascular resistance predicts early mortality in patients with diffuse fibrotic lung disease and suspected pulmonary hypertension. Thorax 2009;64:883–888.
28 Renzoni EA, Walsh DA, Salmon M, Wells AU, Sestini P, Nicholson AG, Veeraraghavan S, Bishop AE, Romanska HM, Pantelidis P, Black CM, Du Bois RM: Interstitial vascularity in fibrosing alveolitis. Am J Respir Crit Care Med 2003;167:438–443.
29 Ebina M, Shimizukawa M, Shibata N, Kimura Y, Suzuki T, Endo M, Sasano H, Kondo T, Nukiwa T: Heterogeneous increase in CD34-positive alveolar capillaries in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 2004;169:1203–1208.
30 Judge EP, Fabre A, Adamali HI, Egan JJ: Acute exacerbations and pulmonary hypertension in advanced idiopathic pulmonary fibrosis. Eur Respir J 2012;40:93–100.
31 Farkas L, Gauldie J, Voelkel NF, Kolb M: Pulmonary hypertension and idiopathic pulmonary fibrosis: a tale of angiogenesis, apoptosis, and growth factors. Am J Respir Cell Mol Biol 2011;45:1–15.
32 Farkas L, Farkas D, Ask K, Möller A, Gauldie J, Margetts P, Inman M, Kolb M: VEGF ameliorates pulmonary hypertension through inhibition of endothelial apoptosis in experimental lung fibrosis in rats. J Clin Invest 2009;119:1298–1311.
33 Seimetz M, Parajuli N, Pichl A, Veit F, Kwapiszewska G, Weisel FC, Milger K, Egemnazarov B, Turowska A, Fuchs B, Nikam S, Roth M, Sydykov A, Medebach T, Klepetko W, Jaksch P, Dumitrascu R, Garn H, Voswinckel R, Kostin S, Seeger W, Schermuly RT, Grimminger F, Ghofrani HA, Weissmann N: Inducible NOS inhibition reverses tobacco-smoke-induced emphysema and pulmonary hypertension in mice. Cell 2011;147:293–305.
34 Henno P, Boitiaux JF, Douvry B, Cazes A, Lévy M, Devillier P, Delclaux C, Israël-Biet D: Tobacco-associated pulmonary vascular dysfunction in smokers: role of the ET-1 pathway. Am J Physiol Lung Cell Mol Physiol 2011;300:L831–L839.
35 Pak O, Aldashev A, Welsh D, Peacock A: The effects of hypoxia on the cells of the pulmonary vasculature. Eur Respir J 2007;30:364–372.
36 Sumikawa H, Johkoh T, Colby TV, Ichikado K, Suga M, Taniguchi H, Kondoh Y, Ogura T, Arakawa H, Fujimoto K, Inoue A, Mihara N, Honda O, Tomiyama N, Nakamura H, Muller NL: Computed tomography findings in pathological usual interstitial pneumonia: relationship to survival. Am J Respir Crit Care Med 2008;177:433–439.
37 Cottin V, Le Pavec J, Prevot G, Mal H, Humbert M, Simonneau G, Cordier JF: Pulmonary hypertension in patients with combined pulmonary fibrosis and emphysema syndrome. Eur Respir J 2010;35:105–111.
38 Mejia M, Carrillo G, Rojas-Serrano J, Estrada A, Suarez T, Alonso D, Barrientos E, Gaxiola M, Navarro C, Selman M: Idiopathic pulmonary fibrosis and emphysema: decreased survival associated with severe pulmonary arterial hypertension. Chest 2009; 136:10–15.