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Cigarette Smoking Decreases Dynamic Inspiratory Capacity during Maximal Exercise in Patients with Type 2 Diabetes

Yoshihiro KITAHARA[1,*], Noboru HATTORI[1], Akihito YOKOYAMA[2], Kiminori YAMANE[1], Kiyokazu SEKIKAWA[3], Tsutomu INAMIZU[3] and Nobuoki KOHNO[1]

1) Department of Molecular and Internal Medicine, Graduate School of Biochemical Sciences, Hiroshima University, Hiroshima, Japan
2) Department of Hematology and Respiratory Medicine, Kochi University, Nankoku, Japan
3) Department of Sports Medicine, Graduate School of Health Sciences, Hiroshima University, Hiroshima, Japan

ABSTRACT

To investigate the influence of cigarette smoking on exercise capacity, respiratory responses and dynamic changes in lung volume during exercise in patients with type 2 diabetes.

Forty-one men with type 2 diabetes without cardiopulmonary disease were recruited and divided into 28 non-current smokers and 13 current smokers. All subjects received lung function tests and cardiopulmonary exercise testing using tracings of the flow-volume loop. Exercise capacity was compared using the percentage of predicted oxygen uptake at maximal workload (%$\dot{V}O_2$max). Respiratory variables and inspiratory capacity (IC) were compared between the two groups at rest and at 20%, 40%, 60%, 80% and 100% of maximum workload.

Although there was no significant difference in lung function tests between the two groups, venous carboxyhemoglobin (CO-Hb) levels were significantly higher in current smokers. %$\dot{V}O_2$max was inversely correlated with CO-Hb levels. Changing patterns in respiratory rate, respiratory equivalent and IC were significantly different between the two groups. Current smokers had rapid breathing, a greater respiratory equivalent and a limited increase in IC during exercise.

Cigarette smoking diminishes the increase in dynamic IC in patients with type 2 diabetes. As this effect of smoking on dynamic changes in lung volume will exacerbate dynamic hyperinflation in cases complicated by chronic obstructive pulmonary disease, physicians should consider smoking habits and lung function when evaluating exercise capacity in patients with type 2 diabetes.

Key words: *Cardiopulmonary exercise testing, Cigarette smoking, Inspiratory capacity, Type 2 diabetes*

Previous epidemiological studies have demonstrated that there is a dose-response relationship between the number of cigarettes smoked per day and the incidence of type 2 diabetes, and that cessation of cigarette smoking diminishes the risk of developing this disease[27,31]. Large population-based studies have also shown that glycosylated hemoglobin (HbA_{1C}) levels were lowest in persons who had never smoked, intermediate in former smokers and highest in current smokers. These results suggest a close association between cigarette smoking and type 2 diabetes and emphasize the importance of smoking cessation for the prevention and management of type 2 diabetes[27,31]. However, in a practical clinical setting, physicians treat many diabetic patients who are unable to stop smoking.

Although aerobic exercise is an important strategy for controlling hyperglycemia in patients with type 2 diabetes[14,28,29], little is known about

*Address for correspondence: Yoshihiro Kitahara, MD, Ph.D.
Department of Molecular and Internal Medicine, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan
Phone: (81) 82-257-5196, Fax: (81) 82-255-7360, E-mail: mayuchina@hotmail.co.jp

the influence of cigarette smoking on exercise capacity in these patients. In healthy subjects, cigarette smoking is known to impair exercise capacity, mainly due to an elevation in blood carboxyhemoglobin (CO-Hb) levels which reduce the oxygen carrying capacity of blood and lead to relative tissue hypoxia[7,8,12,17,19,25,26]. On the other hand, in the previous study which focused on HbA_{1C} and exercise capacity in diabetic patients, HbA_{1C} but not cigarette smoking was shown to affect exercise capacity, with HbA_{1C} levels being significantly higher in current smokers[5]. We therefore consider it is necessary to investigate precisely the influence of cigarette smoking on exercise capacity in diabetic patients.

Recent studies have demonstrated that dynamic changes in lung volume affect exercise capacity. In particular, dynamic hyperinflation in patients with chronic obstructive pulmonary disease (COPD) is considered to be a major reason for impaired exercise capacity and dyspnea on exertion[6,21,22]. Cigarette smoking increases pulmonary airway resistance leading to an increase in the oxygen cost of breathing during exercise. In addition, cigarette smoking causes mucosal swelling and bronchoconstriction, resulting in an increase in the diffusion distance of oxygen across alveolar walls and a decrease in arterial oxygen content[17,20]. Cigarette smoking therefore may have a considerable influence on respiratory responses and dynamic changes in lung volume during exercise.

On the basis of these observations, we hypothesized that cigarette smoking may affect exercise capacity in diabetic patients by influencing dynamic changes in lung volume during exercise. This study was conducted to clarify these points.

PATIENTS AND METHODS

Subject recruitment

Male patients with type 2 diabetes without diabetic complications were recruited between January and December 2004. In all subjects, HbA_{1C} was < 10% and no subject had a history of cardiopulmonary disease, such as bronchial asthma or chronic heart failure. Smoking habits were assessed by venous CO-Hb levels and responses to a questionnaire. Subjects with CO-Hb levels ≥ 3% who had continued cigarette smoking at study entry were classified as current smokers, while subjects with CO-Hb levels < 3% who had never smoked cigarettes or had stopped cigarette smoking for longer than 3 months prior to study entry were classified as non-current smokers. Subjects who had only stopped smoking within the 3-month period prior to the study were excluded from enrollment. The subjects were asked about their physical activity habits and were classified as regular exercisers if they undertook aerobic exercise continuously for longer than 30 min more than once a week.

The lung function tests were conducted in triplicate using a portable spirometer (SUPER SPIRO DISCOM-21 FXII®; Chest Co., Tokyo, Japan) according to the guidelines of the American Thoracic Society[2]. The formulas developed by Baldwin[3] and Berglund[4] were used to calculate vital capacity (VC), forced vital capacity (FVC) and forced expiratory volume in one second (FEV_1). Subjects with a ratio of FEV_1 to FVC (FEV_1/FVC) < 70% or a percentage of predicted VC or FVC (%VC or %FVC) < 80% were excluded from the study. All subjects were given informed consent information and the study protocol was approved by the ethics committee of Hiroshima University.

Cardiopulmonary exercise testing (CPET) and inspiratory capacity (IC) during exercise

All eligible subjects were instructed to consume a light meal and take medications at least 3 hr before CPET. CPET was conducted using an electrically braked cycle ergometer (STB-2400®; Nihon Kohden Co., Tokyo, Japan) with an incremental ramp protocol. For safety, the target heart rate was set at 210-age (beats/min). Oxygen uptake ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) were measured breath-by-breath using a computerized expired gas analyzing system (Aeromonitor AE-300RC®; Minato Medical Science Inc., Osaka, Japan). During a 3-min rest period, the subjects were instructed to perform an IC maneuver that consisted of deep breathing from resting expiratory level to maximal inspiratory level (Fig. 1). After a 1-min warm-up period at 10 watts per minute (W/min), the workload was increased at a slope of 20 W/min. The subjects were instructed to maintain 50 to 60 revolutions per minute (rpm). During CPET, the flow-volume loop was continuously traced breath by breath, while tidal volume (VT), respiratory rate (RR), minute ventilation ($\dot{V}E$), respiratory equivalent ($\dot{V}E/\dot{V}CO_2$), blood pressure, heart rate, percutaneous arterial oxygen saturation (SpO_2) and 12-lead electrocardiogram were recorded continuously. CPET was terminated when one of the following criteria was satisfied: 1) unable to maintain 50 rpm for any reason, 2) reached target heart rate, 3) desaturation defined as SpO_2 < 90% and 4) appearance of ischemic changes or severe arrhythmia on the electrocardiogram. Degrees of chest discomfort and leg fatigue were evaluated using the modified Borg scale[11]. Workload at the time of CPET termination was defined as maximal workload (Wmax), while $\dot{V}O_2$ at Wmax was defined as $\dot{V}O_2$max. We used the percentage of predicted $\dot{V}O_2$max (%$\dot{V}O_2$max) as an index of individualized exercise capacity, calculated as the ratio of $\dot{V}O_2$max to predicted $\dot{V}O_2$max.

Study subjects were not familiar with the IC

maneuver during incremental exercise: performing maximal breathing from the end-expiratory level. Since it is assumed that total lung capacity remains constant during exercise, we defined IC during CPET as the difference between end-expiratory level and resting maximal inspiratory level (Fig. 1)[6,21,22]. IC was calculated at 20%, 40%, 60%, 80% and 100% of Wmax and the average values of three breaths were defined as IC at each percentage of Wmax.

Statistical analysis

The data were analyzed using the SPSS for Windows statistical program (version 11.0; SPSS; Chicago, IL, USA). The data are presented as mean ± SEM. The characteristics of the two groups were compared using the Mann-Whitney U test. Repeated measure one-way analysis of variance was used to compare the responses of respiratory variables in the two groups. When there was a statistically significant difference in the responses of these respiratory variables between the groups, the Mann-Whitney U test was used to compare the difference in values at each time point. Relationships between variables were examined using Spearman's rank correlation test. A p-value of < 0.05 was considered statistically significant.

RESULTS

Patient characteristics

Table 1 shows the baseline characteristics of the two groups. Current smokers tended to be younger than non-current smokers, although this difference was not statistically significant. There was no statistically significant difference in body mass index, duration of diabetes, exercise habits, HbA_{1C} or plasma brain natriuretic peptide levels between the groups. The Brinkmann index: average number of cigarettes smoked per day × the number of years smoked, and venous CO-Hb levels were significantly higher in current smokers.

Table 1. Patient characteristics

	Non-current smoker (n = 28)	Current smoker (n = 13)	p value
Age (years)	55.6 ± 1.6	50.3 ± 2.2	0.066
Body mass index (kg/m²)	24.5 ± 0.7	25.5 ± 0.7	0.538
Duration of diabetes (years)	6.7 ± 0.9	7.2 ± 1.7	0.682
Regular exercise habit (yes/no)	21 / 7	9 / 4	0.772
Brinkmann index	224.8 ± 52.7	732.2 ± 97.6	<0.001
HbA_{1C} (%)	6.5 ± 0.2	6.8 ± 0.4	0.989
Brain natriuretic peptide (pg/mL)	15.4 ± 3.0	11.7 ± 3.2	0.271
Carboxyhemoglobin (%)	1.4 ± 0.1	4.9 ± 0.7	<0.001

Values are mean ± SEM.
HbA_{1C}; glycosylated hemoglobin

Lung function tests at rest

Table 2 shows the results of lung function tests at rest. The values of VC, FVC, FEV_1 and FEV_1/FVC were similar in the two groups. The values of %VC, %FVC, percentage of predicted FEV_1 (%FEV_1), expiratory flow rate at 50% of FVC ($\dot{V}_{50}$)

Table 2. Lung function test results at rest

	Non-current smoker (n = 28)	Current smoker (n = 13)	p value
VC (L)	4.1 ± 0.1	4.1 ± 0.2	0.978
%VC	116.1 ± 2.4	112.0 ± 3.9	0.385
FVC (L)	3.8 ± 0.1	3.7 ± 0.2	0.779
%FVC	107.9 ± 2.5	101.4 ± 3.5	0.085
FEV_1 (L)	3.1 ± 0.1	3.0 ± 0.2	0.758
FEV_1/FVC (%)	79.7 ± 0.9	80.2 ± 1.3	0.801
%FEV_1 (%)	106.9 ± 3.1	97.5 ± 3.1	0.098
%$\dot{V}_{50}$ (%)	83.6 ± 4.6	73.5 ± 5.8	0.327
%$\dot{V}_{25}$ (%)	54.5 ± 4.2	48.1 ± 4.9	0.385

Values are mean ± SEM.
VC; vital capacity, FVC; forced vital capacity, FEV_1; forced expiratory volume in one second, $\dot{V}_{50}$; expiratory flow rate at 50% of forced vital capacity, $\dot{V}_{25}$; expiratory flow rate at 25% of forced vital capacity

Table 3. Cardiopulmonary exercise test results at maximal workload

	Non-current smoker (n = 28)	Current smoker (n = 13)	p value
Workload (Watt)	149.5 ± 4.4	158.2 ± 6.3	0.408
$\dot{V}O_2$max (mL/kg/min)	28.8 ± 0.8	27.9 ± 0.7	0.538
%$\dot{V}O_2$max (%)	87.7 ± 2.9	80.5 ± 2.4	0.218
$\dot{V}CO_2$ (mL/min)	2229.4 ± 80.0	2322.6 ± 101.6	0.695
Heart rate (beats/min)	151.1 ± 2.9	155.3 ± 4.0	0.355
SpO_2 (%)	96.6 ± 0.4	97.2 ± 0.3	0.872
Lactate (mg/dL)	45.5 ± 3.4	45.3 ± 3.0	0.737
Borg scale			
Chest	5.0 ± 0.3	4.5 ± 0.6	0.953
Leg	5.1 ± 0.4	5.5 ± 0.5	0.518

Values are mean ± SEM.
$\dot{V}O_2$max; oxygen uptake at maximal workload, $\dot{V}CO_2$; carbon dioxide production, SpO_2; percutaneous arterial oxygen saturation

and expiratory flow rate at 25% of FVC ($\dot{V}_{25}$) were not significantly different between the groups.

CPET

Table 3 shows the results of CPET at Wmax. $\dot{V}O_2$max and %$\dot{V}O_2$max values were similar in the two groups. Workload, $\dot{V}CO_2$, heart rate, SpO_2, venous lactate levels and Borg scale at Wmax were not significantly different between the groups. Only one non-current smoker developed significant ischemic changes without chest pain.

Respiratory variables and IC during exercise

Figure 1 shows typical examples of flow-volume loop during CPET in non-current smokers and current smokers. Figure 2 shows the values of VT, RR, $\dot{V}E$, and $\dot{V}E/\dot{V}CO_2$ at each workload. VT was slightly, but not significantly higher, in the non-current smoker group during the entire exercise period (Fig. 2a). The response of RR was significantly different (Fig. 2b), with values at ≥ 40% of Wmax being significantly higher in the current smoker group. The magnitude of changes in RR from rest to Wmax (ΔRR) was significantly different between the groups (ΔRR; 16.6 ± 1.4 in non-current smokers and 24.3 ± 2.8 breaths/min in current smokers, p = 0.017). The response of $\dot{V}E$ was similar in the two groups (Fig. 2c), whereas that of $\dot{V}E/\dot{V}CO_2$ was significantly different, with the current smoker group having significantly higher $\dot{V}E/\dot{V}CO_2$ at Wmax (Fig. 2d).

Figure 3 shows the values of IC at rest and at each workload. IC values at rest were similar in the two groups: [2.5 ± 0.1 (L) in non-current smokers and 2.4 ± 0.1 (L) in current smokers, p = 0.801]. The response of IC was significantly different (Figs. 1 and 3), with IC at Wmax being significantly lower in the current smoker group. The magnitude of changes in IC from rest to Wmax (ΔIC) was significantly different between the groups (0.5 ± 0.1 L in non-current smokers and 0.2 ± 0.1 L in current smokers, p = 0.009).

Table 4. Relations between venous CO-Hb levels and respiratory variables at rest or maximal workload.

	At rest		At maximal workload		Δ each variable	
	r	p	r	p	r	p
VT	-0.135	0.399	-0.180	0.261	-0.112	0.484
RR	0.010	0.951	0.394	0.011†	0.383	0.013†
$\dot{V}E$	0.007	0.964	0.257	0.105	0.292	0.064
$\dot{V}E/\dot{V}CO_2$	0.196	0.219	0.526	<0.001†	0.082	0.608
IC	-0.076	0.636	-0.297	0.059	-0.221	0.165

†; $p < 0.05$
CO-Hb; carboxyhemoglobin, VT; tidal volume, RR; respiratory rate, $\dot{V}E$; minute ventilation, $\dot{V}CO_2$; carbon dioxide production, IC; inspiratory capacity, %$\dot{V}O_2$max; percent predicted of oxygen uptake at maximal workload

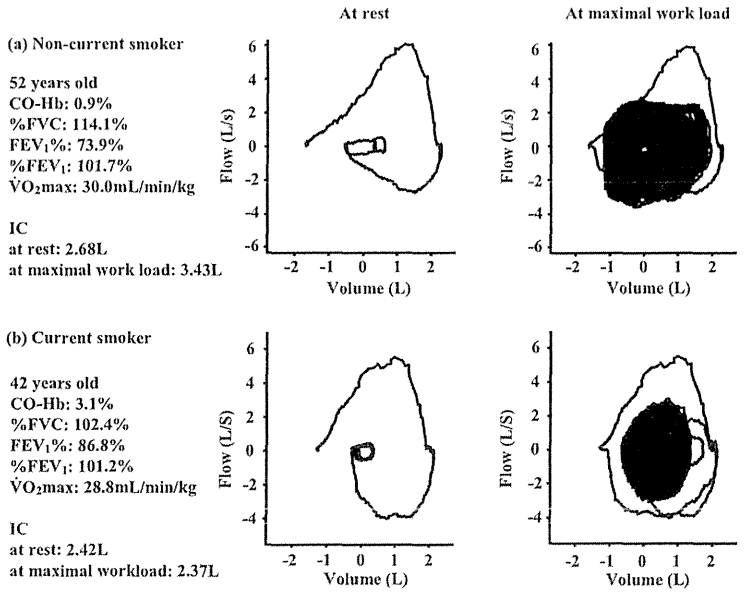

Fig. 1. Inspiratory capacity (IC) at rest and at maximal workload (Wmax) in non-current smokers (panel a) and current smokers (panel b).
A representative example of each group is shown. IC during cardiopulmonary exercise testing was defined as the difference between end-expiratory level and resting maximal inspiratory level. Current smokers had a limited increase in IC during exercise.

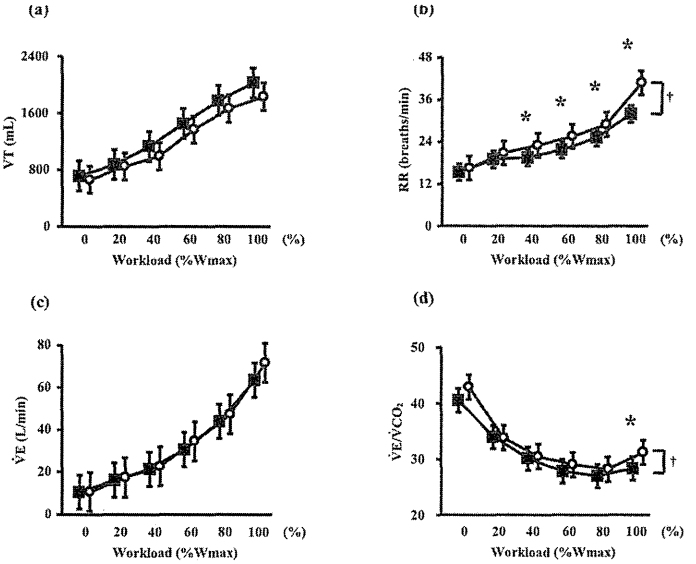

Fig. 2. The values of respiratory variables at different work loads during cardiopulmonary exercise testing (CPET) in non-current smokers (■) and current smokers (○).
Current smokers had significantly more rapid breathing (panel b; $p = 0.005$) and a significantly greater respiratory equivalent, suggesting a decrease in respiratory efficiency (panel d; $p = 0.042$).
VT, tidal volume; RR, respiratory rate; $\dot{V}E/\dot{V}CO_2$, minute ventilation/carbon dioxide production.
†; $p < 0.05$ in repeated measure one-way analysis of variance (ANOVA).
*; $p < 0.05$ in Mann-Whitney U test.

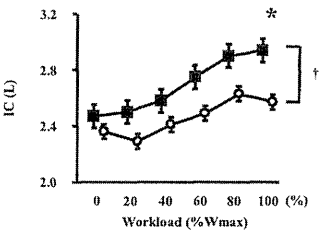

Fig. 3. The values of inspiratory capacity (IC) during cardiopulmonary exercise testing (CPET) at different work loads in non-current smokers (■) and current smokers (○). Current smokers had a limited increase in IC during exercise ($p = 0.003$).
†; $p < 0.05$ in repeated measure one-way analysis of variance (ANOVA).
*; $p < 0.05$ in Mann-Whitney U test.

At Wmax, there was positive correlation between $\dot{V}E/\dot{V}CO_2$ and RR, and an inverse correlation between RR and IC. IC tended to decrease with an increase in $\dot{V}E/\dot{V}CO_2$ (Figs. 4a-c).

Relationship between respiratory variables and exercise capacity and venous CO-Hb levels

We examined the correlation between venous CO-Hb levels and respiratory variables at rest and Wmax and also the magnitude of changes in these variables (Δ for each variable) (Table 4). No respiratory variable at rest correlated with CO-Hb levels. At Wmax, $\dot{V}E/\dot{V}CO_2$ and RR correlated positively with CO-Hb levels, with IC tending to decrease with increasing CO-Hb levels. There was a positive correlation between ΔRR and CO-Hb levels. $\Delta\dot{V}E$ tended to increase and ΔIC tended to

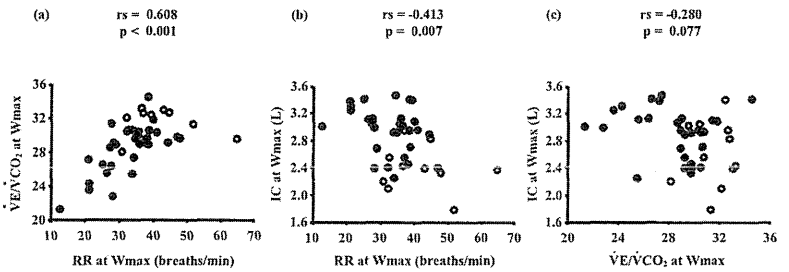

Fig. 4. The relationship between respiratory variables at maximal workload (Wmax). Closed circles (●) and open circles (○) represent non-current smokers and current smokers, respectively. There was a positive correlation between minute ventilation/carbon dioxide production ($\dot{V}E/\dot{V}CO_2$) and respiratory rate (RR) (panel a; $rs = 0.608$, $p < 0.001$), and an inverse correlation between RR and inspiratory capacity (IC) (panel b; $rs = -0.413$, $p = 0.007$). IC tended to decrease with an increase in $\dot{V}E/\dot{V}CO_2$ (panel c; $rs = -0.280$, $p = 0.077$).

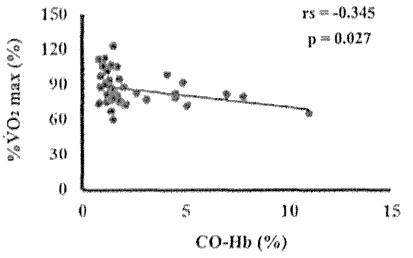

Fig. 5. The relationship between venous carboxyhemoglobin (CO-Hb) levels and the percentage of predicted oxygen uptake at maximal workload (%$\dot{V}O_2$max).
There was an inverse correlation between the two variables (rs = -0.345, p = 0.027)

decrease with increasing CO-Hb levels, although these changes were not statistically significant. Venous CO-Hb levels correlated inversely with %$\dot{V}O_2$max (rs = -0.345, p = 0.027) (Fig. 5).

DISCUSSION

As far as we are aware, this is the first report that has focused on the direct influence of cigarette smoking on both exercise capacity and dynamic changes in lung volume in patients with type 2 diabetes.

The mean venous CO-Hb levels in current smokers in our study was 4.9%, which is almost the same level reported by previous studies[10,17]. In addition,%$\dot{V}O_2$max was found to be correlated inversely with CO-Hb levels in diabetic patients, which is consistent with reports in healthy subjects[1,7-9,12,17,19,26]. The ATS guidelines define a $\dot{V}O_2$max > 84% of predicted values as normal[1]. Based on this criterion, the mean %$\dot{V}O_2$max in current smokers was lower than the normal range, whereas in non-current smokers it was within the normal range. These results suggest the importance of paying attention to smoking habits when interpreting CPET results in diabetic patients[1,7-9,12,17,19,26].

We found some differences between our findings and the results of a previous study which concluded that HbA_{1C} levels, but not cigarette smoking, affected exercise capacity in diabetic patients[5]. This discrepancy may be explained by the selection criteria of patients in our study, in which subjects with a high HbA_{1C} level were excluded.

Another new finding in the current study was that the changing patterns in RR, respiratory equivalent and IC during incremental exercise were significantly different between non-current smokers and current smokers with type 2 diabetes. Interestingly, current smokers had rapid breathing, decreased respiratory efficiency and a limited increase in IC, while lung function was similar between the two groups. Venous CO-Hb levels were associated closely with RR, $\dot{V}E/\dot{V}CO_2$ and IC at Wmax, suggesting that cigarette smoking has a marked influence on respiratory responses and dynamic changes in lung volume during exercise. Moreover, based on our finding of significant correlations between these respiratory variables at Wmax, we speculate that current smokers have physiological increases in RR during exercise in order to compensate for reduced respiratory efficiency[13,19,20]. This rapid breathing must lead to a reduction in expiratory time and a limited increase in IC during exercise.

Previous studies have shown that COPD is a risk factor for development of diabetes[24], and that diabetes is an established common co-morbidity in COPD patients[16]. Conversely, the Framingham Heart Study showed that a diagnosis of diabetes or of higher levels of fasting glucose was associated with lower levels of lung function[30]. Considering these close associations between diabetes and COPD, diabetic patients with the complication of COPD are not a rare population. Since dynamic hyperinflation and a decrease in IC are known to occur during exercise even in patients with stage I COPD, ($FEV_1/FVC < 70\%$ and $\%FEV_1 \geq 80\%$)[23], cigarette smoking must play an additional role in the dynamic hyperinflation and deterioration of exercise capacity in diabetic patients with the complication of COPD.

We recognize a number of limitations in our study. Firstly, the number of patients in the study group, especially the current smoking group, was too small to show a statistically significant difference in %$\dot{V}O_2$max between the two groups. Secondly, the study population was limited to male patients with type 2 diabetes and therefore it remains to be established whether the results are applicable to non-diabetic and female subjects. Thirdly, we could not evaluate the diffusing capacity of the lung for carbon monoxide (DLco), which is well known to be reduced in diabetic patients[15]. A reduction in DLco would lead to a ventilation-perfusion mismatch and an increase in RR during exercise[18]. Finally, as this study was a cross sectional investigation, the effect of smoking cessation on exercise capacity and respiratory responses could not be analyzed. Further studies are therefore required to clarify these points.

In conclusion, we found that cigarette smoking not only impairs exercise capacity but also diminishes increases in IC during incremental exercise in patients with type 2 diabetes. Since this effect of smoking on dynamic change in lung volume must exacerbate dynamic hyperinflation and lead to further impairment of exercise in cases complicated by COPD, physicians should consider smoking habits and lung function when evaluating exercise capacity in patients with type 2 diabetes.

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Conflict of interest statement:
Yoshihiro Kitahara, Noboru Hattori, Akihito Yokoyama, Kiminori Yamane, Kiyokazu Sekikawa, Tsutomu Inamizu, and Nobuoki Kohno have no conflict of interest to disclose.

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Different *MUC1* gene polymorphisms in German and Japanese ethnicities affect serum KL-6 levels

Yasushi Horimasu [a], Noboru Hattori [a,*], Nobuhisa Ishikawa [a], Shigeo Kawase [a], Sonosuke Tanaka [a], Koji Yoshioka [a], Akihito Yokoyama [b], Nobuoki Kohno [a], Francesco Bonella [c], Josune Guzman [d], Shinichiro Ohshimo [a], Ulrich Costabel [c]

[a] *Department of Molecular and Internal Medicine, Graduate School of Biomedical Science, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan*
[b] *Department of Hematology and Respiratory Medicine, Kochi Medical School, Kochi University, Kohasu Oko-cho, Nankoku, Kochi 783-8505, Japan*
[c] *Department of Pneumology/Allergy, Ruhrlandklinik, University Hospital Essen, Tueschener Weg 40, 45239 Essen, Germany*
[d] *General and Experimental Pathology, Faculty of Medicine, Ruhr-University Bochum, Universitätsstraße 150, 44801 Bochum, Germany*

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KEYWORDS
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Interstitial pneumonia;
Mucin-1;
Single nucleotide polymorphism

Summary
Background: KL-6 is a high-molecular-weight glycoprotein classified as human Mucin-1 (MUC1). KL-6 has been reported to be a sensitive biomarker for interstitial lung diseases (ILDs) in the Japanese population. It is also known that polymorphisms in the MUC1 gene affect serum levels of KL-6. This study was conducted to evaluate serum levels of KL-6 and MUC1 polymorphisms in both German and Japanese populations.
Methods: Serum levels of KL-6 were measured in 267 patients with ILDs (152 German and 115 Japanese) and 186 healthy subjects (HS) (76 German and 110 Japanese). In addition, rs4072037 single nucleotide polymorphisms (SNPs) were genotyped by polymerase chain reaction. The optimal cutoff values for discriminating patients with ILDs from HS was determined by receiver operating characteristic analysis based on ethnicity and rs4072037 genotypes.
Results: The serum KL-6 levels in patients with ILDs were significantly higher compared with HS in both the German and the Japanese cohorts (both $p < 0.001$). The discriminating cutoff value of serum KL-6 in the German cohort was significantly higher than the value in the Japanese cohort. The difference in the serum levels of KL-6 was significantly associated with the rs4072037 genotype distribution.
Conclusions: Even in the German cohort, the serum KL6 levels were significantly higher in patients with ILDs than HS. Because of differences in the genotype distribution of

* Corresponding author. Tel.: +81 82 257 5196; fax: +81 82 255 7360.
E-mail address: nhattori@hiroshima-u.ac.jp (N. Hattori).

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rs4072037, the KL-6 cutoff value for the German cohort that discriminated patients with ILDs from HS was significantly higher than the value in the Japanese cohort.

Background

The idiopathic interstitial pneumonias (IIPs) are a group of diffuse parenchymal lung diseases characterized by interstitial involvement resulting from various patterns of inflammation and fibrosis of unknown cause. The prevalence of IIPs has been generally reported to be 5–20 per 100,000 persons.[1–4] Since therapy with corticosteroids and/or immunosuppressants is largely ineffective for advanced stages of interstitial lung diseases (ILDs), early diagnosis is of utmost importance.[5,6] High-resolution computed tomography (HRCT) and/or surgical lung biopsy (SLB) are at present the fundamental modalities for definitive diagnosis of IIPs.[7,8] Compared to these diagnostic methods, an optimal biomarker for discriminating patients with IIPs from healthy subjects should be less invasive, more rapid and reproducible, and easier to obtain from patients.[9]

We previously developed a murine IgG1 monoclonal antibody that recognized a sialylated sugar chain, designated Krebs von den lungen-6 (KL-6). KL-6 is a high-molecular-weight glycoprotein and has been classified as a human Mucin-1 (MUC1).[10–13] We have demonstrated that KL-6 is a useful biomarker for discriminating ILDs from other benign lung diseases, evaluating disease activity, predicting disease outcome, and monitoring the clinical course.[10,13,14] Because of these findings, KL-6 was approved in 1999 by the Japanese Health Insurance Program as a diagnostic biomarker for ILDs, and has been measured in over 1,800,000 samples per year in Japan. In most countries, however, KL-6 is currently unavailable for clinical practice, and the use of KL-6 as a serum biomarker for ILDs among non-Japanese populations remains limited. We recently found indications that circulating levels of KL-6 were higher in European cohorts than levels reported earlier from the Japanese population.[15,16] Furthermore, it has been reported that the serum levels of KL-6 in patients with sarcoidosis vary according to the status of rs4072037, a single nucleotide polymorphism (SNP) on exon 2 of the *MUC1* gene.[17] KL-6 levels were highest for the G/G genotype, lowest for the A/A genotype, and at intermediate levels for the A/G genotype. In addition, available data from the HapMap database indicate that the genotypes of this polymorphism are found at different frequencies in Caucasian and Japanese ethnic groups.[18]

Based on these observations, we hypothesized that there might be differences in the circulating KL-6 levels of patients with ILDs and healthy subjects between European (e.g., German) and Japanese populations. To test this hypothesis, we evaluated the serum KL-6 levels and determined the rs4072037 genotypes in *MUC1* in both German and Japanese cohorts that included patients with ILDs and healthy subjects. The cutoff values for serum KL-6 levels that discriminated patients with ILDs from healthy subjects were determined and compared between the German and Japanese cohorts, and associations between serum KL-6 levels and rs4072037 genotypes in *MUC1* were also analyzed.

Methods

Study subjects

Between February 2007 and December 2011, 152 consecutive patients with ILDs at Ruhrlandklinik, University Hospital (Essen, Germany) and 115 patients with ILDs at Hiroshima University Hospital (Hiroshima, Japan) were enrolled in this study. Seventy-six German and 110 Japanese healthy volunteers were also included as healthy control subjects. Each patient with ILD underwent a physical examination, pulmonary function tests, chest computed tomography (CT), and/or SLB. Diagnoses of IIPs were made based on the criteria of the American Thoracic Society (ATS)/European Respiratory Society (ERS) published in 2002, which included characteristic clinical and/or CT findings and/or histological findings.[7] Each healthy volunteer underwent pulmonary function tests and chest X-ray studies, and those with apparent lung disease, such as ILDs or chronic obstructive pulmonary disease (COPD), were excluded. This study was approved by the Ethics Committees of Ruhrlandklinik (IRB 06-3170) and Hiroshima University Hospital (IRB 326) and conducted in accordance with the ethical standards established in the Helsinki Declaration of 1975. All patients and healthy volunteers gave informed consents in writing and permission to use their samples.

Lung function values

Physiologic assessment included measurements of thoracic gas volume, total lung capacity, forced vital capacity (FVC), forced expiratory volume in 1 second (FEV_1), and single-breath diffusing capacity of the lung for carbon monoxide (DL_{CO}), as previously described.[16] The protocol for lung function measurements conformed to ATS recommendations.[19]

Measurement of serum KL-6 levels

Serum samples were obtained from 267 patients with ILDs and 186 healthy subjects and stored at −80 °C until analyzed. Serum KL-6 levels were measured by a sandwich-type electrochemiluminescence immunoassay (ECLIA) using a Picolumi 8220 Analyzer (EIDIA Co. Ltd. Tokyo, Japan), as previously described.[20,21]

DNA preparation and genotype analyses of *MUC1* rs4072037

We extracted DNA from peripheral whole venous blood samples using the phenol-chloroform extraction and ethanol precipitation methods, as previously described.[22] The rs4072037 genotype was determined using a real-time polymerase chain reaction (RT-PCR) method. We used a commercially available SNP genotyping assay (TaqMan SNP Genotyping Assay C 27532642-10; Life Technologies

Corp., Carlsbad, California, USA) and the Applied Biosystems 7500 Fast RT-PCR System (Life Technologies Corp.).

Statistical analysis

Individual variables for two groups were analyzed by the Mann–Whitney *U*-test or chi-square test. The significance levels for multiple pairwise comparisons were set according to Bonferroni's correction. Linear regression analysis was conducted to study the independent effect of age, gender, smoking status, ethnicity, diagnostic category, lung function values, rs4072037 genotype, and presence or absence of ILD on serum KL-6 levels. The usefulness of serum KL-6 as a diagnostic biomarker for ILDs was assessed by receiver operating characteristic (ROC) analysis, and the optimal cutoff values for discriminating the patients with ILDs from healthy subjects were determined. All statistical analyses were performed using SPSS for Windows, version 18.0 (SPSS Inc. Chicago, USA).

Results

Serum KL-6 levels were significantly higher in healthy German subjects than in healthy Japanese subjects

The clinical characteristics of 267 patients with ILDs (152 Germans and 115 Japanese) and 186 healthy subjects (76 Germans and 110 Japanese) are shown in Table 1. Serum KL-6 levels in patients with ILDs and in healthy subjects are shown in Fig. 1a. The mean serum KL-6 levels in healthy German subjects (German HS), German patients with ILDs (German ILDs), healthy Japanese subjects (Japanese HS), and Japanese patients with ILDs (Japanese ILDs) were 331.5 ± 13.6 U/ml (mean ± SEM), 1831.0 ± 110.4 U/ml, 233.7 ± 8.1 U/ml, and 1519.0 ± 97.9 U/ml, respectively. In both German and Japanese cohorts, the serum KL-6 levels were significantly higher in patients with ILDs than in healthy subjects ($p < 0.001$ and $p < 0.001$, respectively). Furthermore, serum KL-6 levels in German HS were found to be significantly higher than those in Japanese HS ($p < 0.001$). The serum KL-6 levels in German ILDs tended to be higher than the levels in Japanese ILDs, although the difference was not statistically significant. We also compared serum KL-6 levels between German and Japanese patients within each of the following diagnostic categories of ILD: idiopathic pulmonary fibrosis (IPF), nonspecific interstitial pneumonia (NSIP), cryptogenic organizing pneumonia (COP), and drug-induced ILD. There were no significant differences in serum KL-6 levels according to the ethnicities of patients with each type of ILD (Fig. 1b).

The optimal cutoff value for serum KL-6 levels that discriminated patients with ILDs from healthy subjects was higher in the German cohort than in the Japanese cohort

To evaluate the ability of the serum KL-6 level for discriminating patients with ILDs from HS, ROC curves were drawn for each cohort. The area under the ROC curve (AUC) was 0.967 ($p < 0.001$) for the German cohort (Fig. 1c) and 0.987 ($p < 0.001$) for the Japanese cohort (Fig. 1d). Based on these ROC analyses, the optimal cutoff value for the serum KL-6 level showing the best sensitivity and specificity was 659 U/ml for the German cohort and 461 U/ml for the Japanese cohort (Table 2).

Table 1 The clinical characteristics of study subjects.

Patients with ILDs	German	Japanese	*p* value
Number of subjects	152	115	
Age, years	67.4 ± 0.8	67.5 ± 0.8	0.659
Gender, male/female	94/58	77/38	0.388
Smoking status, non/Ex/Cu/ unknown	68/56/15/13	44/55/14/2	0.282
VC, percent predicted	67.3 ± 1.6	72.0 ± 2.1	0.103
DL_{CO}, percent predicted	48.3 ± 1.5	47.5 ± 1.8	0.642
Diagnostic categories of ILDs			
IPF, *n*	92	61	0.320
NSIP, *n*	44	34	
COP, *n*	6	10	
Drug-induced ILD, *n*	10	10	
Healthy subjects	**German**	**Japanese**	***p* Value**
Number of subjects	76	110	
Age, years	45.1 ± 1.2	45.8 ± 0.7	0.345
Gender, male/female	33/43	62/48	0.083
Smoking, non/Ex/ Cu/ unknown	36/9/19/12	66/15/29	0.876

Data are shown as mean ± SEM. Statistical significance was tested by Mann–Whitney *U* test or Chi-square test. ILD, interstitial lung disease; Non, non-smoker; Ex, ex-smoker; Cu, current smoker; VC, vital capacity; DL_{CO}, diffusing capacity of the lung for carbon monoxide; IPF, idiopathic pulmonary fibrosis; NSIP, nonspecific interstitial pneumonia; COP, cryptogenic organizing pneumonia.

Distribution of the rs4072037 genotypes in *MUC1* was different in the German and Japanese cohorts

DNA was extracted from blood samples in 193 out of 267 patients with ILDs (113 Germans and 80 Japanese) and 145 out of 186 HS (35 Germans and 110 Japanese), and the rs4072037 genotypes of *MUC1* were determined. The distributions of the SNPs in the German and Japanese cohorts are shown in Table 3. In both the German and Japanese cohorts, the distributions of the rs4072037 genotypes were in Hardy–Weinberg equilibrium ($p = 0.679$ and $p = 0.839$, respectively). In addition, the distributions of the rs4072037 genotypes in both HS and patients with ILDs varied with ethnicity (Table 3, $p < 0.001$ and $p < 0.001$, respectively); the frequency of the G/G genotype was

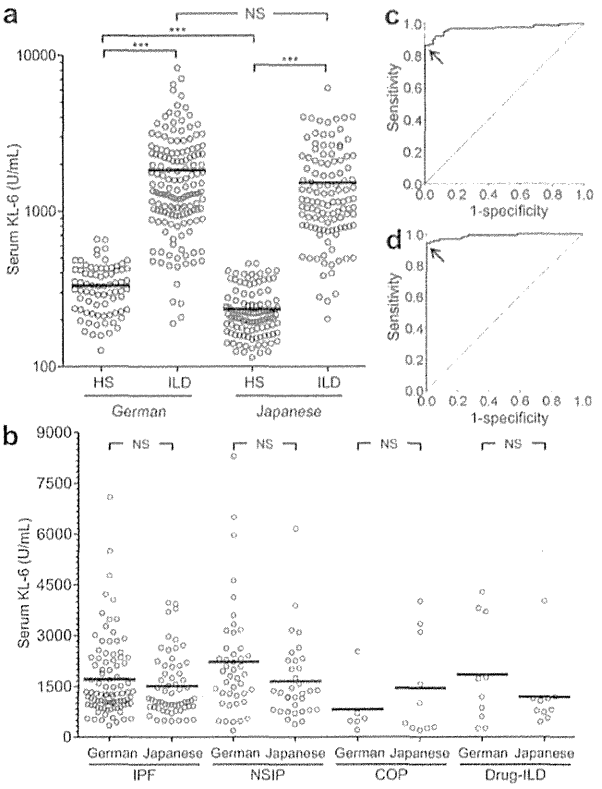

Figure 1 Serum levels of KL-6 according to ethnicity, (a) compared between healthy subjects and patients with interstitial lung diseases (ILDs), and (b) compared within each diagnostic category of ILD. Each point represents the serum KL-6 level in the studied subjects (HS, healthy subjects; ILDs, patients with interstitial lung diseases; IPF, idiopathic pulmonary fibrosis; NSIP, nonspecific interstitial pneumonia; COP, cryptogenic organizing pneumonia; drug-ILD, drug-induced ILD; NS, not significant). The horizontal bars represent the mean values. (a) The significance level was set at $\alpha = 0.0125$ (four comparisons in four groups). ***$p < 0.001$ (Mann–Whitney U test). Receiver operating characteristic (ROC) analyses were performed for patients with ILDs and healthy subjects in (c) German and (d) Japanese cohorts. Arrows at the top left corner show the points with best sensitivities and specificities.

Table 2 Optimal cutoff values of serum KL-6 according to ethnicity or rs4072037 genotype.

	Ethnicity		rs4072037 Genotype		
	German	Japanese	A/A	A/G	G/G
Cutoff value, U/ml	659	461	397	461	503
Sensitivity	0.862	0.939	0.958	0.986	1.000
Specificity	1.000	1.000	1.000	0.981	1.000
Accuracy	0. 908	0.969	0.977	0.984	1.000

The optimal cutoff values are determined as the serum levels of KL-6 which show the best sensitivity and specificity in ROC analysis. Accuracy = (true positives + true negatives)/total subjects.

significantly higher and the frequency of the A/A genotype was significantly lower in the German cohort than in the Japanese cohort. However, the distributions of the rs4072037 genotypes did not differ between HS and patients with ILDs in either the German or Japanese cohorts.

The optimal cutoff values for serum KL-6 levels that discriminated patients with ILDs from healthy subjects were different, depending on the rs4072037 genotype

To determine the effects of rs4072037 SNPs on serum KL-6 levels in healthy subjects, serum KL-6 levels were compared between the patients with rs4072037 A/A, A/G, and G/G genotypes. Because there were no significant ethnic differences between the serum KL-6 levels of patients with each genotype (data not shown), both German and Japanese subjects were included in each genotype analysis. The mean serum KL-6 levels in HS were determined to be 210.4 ± 7.6 U/ml for the A/A genotype, 286.9 ± 13.8 U/ml for the A/G genotype, and 354.5 ± 361 U/ml for the G/G genotype. As shown in Fig. 2a, the serum KL-6 levels in the HS with A/G or G/G genotypes were significantly higher than the serum KL-6 levels in the subjects with the A/A genotype ($p < 0.001$ and $p = 0.001$, respectively).

The serum KL-6 levels were then compared between the ILD patients with rs4072037 A/A, A/G, and G/G genotypes. The mean serum KL-6 levels in patients with ILDs were 1421.2 ± 99.7 U/ml for the A/A genotype, 2129.0 ± 176.9 U/ml for the A/G genotype, and 1915.6 ± 244.8 U/ml for the G/G genotype. As shown in Fig. 2a, the serum KL-6 levels were significantly higher in ILD patients with the A/G genotype than in patients with the A/A genotype ($p \leq 0.001$). We also compared the serum KL-6 levels between patients with rs4072037 A/A, A/G, and G/G genotypes within each diagnostic category of ILD (IPF, NSIP, COP, and drug-induced ILD). There were no significant differences in serum KL-6 levels according to rs4072037 genotypes of patients with each type of ILD (Fig. 2b).

To evaluate the ability of serum KL-6 levels to discriminate patients with ILDs from HS within each genotype, ROC curves were drawn for each rs4072037 genotype. The AUC values were 0.991 (95% CI, 0.000–1.000, $p < 0.001$) for the A/A genotype (Fig. 2c), 0.990 (95% CI, 0.000–1.000, $p < 0.001$) for the A/G genotype (Fig. 2d), and 1.000 (95% CI, 1.000––1.000, $p < 0.001$) for the G/G genotype (Fig. 2e). As shown in Table 2, the optimal cutoff values of serum KL-6 levels that discriminated patients with ILDs from HS were determined to be 397 U/ml for the A/A genotype, 461 U/ml for the A/G genotype, and 503 U/ml for the G/G genotype.

Serum KL-6 levels are independently correlated with the rs4072037 genotype in *MUC1*

To obtain more information on variables that might affect serum KL-6 levels, correlations between the serum level of KL-6 and clinical characteristics, including age, gender, smoking status, ethnicity, specific rs4072037 genotype, and the presence or absence of ILD, were examined using linear regression analysis. Univariate analysis confirmed that older

Table 3 Frequency of each genotype of rs4072037.

Patients with ILDs	Total	A/A	(%)	A/G	(%)	G/G	(%)
German							
Number of subjects	113	38	(33.6)	49	(43.4)	26	(23.0)
Standardized residuals		−5.3		2.4		4.3	
Japanese							
Number of subjects	80	58	(72.5)	21	(26.3)	1	(1.2)
Standardized residuals		5.3		−2.4		−4.3	
Healthy subjects	Total	A/A	(%)	A/G	(%)	G/G	(%)
German							
Number of subjects	35	9	(25.7)	17	(48.6)	9	(25.7)
Standardized residuals		−4.0		1.7		4.3	
Japanese							
Number of subjects	110	71	(64.5)	36	(32.8)	3	(2.7)
Standardized residuals		4.0		−1.7		−4.3	

$p < 0.001$, tested by chi-square test.
ILDs, interstitial lung diseases.

age (regression coefficient [*B*] = 35.413, standard error [SE] = 4.090, $p \leq 0.001$), German ethnicity ($B = 730.926$, SE = 125.844, $p \leq 0.001$), the rs4072037 G/G genotype ($B = 333.670$, SE = 93.596, $p < 0.001$), and the presence of ILD ($B = 1489.534$, SE = 104.439, $p \leq 0.001$) were significantly correlated with higher serum KL-6 levels (Table 4a); and multivariate analysis revealed that the rs4072037 G/G genotype ($B = 213.973$, SE = 81.802, $p = 0.009$) and the presence of ILD ($B = 1533.652$, SE = 159.022, $p \leq 0.001$) were independently correlated with higher serum KL-6 levels. To further evaluate the correlation between the serum KL-6 levels and clinical characteristics, we performed subgroup analyses based on subjects with or without ILDs. For the HS, univariate analysis confirmed that older age ($B = 2.648$, SE = 0.853, $p = 0.002$), German ethnicity ($B = 63.969$, SE = 18.190, $p = 0.001$) and rs4072037 G/G genotype ($B = 69.949$, SE = 11.162, $p \leq 0.001$) were significantly correlated with higher serum KL-6 levels (Table 4b); and multivariate analysis revealed that older age (B = 3.035, SE = 0.741, $p < 0.001$) and the rs4072037 G/G genotype ($B = 64.924$, SE = 11.589, $p < 0.001$) were independently correlated with higher serum KL-6 levels. For the patients with ILDs, univariate analysis demonstrated that decreased %VC ($B = 13.082$, SE = 5.681, $p = 0.006$), decreased $\%DL_{CO}$ ($B = 12.664$, SE = 5.681, $p = 0.027$), and rs4072037 G/G genotype ($B = 358.590$, SE = 124.140, $p = 0.004$) were significantly correlated with higher serum KL-6 levels (Table 4c); and multivariate analysis revealed that only the rs4072037 G/G genotype ($B = 301.665$, SE = 130.177, $p = 0.022$) was independently correlated with higher serum KL-6 levels.

Discussion

In this study, we found in both the German and Japanese cohorts that serum KL-6 levels were significantly higher in patients with ILDs than in healthy subjects. We also found that the optimal cutoff value for the serum KL-6 level that discriminated patients with ILDs from healthy subjects was higher in the German cohort than in the Japanese cohort. The distribution of the rs4072037 genotypes in *MUC1* was shown to differ between the German and Japanese cohorts. In addition, multivariate regression analyses, which included both German and Japanese subjects, revealed that a specific rs4072037 genotype in *MUC1* and the presence of an ILD independently affected serum KL-6 levels.

Our study showed that the difference in the cutoff values for serum KL-6 levels of German and Japanese cohorts that discriminated patients with ILDs from healthy subjects was at least in part due to different distributions of the rs4072037 genotypes in *MUC1*. The G/G genotype was more common in the German cohort, and the A/A genotype was more common in the Japanese cohort. The distributions of the rs4072037 genotypes in the German and Japanese cohort were shown to be similar to the respective distributions in CEU (Utah residents with ancestry from northern and western Europe) and in JPT (Japanese in Tokyo, Japan) populations, which are reported in the HapMap database.[18]

The rs4072037 SNP is located in exon 2 of *MUC1*. Previous studies reported that this SNP regulates splicing site selection and is in strong linkage disequilibrium with variable numbers of tandem repeats (TRs) in *MUC1*. The G allele correlates with a large number of TRs and the A allele correlates with a small number of TRs.[23,24] Because the sialylated sugar chains on MUC1, which are believed to be recognized by the anti-KL-6 monoclonal antibody ,[11,12] are known to be abundant in the TR domain, larger numbers of TRs may contain more KL-6 antigen, resulting in higher serum KL-6 levels.[25–27] In agreement with these previous reports, our data showed that the serum KL-6 levels in the A/G and G/G genotype cohorts were higher than those in the A/A genotype cohort.[17] Based on these results, we hypothesize that the higher levels of serum KL-6 in the German compared with the Japanese cohort may be caused

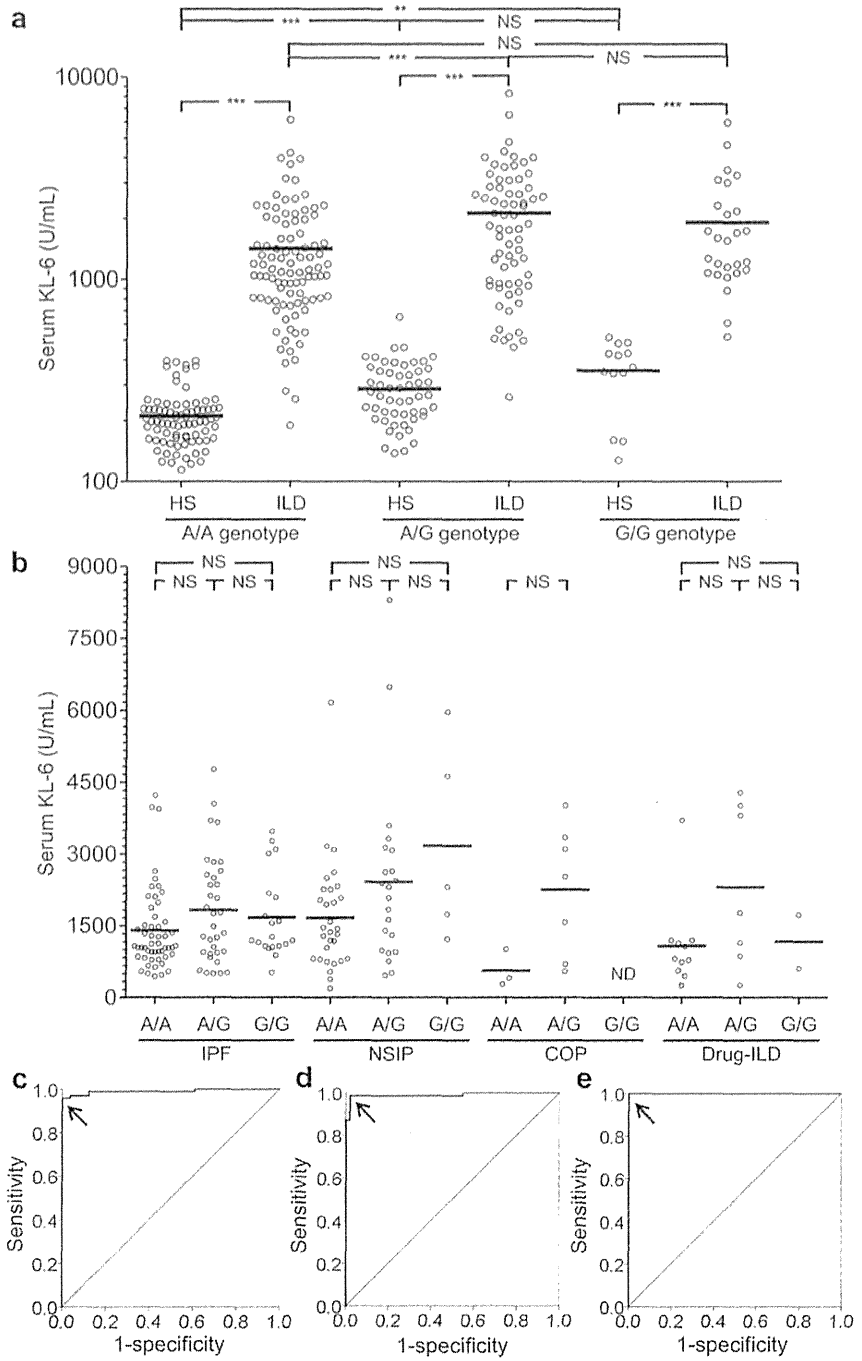

Figure 2 Serum levels of KL-6 according to rs4072037 genotype, (a) compared between healthy subjects and patients with interstitial lung diseases (ILDs), and (b) compared within each diagnostic category of ILD. Each point represents the serum KL-6 level in the studied subjects (HS, healthy subjects; ILDs, patients with interstitial lung diseases; IPF, idiopathic pulmonary fibrosis; NSIP, nonspecific interstitial pneumonia; COP, cryptogenic organizing pneumonia; drug-ILD, drug-induced ILD; NS, not significant). The horizontal bars represent the mean values. (a) The significance level was set at $\alpha = 0.0056$ (nine comparisons in six groups). $^{**}p < 0.0056$, $^{***}p < 0.001$ (Mann–Whitney U test). (b) The significance level was set at $\alpha = 0.017$ (three comparisons in three groups) for IPF, NSIP, and drug-ILD, and at $\alpha = 0.05$ (Bonferroni's correction was not adopted) for COP. Receiver operating characteristic (ROC) analyses were performed between patients with ILDs and healthy subjects in (c) A/A cohort, (d) A/G cohort, and (e) G/G cohort of rs4072037. Arrows at the top left corner show the points with best sensitivities and specificities.

Table 4 Linear regression analysis for serum KL-6.

a. Whole studied subjects	Regression coefficient	Standard error	*p* Value
Univariate analysis			
Age, years	35.413	4.090	<0.001
Gender, female vs male	135.546	133.043	0.309
Smoking, Non vs Ex vs Cu	23.001	88.610	0.795
Ethnicity, Japanese vs German	730.926	125.844	<0.001
rs4072037, A/A vs A/G vs G/G	333.670	93.596	<0.001
With or without ILDs, HS vs ILDs	1489.534	104.439	<0.001
Multivariate analysis			
Age, years	4.989	5.263	0.344
Ethnicity, Japanese vs German	117.823	120.173	0.328
rs4072037, A/A vs A/G vs G/G	213.973	81.802	0.009
With or without ILDs, HS vs ILDs	1533.652	159.022	<0.001

b. Healthy subjects	Regression coefficient	Standard error	*p* Value
Univariate analysis			
Age, years	2.648	0.853	0.002
Gender, female vs male	11.760	16.229	0.470
Smoking, Non vs Ex vs Cu	1.159	9.300	0.901
Ethnicity, Japanese vs German	63.969	18.190	0.001
rs4072037, A/A vs A/G vs G/G	69.949	11.162	<0.001
Multivariate analysis			
Age, years	3.035	0.741	<0.001
Ethnicity, Japanese vs German	28.572	17.447	0. 104
rs4072037, A/A vs A/G vs G/G	64.924	11.589	<0.001

c. Patients with ILDs	Regression coefficient	Standard error	*p* Value
Univariate analysis			
Age, years	10.616	8.997	0.239
Gender, female vs male	55.201	188.244	0.770
Smoking, Non vs Ex vs Cu	111.173	135.264	0.412
VC, percent predicted	13.082	4.722	0.006
DL_{CO}, percent predicted	12.664	5.681	0.027
Ethnicity, Japanese vs German	355.953	181.940	0.052
Diagnostic category, drug-ILD vs COP vs IPF vs NSIP	195.010	102.851	0.059
rs4072037, A/A vs A/G vs G/G	358.590	124.140	0.004
Multivariate analysis			
VC, percent predicted	2.219	5.72	0.699
DL_{CO}, percent predicted	11.682	6.366	0.069
rs4072037, A/A vs A/G or G/G	301.665	130.177	0.022

Non, non-smoker; Ex, ex-smoker; Cu, current smoker; HS, Healthy subjects; ILDs, interstitial lung diseases; VC, vital capacity; DL_{CO}, diffusing capacity of the lung for carbon monoxide; drug-ILD, drug-induced interstitial lung disease; COP, cryptogenic organizing pneumonia; IPF, idiopathic pulmonary fibrosis; NSIP, nonspecific interstitial pneumonia.

by higher frequencies of rs4072037 A/G and G/G genotypes in the German than in the Japanese cohort.

To evaluate the ability of serum KL-6 levels based on ethnicity and genotype for discriminating patients with ILDs from healthy subjects, we performed ROC analysis for each rs4072037 genotype. Importantly, the AUC value for each rs4072037 genotype (Fig. 2c–e; 0.991 for A/A, 0.990 for A/G, and 1.000 for G/G, respectively) was higher than the AUC value for each ethnicity (Fig. 1c, d; 0.967 for German and 0.987 for Japanese cohort, respectively). In addition, the sensitivity, specificity, and accuracy tended to be higher in genotype-based analysis than in ethnicity-based analysis (Table 2). These data suggest that the cutoff values of serum KL-6 levels based on rs4072037 genotypes show better abilities of discriminating patients with ILDs from healthy subjects than the cutoff values based on ethnicity. Furthermore, the rs4072037 G/G genotype was independently correlated with serum KL-6 levels, whereas ethnicity was not. Based on these results, we can conclude that the different cutoff values for serum KL-6 levels in the German and Japanese cohorts that discriminated patients with ILDs from healthy subjects were at least partially a result of the different distributions of rs4072037 genotypes in these cohorts; and, ideally, a cutoff value for the serum KL-6 levels that discriminates patients with ILDs from healthy subjects should be based on the rs4072037 genotype. However, primarily because of the expense, it may not be clinically feasible to determine the rs4072037 genotype of every patient with ILD. Therefore, we believe that cutoff values of serum KL-6 levels that discriminate patients with ILDs from healthy individuals should be determined on the basis of ethnicity.

Another interesting finding of this study was that the serum KL-6 levels in patients with ILDs were inversely correlated with the values for %DLco and %VC (Table 4c). This finding is consistent with the results of previous studies demonstrating the correlation between serum KL-6 and parameters of lung function in patients with various types

of ILDs.[28–31] Interestingly, previous studies from our laboratory demonstrated that KL-6 itself has chemotactic and antiapoptotic effects on fibroblasts, and additive effects on the proliferative and antiapoptotic activity of transforming growth factor-β toward fibroblasts.[11,32] These observations suggest that KL-6 may be involved in the fibrotic processes in the lung that lead to decreased values of %DLco and %VC.

On the other hand, there were no significant differences in the distributions of rs4072037 genotypes between healthy subjects and patients with ILDs. This finding indicates that there is no correlation between rs4072037 genotype and susceptibility to ILDs, although correlations between rs4072037 genotypes and susceptibility to dry-eye syndrome and gastric cancer have been reported.[33,34]

There are a number of limitations to this study. First, the sizes of the cohorts were relatively small, particularly with regard to the number of subjects available for analysis of rs4072037 genotypes. In addition, the patients with ILDs were older than the healthy subjects. However, multivariate analysis of all the subjects demonstrated that serum KL-6 levels did not correlate with age. Second, the relationship between radiographic manifestations of chest CT images and KL-6 levels was not analyzed. This was because we were unable to assess the extent of fibrosis using standardized criteria for both German and Japanese patients with ILDs, since different CT scanners with different slice thicknesses were used at the Ruhrlandklinik in Germany and the Hiroshima University Hospital in Japan. Third, only German and Japanese populations were studied. It remains to be seen whether the findings of the present study can be applied to other ethnic groups such as African Americans. Fourth, there were no follow-up data available for assessing prognostic values. A prospective follow-up study to investigate the association between serum KL-6 levels and prognosis is now being performed for both the German and Japanese cohorts.

Conclusions

In conclusion, we were able to demonstrate that the serum KL6 levels were significantly higher in patients with ILDs than in healthy subjects in both the German and Japanese cohorts. Because of differences in the distribution of rs4072037 genotypes in the German and Japanese cohorts, the cutoff value of KL-6 that discriminated patients with ILDs from healthy subjects was significantly higher in the German cohort than in the Japanese cohort. Although promising data were obtained in the present study, further investigations are needed to determine whether KL-6 can be used as a diagnostic biomarker in ethnic groups other than the Japanese.

Author contributions

YH drafted and finalized the manuscript, and performed some of the serum measurements, genotyping, and statistical analyses. NH, NI, AY, NK, and UC conceived the study, participated in its design and coordination, and helped to draft and finalize the manuscript. SK performed part of the statistical analysis. ST and KY performed some of the serum measurements and genotyping. FB, JG, and SO recruited the study subjects and determined their diagnoses.

Conflict of interest statement

Nobuoki Kohno holds a patent on KL-6. The remaining authors have no conflicts of interest.

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KL-6, a Human MUC1 Mucin, as a prognostic marker for diffuse alveolar hemorrhage syndrome

Yoshiko Kida[1], Shinichiro Ohshimo[1*], Kohei Ota[1], Tomoko Tamura[1], Tadatsugu Otani[1], Kazunobu Une[1], Takuma Sadamori[1], Yasumasa Iwasaki[1], Francesco Bonella[2], Noboru Hattori[3], Nobuyuki Hirohashi[1], Josune Guzman[4], Ulrich Costabel[2], Nobuoki Kohno[3] and Koichi Tanigawa[1]

Abstract

Background: Diffuse alveolar hemorrhage syndrome is a life threatening condition with diverse etiologies. Sensitive prognostic markers for diffuse alveolar hemorrhage have not been well investigated. Serum KL-6 is a biomarker for various interstitial lung disease associated with disease activity and prognosis. The purpose of the present study was to evaluate the clinical utility of serum KL-6 level as a prognostic marker for diffuse alveolar hemorrhage.

Methods: We retrospectively collected 41 consecutive patients clinically diagnosed as having diffuse alveolar hemorrhage who were admitted to the Intensive Care Unit of Hiroshima University Hospital between 2004 and 2011. Correlation between prognosis and age, sex, laboratory findings including serum KL-6, radiological findings, ventilatory modes or therapeutic regimens were evaluated.

Results: Baseline and peak serum KL-6 levels were significantly higher in non-survivors compared with survivors. An increase in KL-6 levels during the initial week was associated with a subsequent deterioration of the oxygenation index. Higher baseline KL-6 levels and higher peak KL-6 levels were strongly correlated with death. With a cut-off level of 700 U/mL for peak KL-6, the sensitivity, specificity and accuracy for non-survival were 75%, 85% and 78%, respectively. In the multivariate analysis, only the peak KL-6 level ≥700 U/ml was an independent poor prognostic factor for diffuse alveolar hemorrhage.

Conclusions: Peak serum KL-6 level ≥700 U/ml may become a clinically useful marker of poor prognosis for diffuse alveolar hemorrhage.

Keywords: Prognosis, Survival, Outcome, Biomarker, Interstitial lung disease, Diffuse alveolar damage

Background

Diffuse alveolar hemorrhage (DAH) syndrome is a life threatening condition associated with diverse etiologies including infection, excessive anticoagulation and vasculitis [1]. Repeated episodes of DAH may lead to irreversible pulmonary fibrosis [1] and progressive obstructive pulmonary dysfunction [2]. Low PaO_2/F_IO_2 (P/F) ratio, high multi-organ dysfunction score and non-autoimmune etiology have been reported to be poor prognostic factors for DAH [3]. However, reliable prognostic markers of DAH have not been well investigated.

KL-6, a complex sialo-carbohydrate glycoprotein present in the human MUC1 mucin, is expressed on the surface of type II pneumocytes in the alveolar space [4]. KL-6 is a sensitive serum marker for various interstitial lung diseases including idiopathic pulmonary fibrosis, radiation pneumonitis, drug-induced pneumonitis, collagen vascular disease-associated interstitial pneumonitis, extrinsic allergic alveolitis, pulmonary sarcoidosis, pulmonary alveolar proteinosis and cystic fibrosis [5-7]. Serial changes of serum KL-6 were useful to predict the short-term prognosis in rapidly progressive idiopathic pulmonary fibrosis [8], and baseline serum KL-6 levels were related to the long-term survival in idiopathic pulmonary fibrosis [9]. Serum KL-6 levels are therefore likely to be of use for evaluating the prognosis of DAH.

* Correspondence: ohshimos@hiroshima-u.ac.jp
[1]Department of Emergency and Critical Care Medicine, Graduate School of Biomedical Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan
Full list of author information is available at the end of the article