Pulmonary alveolar proteinosis (PAP) is a syndrome involving altered surfactant homeostasis, characterized by the accumulation of periodic-acid-Schiff-positive proteinaceous material in the alveoli, which blocks gas exchange in the lungs. PAP can lead to life-threatening respiratory failure (1-3). Whole lung lavage (WLL) can remove proteinaceous material in the alveoli, and is considered the most effective treatment for PAP.

KL-6 is a mucin-like glycoprotein present in human MUC1 mucin, and is mainly secreted by proliferating, stimulated, or damaged alveolar type II epithelial cells. Serum KL-6 level is significantly elevated in Japanese patients with PAP (6,067±894.5 U/mL) (4). Recently, Bonella et al. (5)
reported that serum KL-6 level is also elevated in Caucasian patients with PAP (2,049±1,893 U/mL), and that this value may be used as a biomarker to evaluate whether the patients needed WLL. However, the results of these two studies (4,5) revealed that there was a significant difference in the mean serum KL-6 level between Japanese and Caucasian PAP patients, despite the fact that the same method and reagents were used for the measurements.

On the basis of this background, we hypothesized that serum KL-6 levels in Chinese patients with PAP may differ from those in patients of a different ethnicity and that such differences might have important implications on the need for WLL. To examine this hypothesis, we measured serum KL-6 levels in Chinese patients with PAP, using similar methods as described in the above two studies (4,5), and compared the results with the serum levels reported in patients of other ethnicities. We also explored the clinical implications of serum KL-6 level in Chinese PAP patients.

Methods

Patients

We prospectively studied 37 Chinese patients who had fulfilled the diagnostic criteria for PAP and were examined between December 2014 and May 2016. In all the patients, the diagnosis of PAP was confirmed by histopathological findings in lung tissues obtained by transbronchial or open-lung biopsy. Exclusion criteria were as follows: (I) combined with other interstitial lung disease (ILD); (II) combined with lung cancer; (III) combined with acute respiratory distress syndrome; and (IV) combined with other malignant diseases. Permission was obtained from our institutional ethics committee (approval number 2014-49), and informed consent was obtained from all the study participants.

Treatment

PAP patients without infection or heart failure were subjected to WLL if they exhibited deterioration of symptoms (dyspnea and cough) or chest imaging findings (increase in previous findings or appearance of new infiltrates characteristic of PAP), and met one of the following criteria: (I) arterial oxygen partial pressure (PaO₂) <60 mmHg on room air for >30 min; or (II) lung diffusion capacity for carbon monoxide (DLCO) <60%. The observed group included patients who did not exhibit any new PAP symptoms or with no worsening of previous symptoms (see above), no new radiological infiltrates, or PaO₂ ≥60 mmHg on room air for >30 min and DLCO ≥60%.

Outcome of WLL

The outcomes of WLL were defined as follows: (I) effective group: patients were in remission for >6 months after WLL; (II) ineffective group: patients were in remission for <6 months after WLL. Disease progression was defined when patient met one of the following criteria comparing to last measurement: (I) PaO₂ decrease more than 10 mmHg; (II) DLCO% pred decrease more than 10%; (III) the alveolar-arterial gradient of partial pressure of oxygen [(P(A-a)O₂)] increase more than 10 mmHg. Or else we defined it remission.

Measurement of KL-6 and granulocyte–macrophage colony-stimulating factor (GM-CSF) autoantibody levels, and other laboratory assays

Serum samples were obtained at the time of first evaluation, 1 week after WLL, and at every follow-up period. Blood supernatants were frozen and stored at −20 °C until further analysis. The levels of KL-6 in the serum were measured by enzyme-linked immunosorbent assay (ELISA; Eisai Co. Ltd., Tokyo, Japan) as described previously (4,5) (KL-6 level was 276±181 U/mL, as determined in 30 Chinese healthy individuals).

GM-CSF autoantibody concentration was measured by ELISA as described previously (5), with some modifications. Microtiter plates (3590, Costar) were coated with 1 μg/mL recombinant human GM-CSF (300-30; Peprotech) at 4 °C overnight, washed with phosphate-buffered saline (PBS) containing 0.1% Tween 20, and blocked with 200 μL of 1% bovine serum albumin (BSA; room temperature, 1 h, on a horizontal orbital microplate shaker). Serum and BALF samples were diluted 1/3,000 in sample dilution buffer (PBS, 1% w/v BSA, 0.1% v/v Tween 20), and 100 μL of sample was incubated in duplicate wells (room temperature, 2 h, on a horizontal orbital microplate shaker). Plates were washed and incubated with 100 μL of ammonium acetate (10 mM, pH 5.0, room temperature, 15 min, on a horizontal orbital microplate shaker) to eliminate nonspecific binding. Bound IgGs were detected with goat anti-human IgG–horseradish peroxidase (A0293; Sigma-Aldrich) diluted 1/3,000 with sample dilution buffer (room temperature, 1 h, on a horizontal orbital microplate shaker) and imaged with 3,3′,5,5′-tetramethylbenzidine substrate solution.
(100 μL; T8665; Sigma-Aldrich). H₂SO₄ (1 N) was added to the samples, and the absorbance was read at excitation and emission wavelengths of 450 and 630 nm, respectively.

Arterial blood gas, carcinoembryonic antigen (CEA), and lactate dehydrogenase (LDH) levels were measured in blood samples, after the patients were made to breathe room air for >30 min in a supine position.

Pulmonary function variables, including vital capacity (VC), total lung capacity (TLC), and DLCO, were expressed as percentages of predicted normal values.

Database search

We searched the GenBank database (http://www.ncbi.nlm.nih.gov/gene) for MUC1, and found a single nucleotide polymorphism (SNP) position with a minimum allele frequency of >0.05. Additionally, we searched the dbSNP database (http://www.ncbi.nlm.nih.gov/snp) for the distribution of the allele and the genotype frequency of the SNP position in China, Japan, and Germany.

Statistical analysis

All statistical analyses were performed using SPSS version 13.0 software package (SPSS Inc., Chicago, IL, USA). Numerical data are presented as means ± standard deviation (SD). Categorical data are presented numerically or as a percentage of the total. Comparison between two groups was performed with the Student’s t-test or Wilcoxon’s rank test for continuous variables, paired t-tests for paired data, and Fisher’s exact test for categorical variables. Spearman’s or Pearson’s correlation coefficient was obtained for correlations. Receiver operating curves (ROC) analysis was used to predict the need for WLL. The χ² test was used to compare allele and genotype frequencies between the two groups. All tests were two-sided, and P<0.05 was considered to indicate statistical significance.

Results

Patient characteristics

Between December 2014 and May 2016, 39 Chinese PAP patients were screened, and 37 patients were enrolled in the study. Of the two patients who were excluded from the study, one had PAP combined with leukemia and the other was Vietnamese. Two were secondary PAP and others were APAP. Eighteen patients may had exposited to potential pneumotoxic substance, which were coal, chalk dust, dye, metal chip, macadam, paint and gasoline inhalation. The demographics and baseline characteristics of the patients are shown in Table 1. All 14 patients experiencing disease progression were treated with WLL. In two patients (14.3%), remission time was <6 months, and they needed a repeat WLL. Because of the seriousness of their condition, some patients could not complete the pulmonary function tests. Twenty-nine patients completed the pulmonary ventilation function and diffusion function tests, and three patients completed only the pulmonary ventilation function test.

Correlation between initial KL-6 serum levels, clinical-severity markers, and GM-CSF autoantibody concentration

Initial KL-6 serum levels were directly correlated with P(A-a)O₂ (r=0.554, P<0.001) and LDH (r=0.73, P<0.001) levels, and inversely correlated with PaO₂ (r=-0.608, P<0.001), forced vital capacity (FVC; r=-0.592, P<0.001), and DLCO (r=-0.668, P<0.001). No correlations were detected between the initial serum KL-6 level and GM-CSF autoantibody concentration (Table 2).

Changes in KL-6 serum levels over time

Fourteen patients back to hospital to reexamination, including 10 in the WLL group and 4 in the observation group. The correlations between changes in serum KL-6 levels and changes in serum LDH levels are shown in Figure 1. Patients who showed improvement during follow-up exhibited decreased serum LDH and KL-6 levels. No correlations were detected between changes in serum KL-6 levels and changes in PaO₂, P(A-a)O₂, FVC, and DLCO (data not shown). Others were telephone follow-up, they all in good condition and refused returning to hospital.

Correlation between initial serum KL-6 level and the need for WLL

The mean initial serum KL-6 level in the WLL group was significantly higher than that in the observed group (Table 1). ROC analysis was performed to test whether the initial serum KL-6 levels could predict the need for WLL. At a cut-off level of ≥6,512 U/mL, serum KL-6 levels yielded a sensitivity of 92.9% and a specificity of 100% to predict the need for WLL (AUC⁹⁸²=0.988; 95% CI, 0.96–1.016; P<0.001; Figure 2). The results of the ROC analysis for
Table 1 Demographics and baseline characteristics of all patients and subgroups according to treatment (all data were collected at baseline)

<table>
<thead>
<tr>
<th>Variables</th>
<th>All patients</th>
<th>Treatment</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total number (%)</td>
<td>Observed group</td>
<td>WLL group</td>
</tr>
<tr>
<td></td>
<td>37 (100.00)</td>
<td>23 (62.16)</td>
<td>14 (37.84)</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>19/18</td>
<td>14/9</td>
<td>5/9</td>
</tr>
<tr>
<td>Age (years)</td>
<td>46±12</td>
<td>45±11</td>
<td>48±15</td>
</tr>
<tr>
<td>Smoking habits</td>
<td>Never</td>
<td>27</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Ex</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Current</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.11±3.79</td>
<td>23.46±3.51</td>
<td>22.53±4.29</td>
</tr>
<tr>
<td>PaO₂ (mmHg)</td>
<td>73.94±19.07</td>
<td>84.96±14.06</td>
<td>55.84±10.22</td>
</tr>
<tr>
<td>P(A-a)O₂ (mmHg)</td>
<td>39.66±30.58</td>
<td>25.17±11.76</td>
<td>63.45±37.07</td>
</tr>
<tr>
<td>FVC (%pred)</td>
<td>78.55±16.99</td>
<td>85.08±13.69</td>
<td>61.87±12.95</td>
</tr>
<tr>
<td>TLC (%pred)</td>
<td>73.96±12.97</td>
<td>79.36±8.99</td>
<td>56.99±7.66</td>
</tr>
<tr>
<td>DLCO (%pred)</td>
<td>63.99±23.35</td>
<td>73.91±16.69</td>
<td>32.81±8.64</td>
</tr>
<tr>
<td>KL-6 (U/mL)</td>
<td>8.248±9.544</td>
<td>2.178±1.785</td>
<td>18.219±8.639</td>
</tr>
<tr>
<td>LDH (IU/L)</td>
<td>258.4±111.9</td>
<td>189.9±30.1</td>
<td>363.8±110.1</td>
</tr>
<tr>
<td>CEA (ng/mL)</td>
<td>11.34±11.76</td>
<td>5.06±4.07</td>
<td>21.66±13.06</td>
</tr>
<tr>
<td>GM-CSF autoantibody (OD value)</td>
<td>0.97±1.05</td>
<td>1.11±1.24</td>
<td>0.75±0.63</td>
</tr>
</tbody>
</table>

*Fisher’s exact test was used for comparisons. BMI, body mass index; CEA, carcinoembryonic antigen; DLCO, lung diffusion capacity for carbon monoxide; GM-CSF, granulocyte-macrophage colony-stimulating factor; F, female; FVC, forced vital capacity; KL-6, Kerbs von Lungren 6 antigen; LDH, lactate dehydrogenase; M, male; OD, optical density; PaO₂, arterial oxygen partial pressure; P(A-a)O₂, alveolar-arterial gradient of partial pressure of oxygen; TLC, total lung capacity; WLL, whole lung lavage

Table 2 Correlation between the initial KL-6 serum levels, clinical severity markers, and GM-CSF autoantibody concentration in Chinese patients with PAP

<table>
<thead>
<tr>
<th>Variables</th>
<th>Serum KL-6 (U/mL)</th>
<th>N</th>
<th>r</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>PaO₂ (mmHg)</td>
<td>−0.608</td>
<td>37</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>P(A-a)O₂ (mmHg)</td>
<td>0.554</td>
<td>37</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>FVC (%pred)</td>
<td>−0.592</td>
<td>32</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>DLCO (%pred)</td>
<td>−0.668</td>
<td>29</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>LDH (IU/L)</td>
<td>0.73</td>
<td>33</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>GM-CSF autoantibody</td>
<td>−0.224</td>
<td>37</td>
<td>0.233</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1 Correlation between changes in serum KL-6 and lactate dehydrogenase (LDH) levels over time.
other markers that might indicate the severity of PAP and their evaluation values are shown in Table 3.

**Serum KL-6 levels in Chinese and German PAP patients and subgroups according to treatment protocol**

To establish whether the serum KL-6 levels in Chinese PAP patients were different from those in PAP patients of other ethnicities, we searched the literature for studies investigating the correlation between PAP and KL-6. Bonella et al. (5) have reported the levels of KL-6 in German Caucasian PAP patients. We found that the serum KL-6 level was significantly higher in Chinese patients with PAP than in German patients (Chinese group: 8,248±9,544 U/mL, German group: 2,049±1,893 U/mL, P<0.001; Table 4), despite the fact that similar methods were used to estimate the KL-6 levels; no significant differences in disease severity were observed [PaO\(_2\), P(A-a)O\(_2\), FVC, DLCO, and LDH levels].

Next, we compared the subgroups according to treatment protocol.

### Table 3: Cut-off values of different markers employed to assess the need for WLL

<table>
<thead>
<tr>
<th>Variables</th>
<th>Cut-off value (U/mL)</th>
<th>AUC(^{ROC} )</th>
<th>P</th>
<th>Se (%)</th>
<th>Sp (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
<th>Accuracy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum KL-6</td>
<td>6,512</td>
<td>0.988</td>
<td>&lt;0.001</td>
<td>92.9</td>
<td>100.0</td>
<td>100.0</td>
<td>95.8</td>
<td>97.0</td>
</tr>
<tr>
<td>PaO(_2) (mmHg)</td>
<td>66.8</td>
<td>0.963</td>
<td>&lt;0.001</td>
<td>92.9</td>
<td>91.3</td>
<td>86.7</td>
<td>95.5</td>
<td>91.9</td>
</tr>
<tr>
<td>PA-aO(_2) (mmHg)</td>
<td>38.0</td>
<td>0.947</td>
<td>&lt;0.001</td>
<td>92.9</td>
<td>87.0</td>
<td>81.3</td>
<td>95.2</td>
<td>89.2</td>
</tr>
<tr>
<td>FVC (%pred)</td>
<td>79.6</td>
<td>0.894</td>
<td>0.001</td>
<td>100.0</td>
<td>73.9</td>
<td>60.0</td>
<td>100.0</td>
<td>81.3</td>
</tr>
<tr>
<td>TLC (%pred)</td>
<td>68.3</td>
<td>0.987</td>
<td>&lt;0.001</td>
<td>100.0</td>
<td>95.5</td>
<td>87.5</td>
<td>100.0</td>
<td>96.5</td>
</tr>
<tr>
<td>DLCO (%pred)</td>
<td>52.4</td>
<td>0.987</td>
<td>&lt;0.001</td>
<td>100.0</td>
<td>90.9</td>
<td>77.8</td>
<td>100.0</td>
<td>93.0</td>
</tr>
<tr>
<td>LDH (IU/L)</td>
<td>237.0</td>
<td>0.958</td>
<td>&lt;0.001</td>
<td>92.3</td>
<td>95.0</td>
<td>92.3</td>
<td>95.0</td>
<td>94.0</td>
</tr>
<tr>
<td>CEA (ng/mL)</td>
<td>11.9</td>
<td>0.927</td>
<td>&lt;0.001</td>
<td>85.7</td>
<td>91.3</td>
<td>85.7</td>
<td>91.3</td>
<td>89.2</td>
</tr>
</tbody>
</table>

NPV, negative predictive value; PPV, positive predictive value; Se, sensitivity; Sp, specificity.

### Table 4: Serum KL-6 levels in Chinese and German PAP patients

<table>
<thead>
<tr>
<th>Variables</th>
<th>Chinese group (n=37)</th>
<th>German group* (n=33)</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>PaO(_2) (mmHg)</td>
<td>73.94±19.07</td>
<td>72±15</td>
<td>0.47</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>PA-aO(_2) (mmHg)</td>
<td>39.66±30.58</td>
<td>37±14</td>
<td>0.46</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>FVC (%pred)</td>
<td>78.55±16.99</td>
<td>80±16</td>
<td>0.35</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>DLCO (%pred)</td>
<td>63.99±23.35</td>
<td>57±19</td>
<td>1.30</td>
<td>&gt;0.1</td>
</tr>
<tr>
<td>LDH (IU/L)</td>
<td>258.4±111.9</td>
<td>283±93</td>
<td>0.97</td>
<td>&gt;0.2</td>
</tr>
<tr>
<td>Serum KL-6 (U/mL)</td>
<td>8,248±9,544</td>
<td>2,049±1,893</td>
<td>3.87</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± standard error (SE). *, data quoted from Bonella et al. (5).
received; the results are summarized in Tables 5, 6. The serum LDH level was significantly lower in the PAP patients in the observed group (189.9±30.1 IU/L) than in the German patients in the observed group (243±69 IU/L). This finding indicates that the disease severity of our PAP cohort in the observed group was milder than that in the German study.

However, the serum KL-6 levels of Chinese patients in the observed group were higher than that of the German patients. The disease severity of our PAP cohort in the WLL group was similar to that in the German study, but the serum KL-6 levels of Chinese patients in the WLL group were significantly higher than those of the German WLL group.

**Serum KL-6 levels in Chinese and Japanese PAP patients**

Two Japanese studies (6,7) have analyzed the correlation between disease severity and baseline levels of serum KL-6; both studies were conducted by a single research group. We chose the study in which disease severity was similar to that in our study for comparison. Using the similar method, the mean serum KL-6 level of our PAP cohort was similar to that reported by Tazawa et al. (6), and there was no significant difference in LDH level (Table 7); however, Tazawa et al. have not reported the reagent used for the measurement of KL-6 levels in their study.

**Differences in MUC1 gene polymorphisms in Chinese, Caucasian, and Japanese patients**

In GenBank, for MUC1, only rs4072037 had a minimum allele frequency of >0.05. rs4072037 was searched in the dbSNP database, and the frequency of the A/A genotype was found to be significantly higher, and the frequency of the G/G genotype was significantly lower, in Chinese and Japanese cohorts than in Caucasian cohorts (both P<0.001). However, no significant differences in the rs4072037 genotype or allele frequency distributions were detected between the Chinese and Japanese cohorts (P>0.05; Table 8).

**Discussion**

In this study, similar methods as described in previous studies (4,5) were used to compare the serum KL-6 levels of PAP patients of different ethnicities. We found that the mean serum KL-6 level of Chinese PAP patients was significantly higher than that of German PAP patients but close to that of Japanese PAP patients, when there was no significant difference in disease severity.

Consistent with the results of previous studies (5,8,9), our findings confirmed that serum KL-6 is a disease-severity marker for PAP. Serum KL-6 was significantly correlated with markers of disease severity such as PaO2, P(A-a)O2, FVC, and DLCO, and serum LDH levels in patients with PAP. Patients who improved during the follow-up period...
exhibited decreased serum KL-6 and LDH levels. In our PAP cohort, the mean serum KL-6 level (8.248 U/mL) was considerably higher than that reported by Bonella et al. in German PAP patients (2.049 U/mL) (5), when there was no significant difference in disease severity.

Takahashi et al. (4) reported that the mean serum KL-6 level of four Japanese PAP patients was 6067±894.5 U/mL; the methods and reagents used were similar with ours. Because Takahashi et al. did not provide data on disease severity and the sample size used in their study was small, we did not conduct statistical comparison with their results. However, the mean serum KL-6 level was similar to that of our study. On the other hand, Tazawa et al. (6) provided the details of disease severity in their patients. A comparative analysis of their results and the findings in the current study revealed that if disease severity was similar, the mean serum KL-6 level of Chinese and Japanese PAP patients tended to be similar (Table 7).

Why was the serum KL-6 level of Chinese PAP patients significantly higher than that of German patients, and similar to that of the Japanese patients? KL-6 is a mucin-like glycoprotein present in human MUC1 mucin. Are these racial differences in KL-6 levels related to MUC1 gene polymorphism? To address these questions, a thorough search of the GenBank database was performed. The results revealed that only rs4072037 had a minimum allele frequency of >0.05. Janssen et al. (10) reported the serum KL-6 levels and the genotype of rs4072037 in 327 Dutch Caucasian healthy controls and 74 sarcoidosis patients. The study showed no difference in the genotype or allele frequency distributions of rs4072037 between the two groups, and the serum KL-6 level was found to be related to the rs4072037 genotype frequency. Horimasu et al. (11) examined the serum KL-6 level and genotype frequency of rs4072037 in 267 ILD patients (152 cases from Germany and 115 cases from Japan) and 186 healthy controls (76 from Germany and 110 from Japan). Their results revealed that, in the absence of significant differences in demographic characteristics and disease severity, the serum KL-6 levels were higher in the German control and ILD groups than in the Japanese control and ILD groups, and these differences were related to the genotype or allele frequency distributions of rs4072037. In the current study, we found that the distribution of the rs4072037 allele frequencies was A/G 0.854/0.146 in the Chinese population, which was significantly higher than that reported for Caucasian patients (0.589/0.411, P<0.001), and close to that reported for Japanese patients (0.826/0.174, P>0.05). Together, these findings suggest that serum KL-6 levels of PAP patients are associated with the A/G frequency distribution of rs4072037. Therefore, we believe that, in addition to disease severity and detection methods, serum KL-6 levels of PAP patients are also affected by racial differences.

In Chinese PAP patients, serum KL-6 levels may also be used as a serological indicator of the need for WLL. At the cut-off level of ≥6,512 U/mL, serum KL-6 levels yielded a sensitivity of 92.9% and a specificity of 100% in assessing the need for WLL, and the accuracy rate was as high as 97%. In addition, our study showed that other indicators of PAP severity, such as PaO$_2$, P(A-a)O$_2$, TLC, DLCO, and LDH levels, exhibited high sensitivity, specificity, and accuracy in assessing the need for WLL. This result then leads to the following question: “Why is it necessary to use serum KL-6 level to assess the need for WLL?”

Kariman et al. (12,13) suggested that when patients exhibit disease progression, shortness of breath, or activity limitation, with PaO$_2$ <65 mmHg and P(A-a)O$_2$ >40 mmHg or intrapulmonary shunt >10–12% in the resting state, they require WLL. Our study demonstrated that when PaO$_2$ was <66.8 mmHg or P(A-a)O$_2$ was >38 mmHg, the patients needed WLL, thereby corroborating the suggestion put forth by Kariman et al. with objective data. However, the most common complication of PAP is pulmonary infection, and serum LDH and PaO$_2$ levels are easily affected by pulmonary infection. Therefore, increases in serum LDH and PaO$_2$ levels are easily mistaken for disease progression. In addition, from our follow-up process, we found that, in some patients, although PaO$_2$ was <65 mmHg and P(A-a)O$_2$ was >40 mmHg, their condition remained stable. Thus, clinical manifestations need to be considered when determining

### Table 8 Distribution of the MUC1 568 (rs4072037) A/G polymorphism and genotypes in the Chinese, Japanese, and Caucasian cohorts

<table>
<thead>
<tr>
<th>rs4072037</th>
<th>Chinese (n=82)</th>
<th>Japanese (n=172)</th>
<th>Caucasian (n=112)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/G</td>
<td>0.854/0.146</td>
<td>0.826/0.174</td>
<td>0.589/0.411</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AA/AG/GG</td>
<td>0.732/0.244/0.024</td>
<td>0.698/0.256/0.047</td>
<td>0.339/0.500/0.161</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

whether a patient needs WLL. As for pulmonary function
tests, although these tests are non-invasive, the results are
closely related to the extent of patient compliance, and the
reason(s) for poor compliance in clinical practice [serious
medical condition(s) or subjective patient aspects] need to be
identified.

The clinical value of using serum KL-6 level in assessing
the need for WLL is higher than that of other indicators for
the following two main reasons.

First, serum KL-6 is relatively specific to PAP. Patients
with PAP have higher serum KL-6 levels than those with
other diseases such as ILD, acute respiratory distress
syndrome, and pulmonary adenocarcinoma (4,9). Serum
KL-6 level seldom exceeds 3,000 U/mL in these diseases,
and a level >3,000 U/mL indicates PAP. Therefore, PAP
patients in remission might have other diseases, but their
serum KL-6 level seldom exceeds 6,512 U/mL, which is the
threshold used to indicate the need for WLL. The results of
our study also suggest that WLL needs to be administered
to patients whose serum KL-6 level is >6,512 U/mL; the
positive predictive value is 100%. For patients whose serum
KL-6 level is ≤6,512 U/mL, to determine whether WLL is
needed, we recommend using a combination of TLC and
DLCO values, the negative predictive value of which is
100%, with high accuracy.

Second, significant elevation in serum KL-6 level appears
to be specific to Chinese PAP patients. The AUC \( \text{ROC} \) and
accuracy (0.988 and 97%, respectively) of using serum
KL-6 level in assessing the need for WLL were higher in
Chinese patients than in German PAP patients (5) (0.87
and 88%, respectively), suggesting that the clinical value of
serum KL-6 in assessing whether PAP patients need WLL
is higher in Chinese patients than in German patients.
This is because the average serum KL-6 level and the cut-
off for WLL assessment are significantly higher in Chinese
patients than in German PAP patients. Therefore, while
using serum KL-6 level to assess the need for WLL in
PAP patients, different standards should be employed for
different ethnic groups.

This study showed that there is no significant correlation
between serum KL-6 level and serum GM-CSF antibody
concentration, which is consistent with the results of
previous studies (5). This result may be attributed to the
fact that serum GM-CSF antibody concentration is related
to the pathogenesis and etiology of PAP, but is not directly
related to disease severity, while serum KL-6 level is related
to the assessment of disease severity, but not etiology.

Additionally, we found that, in patients with secondary PAP,
the serum GM-CSF antibody level was not high, but the
serum KL-6 level increased, suggesting that KL-6 has no
significant correlation with autoimmunity, but only with
disease severity. This finding indicates that, in PAP patients,
serum GM-CSF antibody concentration and serum KL-6
level are the most important serological markers, the former
for etiology, and the latter for disease severity.

Our study has some limitations. First, our KL-6 data
were compared with historical rather than simultaneously
detected data. Second, genetic data were compared with
data from the GenBank database, rather than the results of
simultaneous rs4072037 genotype testing. A multicenter
study of PAP patients from different countries and
nationalities, which involves simultaneous sample collection,
identical reagents and methods, and simultaneous genetic
testing, will reveal the clinical significance of the expression
level of KL-6 in different ethnicities.

Conclusions

On the basis of our results, the serum KL-6 level of
Chinese PAP patients is significantly correlated with disease
severity, and is consistent with the dynamics of the disease.
Therefore, serum KL-6 level can be used as a biological
indicator in disease monitoring and assessing the need for
WLL. Additionally, the serum KL-6 level of Chinese PAP
patients is significantly higher than that of German PAP
patients, and similar to that of Japanese PAP patients. Such
differences might be related to variations in the rs4072037
genotype distribution among the ethnic groups.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest
to declare.

Ethical Statement: Permission was obtained from our
institutional ethics committee (approval number
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study participants.
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