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Plasma biomarker profiles in acute exacerbation of idiopathic pulmonary fibrosis

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Departments of 1Medicine and 2Anesthesiology and 3Cardiovascular Research Institute, University of California San Francisco, San Francisco; 4Department of Medicine, University of Ulsan, Seoul, South Korea; 5Department of Medicine, Keio University, Tokyo, Japan; and 6Department of Pathology, University of California San Francisco, San Francisco, California

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Collard HR, Calfee CS, Wolters PJ, Song JW, Hong S, Brady S, Ishizaka A, Jones KD, King TE, Jr, Matthay MA, Kim DS. Plasma biomarker profiles in acute exacerbation of idiopathic pulmonary fibrosis. Am J Physiol Lung Cell Mol Physiol 299: L3–L7, 2010. First published April 23, 2010; doi:10.1152/ajplung.90637.2008.—Little is known about the pathobiology of acute exacerbation of idiopathic pulmonary fibrosis (IPF), a condition that shares clinical and histopathological features with acute lung injury. Plasma biomarkers have been well studied in acute lung injury and have provided insight into the underlying disease mechanism. The objective of this study was to determine the plasma biomarker profile of acute exacerbation of IPF and compare this profile with that of stable IPF and acute lung injury. Plasma was collected from patients with stable IPF, acute exacerbation of IPF, and acute lung injury for measurement of biomarkers of cellular activity/injury (receptor for advanced glycation endproducts, surfactant protein D, KL-6, von Willebrand factor), systemic inflammation (IL-6), and coagulation/fibrinolysis (protein C, thrombomodulin, plasmogen activator inhibitor-1). Plasma from patients with acute exacerbation of IPF showed significant elevations in markers of type II alveolar epithelial cell injury and/or proliferation, endothelial cell injury, and coagulation. This profile differed from the biomarker profile in patients with acute lung injury. These findings support the hypothesis that type II alveolar epithelial cells are centrally involved in the pathobiology of acute exacerbation of IPF. Furthermore, they suggest that acute exacerbation of IPF has a distinct plasma biomarker profile from that of acute lung injury.

Idiopathic pulmonary fibrosis; respiratory distress syndrome; adult; biological markers

Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive form of diffuse lung disease with a median survival of 2–3 years (1, 3). The natural history of IPF is complex, and many patients have an unpredictable disease course with periods of relative stability punctuated by episodes of acute and often fatal decline (11). This acute worsening of disease is sometimes attributed to identifiable conditions such as pneumonia or heart failure, but many of these events occur without an identifiable cause. These idiopathic acute worsenings are termed acute exacerbations of IPF (6, 12, 13).

Acute exacerbations of IPF are defined by acute worsening of symptoms, the presence of new ground glass abnormality on computed tomography of the chest, and the absence of an identifiable cause (e.g., infection) (6). Histopathological evaluation most commonly reveals interstitial edema, hyaline membrane formation, and organizing fibrosis, findings characteristic of diffuse alveolar damage (5, 12). This presentation is similar to that seen in patients with acute lung injury (23), and it has been hypothesized that these two conditions may share similar mechanisms of disease. Recent data, however, have suggested acute exacerbation of IPF may instead be due to an acceleration of the underlying fibroproliferative disease process (14).

The pathobiology of acute lung injury is reasonably well understood and includes alveolar epithelial injury, endothelial injury with increased vascular permeability, acute inflammation, and disordered coagulation and fibrinolysis (23). Clinical studies in acute lung injury have defined a biological phenotype, using plasma biomarkers, that reflects these underlying abnormalities (4). Plasma biomarkers previously studied in acute lung injury include Krebs von den Lungen-6 (KL-6) and surfactant protein D (SP-D), markers of type II cell injury and/or proliferation (8, 10), the receptor for advanced glycation endproducts (RAGE), a marker of type I cell injury and/or proliferation (21), and von Willebrand factor (vWF), a marker of endothelial cell injury (22). Inflammatory markers (e.g., IL-6) are also elevated in acute lung injury, reflecting the central importance of acute inflammation in the development of this syndrome (17, 18). Coagulation abnormalities are well described in acute lung injury with evidence of reduced protein C and elevated plasmogen activator inhibitor 1 (PAI-1) levels (24).

The objective of this study was to determine the plasma biomarker profile of acute exacerbation of IPF and compare it to that of stable IPF and acute lung injury. We analyzed biomarkers of type II alveolar epithelial proliferation and/or injury (KL-6, SP-D), type I alveolar epithelial cell injury (RAGE), endothelial injury (vWF), inflammation (IL-6), and coagulation (protein C, thrombomodulin, PAI-1) in plasma from patients with stable IPF, acute exacerbation of IPF, and acute lung injury.

MATERIALS AND METHODS

Study patients. Three cohorts of patients were identified: stable IPF, acute exacerbation of IPF, and acute lung injury. These cohorts were all from a single institution (Asan Medical Center, Univ. of Ulsan, Seoul, South Korea) and were diagnosed based on consensus criteria (1, 2, 6). Written informed consent was obtained from each patient,

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Biomarkers in acute exacerbation of IPF and stable IPF.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Stable IPF</th>
<th>AEx IPF</th>
<th>ALI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 20</td>
<td>n = 47</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KL-6, units</td>
<td>895 (598, 1.428)</td>
<td>1,791 (1,155, 2,866)</td>
<td>0.0003</td>
<td></td>
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<tr>
<td>SP-D, ng/ml</td>
<td>294 (126, 340)</td>
<td>361 (228, 586)</td>
<td>0.01</td>
<td></td>
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<tr>
<td>RAGE, pg/ml</td>
<td>377 (304, 518)</td>
<td>366 (208, 690)</td>
<td>0.79</td>
<td></td>
</tr>
<tr>
<td>Von Willebrand factor, %</td>
<td>41 (24, 87)</td>
<td>89 (59, 180)</td>
<td>0.003</td>
<td></td>
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<tr>
<td>IL-6, pg/ml</td>
<td>5.3 (4.5, 6.2)</td>
<td>10.1 (6.1, 18.8)</td>
<td>0.004</td>
<td></td>
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<tr>
<td>Protein C, %</td>
<td>98 (81, 114)</td>
<td>135 (93, 199)</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>Thrombomodulin, ng/ml</td>
<td>2.6 (2.1, 3.8)</td>
<td>5.0 (3.2, 7.0)</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td>PAI-1, ng/ml</td>
<td>45 (36, 58)</td>
<td>70 (49, 85)</td>
<td>0.0004</td>
<td></td>
</tr>
</tbody>
</table>

SP-D, surfactant protein D; RAGE, receptor for advanced glycation end-products; PAI-1, plasminogen activator inhibitor-1. Values are median (25th percentile, 75th percentile).

and the study protocol was approved by the institutional review board or ethics committee at each center.

Briefly, stable IPF patients were defined as outpatients with IPF who were not experiencing a rapid decline in respiratory function. All acute exacerbation of IPF patients experienced acute worsening of symptoms, demonstrated new ground glass abnormalities, and underwent evaluation for identifiable causes of acute worsening including infection. Infectious causes were evaluated by several mechanisms. Sputum microbiology was performed in all patients; bronchoalveolar lavage culture, polymerase chain reaction for Mycobacterium tuberculosis, direct fluorescence antibody for Pneumocystis jiroveci (PCP), and viral antigen testing (including FITC-conjugated anti-virus antibody testing for respiratory syncytial virus, influenza, paramyxovirus, adenovirus, and human metapneumovirus) were performed in 57% of patients; and direct fluorescent antibodies for CMV antigenemia, aspergillosis antigen, legionella urinary antigen, pneumococcal urinary antigen, and mycoplasma antibody were performed in 70% of patients.

Blood collection and processing. All IPF blood samples were collected at the time of initial presentation (time of diagnosis for stable IPF, time of hospitalization for acute exacerbation of IPF). Acute lung injury samples were collected 1–7 days after admission (defined as early acute lung injury) in a subgroup of patients, and 14–21 days after admission (defined as late acute lung injury) in all patients. All blood samples were immediately centrifuged, and plasma was aliquoted and frozen for analysis. KL-6 levels were measured in a separate laboratory (Keio University and Sanko Junyaku, both Tokyo, Japan). Levels of SP-D, RAGE, vWF, IL-6, total protein C, thrombomodulin, and PAI-1 were measured using commercially available ELISA techniques in a central laboratory (Univ. of California San Francisco, San Francisco, CA). All assays were performed in duplicate, and the mean value was reported.

Statistical analysis. Demographics were compared across study groups. Acute exacerbation of IPF biomarker values were compared with stable IPF values, early acute lung injury values, and late acute lung injury values. Data are presented as means and standard deviation, or median and interquartile range as appropriate. Initial multigroup comparisons were performed using Kruskal-Wallis or Chi-squared methodology, with two-group analyses performed where group comparisons were performed using Wilcoxon two-sample test. Bivariate logistic regression analysis was performed with the dependent variable appropriate using the Wilcoxon two-sample test. Bivariate logistic regression analysis was performed with the dependent variable appropriate using the Wilcoxon two-sample test.

RESULTS

Patient characteristics. There were 47 patients with acute exacerbation of IPF (19 of which were biopsy-proven IPF), 20 patients with stable IPF (all of whom were biopsy-proven IPF), and 20 patients with acute lung injury (Table 1). The acute exacerbation and stable IPF groups were similar across all variables measured, with the exception of prednisone use (51% vs. 20% respectively, P = 0.02). The acute exacerbation of IPF and acute lung injury groups were similar across all variables measured. The mean time from symptom onset to blood draw in patients with acute exacerbation of IPF was 18 days. Early acute lung injury blood samples were available from 10 of the 20 acute lung injury patients.

Twenty-three (49%) patients with acute exacerbation of IPF reported experiencing fever, and 20% reported experiencing myalgias. The average white blood cell count was 10.9 × 10^3 cells/μl; the C-reactive protein level was elevated (average 7.5 mg/dl).

In comparison to acute lung injury, acute exacerbation of IPF demonstrated higher levels of KL-6 and SP-D and lower levels of RAGE, vWF, and IL-6 (Table 3). These differences were all statistically significant when compared against early acute lung injury; in late acute lung injury, RAGE and vWF were not statistically different. Total protein C, thrombomodulin, and PAI-1 levels were significantly higher in acute exacerbation of IPF compared with stable IPF.

Biomarkers in acute exacerbation of IPF and acute lung injury. In comparison to acute lung injury, acute exacerbation of IPF demonstrated higher levels of KL-6 and SP-D and lower levels of RAGE, vWF, and IL-6 (Table 3). These differences were all statistically significant when compared against early acute lung injury; in late acute lung injury, RAGE and vWF were not statistically different. Total protein C was significantly higher in acute exacerbation of IPF compared with both early and late acute lung injury; thrombomodulin was significantly lower.

Biomarker levels, clinical features, and survival in acute exacerbation of IPF. There were no consistent correlations between the clinical variables listed in Table 1 and biomarker levels (data not shown). Bivariate logistic regression analysis evaluating the prognostic value of clinical variables and biomarker levels for survival in acute exacerbation of IPF showed mechanical ventilation and log change in thrombomodulin to be significant predictors of survival (Table 4). No association between other biomarker levels and survival was found.

DISCUSSION

The results of this study suggest that acute exacerbation of IPF is characterized by increased type II alveolar epithelial cell injury and/or proliferation, endothelial cell injury, and coagu-
lation abnormalities. Interestingly, there is little evidence of type 1 alveolar epithelial cell injury or substantial acute inflammation. These findings are consistent with the emerging hypothesis that acute exacerbation of IPF represents the acceleration of the underlying fibroproliferative disorder (6, 14). Abnormalities in type II alveolar epithelial cell function have been proposed as central to the pathogenesis of IPF (7, 20). The biomarker data in this study are consistent with this hypothesis.

Fig. 1. Comparison of serum biomarker profiles in stable idiopathic pulmonary fibrosis (IPF) and acute exacerbation of IPF. A: KL-6; B: SP-D; C: RAGE; D: von Willebrand factor; E: IL-6; F: protein C; G: thrombomodulin; H: plasminogen activator inhibitor 1.
Acute exacerbation of IPF and acute lung injury demonstrated strikingly different biomarker profiles. The acute lung injury biomarker profile reported here is consistent with previously published results (8, 10, 17, 21). In acute exacerbation of IPF, the biomarker pattern suggests a relative predominance of type II alveolar epithelial cell injury and/or proliferation, and an absence of type I alveolar epithelial cell activity compared with patients with acute lung injury. An important consideration in comparing the acute exacerbation of IPF and acute lung injury cohorts is the timing of blood sampling. Although the mean time from onset of symptoms to blood draw in the acute exacerbation of IPF cohort was known (18 days), no data on the time from symptom onset to blood draw was available in the acute lung injury cohort. For this reason, we chose to use two time points in patients with ALI, both measured from the date of hospitalization. The differences between acute exacerbation of IPF and acute lung injury were most pronounced at the earlier of the two time points (day 1–7); differences at the later time point (14–21 days) were less pronounced but qualitatively similar and still significant for five of the eight biomarkers. These differences suggest that acute exacerbation of IPF may have a distinct pathobiological mechanism from acute lung injury.

The differences in coagulation profiles were unexpected, specifically the increase in total protein C levels with acute exacerbation of IPF. In acute lung injury, reduced levels of protein C and in some cohorts elevated levels of PAI-1 have been described (24). The acute lung injury patients included here show similar results. The etiology of the elevation of protein C in acute exacerbation of IPF is unknown. Protein C requires activation by the interaction of protein S, thrombin, thrombomodulin, and the endothelial protein C receptor before it can exert its anti-coagulant effects. Importantly, we could not measure activated protein C levels, only total protein C levels, and it is possible that the elevation in total levels we observed is a consequence of an unrecognized defect in the activation, internalization, or turnover of protein C. Levels of thrombomodulin were reduced in acute exacerbation of IPF compared with acute lung injury, a finding consistent with this hypothesis. Together, with clinical studies that suggest a benefit of anticoagulation therapy in patients experiencing acute exacerbation of IPF (15), these data suggest that alterations in coagulation could play a role in the pathogenesis of acute exacerbations.

Recent evidence suggests that membrane-bound RAGE levels are reduced in the lungs of patients with IPF, and that lower RAGE may contribute to fibroproliferation (9, 19). Lower type I alveolar epithelial cell expression of RAGE may lead to lower plasma levels of RAGE, as there is less available for release during cellular proliferation or injury. However, lower baseline RAGE levels in stable IPF are unlikely to explain the difference in RAGE we observed between acute exacerbation of IPF and acute lung injury, as there is no difference between stable IPF and acute exacerbation of IPF samples. The responses in acute exacerbation of IPF and acute lung injury are qualitatively distinct.

Except for thrombomodulin, no relationship between biomarker levels and short-term survival was found in patients with acute exacerbation of IPF. The significance of thrombomodulin’s association with survival is unclear and should be investigated in future cohorts. Most of these biomarkers have never been investigated in acute exacerbation of IPF. A study from Japan found a lack of prognostic value in baseline KL-6 measurements in 14 patients with rapidly progressive IPF (25). The inclusion criteria for this study were similar to those used in the current study. A more recent, small study published in the Japanese literature contradicts these findings, reporting that baseline KL-6 and SP-D levels predict survival at 180 days (16). This study included just 10 patients, limiting the validity of any conclusions. Identifying biomarkers that predict survival in patients with acute exacerbations of IPF will require future studies designed specifically to test this question.
There are important limitations to this study. First, plasma biomarker profiles provide only a one-time assessment of a dynamic disease process, and it is possible that differences in sampling methods and/or timing of sampling influenced the results. We have addressed this issue through standardized collection of samples drawn from one center and at several time points. Second, it is possible that differences in the baseline substrate of the lung, as well as clinical factors including age, disease severity, prednisone treatment, and mechanical ventilation may affect plasma biomarker levels. However, no correlation between clinical variables and biomarker levels was found in our cohort. Last, our data do not demonstrate a clear association between plasma biomarker levels and survival in acute exacerbation of IPF. However, our study was not designed to fully evaluate such a relationship and was underpowered; the focus was on pathogenesis.

In summary, the results of this study support the hypothesis that type II alveolar epithelial cells are centrally involved in the pathobiology of acute exacerbation of IPF. They also provide evidence of disordered coagulation. Furthermore, these data suggest that acute exacerbation of IPF and acute lung injury, while having clinical similarities, may be pathobiologically distinct conditions. Future research should focus on confirming this plasma biomarker profile and further characterizing the biology of the alveolar epithelial cell, endothelium, and coagulation/fibrinolysis pathway in acute exacerbation of IPF. Studies that correlate peripheral blood biomarker profiles with lung tissue findings in acute exacerbation of IPF may be particularly informative.

REFERENCES


