Elevated levels of plasminogen activator inhibitor-1 in pulmonary edema fluid are associated with mortality in acute lung injury

Priya Prabhakaran, Lorraine B. Ware, Kimberly E. White, Michael T. Cross, Michael A. Matthay, and Mitchell A. Olman. Elevated levels of plasminogen activator inhibitor-1 in pulmonary edema fluid are associated with mortality in acute lung injury. Am J Physiol Lung Cell Mol Physiol 285: L20–L28, 2003. First published May 2, 2003; 10.1152/ajplung.00312.2002.—The alveolar fibrinolytic system is altered in acute lung injury (ALI). Levels of the fibrinolytic protease inhibitor, plasminogen activator inhibitor-1 (PAI-1), are too low in bronchoalveolar lavage to address its prognostic significance. This study was performed to assess whether PAI-1 antigen in undiluted pulmonary edema fluid levels can identify patients with ALI and predict their outcome. PAI-1 antigen levels in both plasma and edema fluid were higher in ALI compared with hydrostatic edema, and edema fluid PAI-1 values identified those with ALI with high sensitivity and specificity. Both the high plasma and edema fluid PAI-1 antigen values were associated with a higher mortality rate and fewer days of unassisted ventilation in patients with ALI. Differences in PAI-1 activity were concordant with levels of PAI-1 antigen. Although the fibrin-derived alveolar D-dimer levels were strikingly similar in both groups, ALI patients had a higher relative proportion of D-monomer. In conclusion, PAI-1 levels in edema fluid and plasma identify those with ALI that have a poor prognosis. The data indicate that fibrin turnover in early ALI is a consequence of a rapid fibrinogen influx and fractional fibrinolytic inhibition.

fibrinolysis; prognosis

CURRENT ESTIMATES INDICATE that the incidence of acute lung injury (ALI) is 20–75 per 100,000 persons, with a mortality rate of 20–60% (48). Prior studies that have attempted to identify prognostic markers in bronchoalveolar lavage (BAL) fluid obtained from patients with ALI in the first several days after intubation have met with only modest success (7, 8, 15, 37, 38). Several obstacles exist in identifying valuable and reliable prognostic markers in ALI. For instance, it is clear that the lung inflammation evolves rapidly in ALI, making the timing of the analysis crucial. Second, there are both soluble activators and inhibitors for numerous cytokines/chemokines in the inflammatory milieu. Third, the BAL procedure itself dilutes the alveolar contents 100-fold, making it difficult to quantify potentially useful markers of ALI. Finally, the heterogeneity of underlying diseases and choice of control groups add to the difficulty in extrapolating the findings to disease pathobiology. We have overcome some of these obstacles by utilizing undiluted pulmonary edema fluid, not BAL fluid, that was obtained from patients with clinical ALI and well-defined hydrostatic pulmonary edema (HYDRO) controls within hours of intubation and initiation of mechanical ventilation.

ALI is characterized by the deposition of fibrin in the alveolar space. Deposition of fibrin in the alveolar space is the net result of an alteration in the balance of coagulation and fibrinolytic proteases [plasmin and urokinase type plasminogen activators (u-PA)] and antiproteases [plasminogen activator inhibitor-1 (PAI-1) and α2-antiplasmin] and the availability of plasma-derived fibrinogen (31, 35). Upon fibrinogen influx into the alveolar compartment, coagulation/fibrinolysis proteases/antiproteases act to generate insoluble cross-linked fibrin and soluble plasmic-cleavage fragments (13, 14, 22, 25, 35). The significance of fibrin turnover in sepsis is underscored by the recent trial demonstrating an improvement in mortality upon administration of the antiagulant, activated protein C (3, 21). Although a fraction of these patients had sepsis-related ALI, the utility of activated protein C in ALI, sepsis related or not, remains to be determined (21).

Several previous studies have demonstrated increased levels of PAI-1 in BAL obtained from patients with ALI, evidence that there is a suppression of the normal profibrinolytic environment in the lung (4, 23). However, PAI-1 antigen levels in the BAL are unde-
detectable in many patients and most controls. Animal models of bleomycin- or hyperoxia-induced lung injury demonstrate a pathogenic role for PAI-1 since genetic deletion of PAI-1 has been shown to be protective against pulmonary fibrosis or lethality, respectively (1, 10).

We hypothesized that the levels of PAI-1 in plasma or undiluted edema fluid obtained within hours of intubation would be significantly higher in patients with pulmonary edema from ALI compared with those with HYDRO. We also hypothesized that the magnitude of elevation in PAI-1 antigen would have important prognostic implications in patients with ALI. Last, to understand the effects of altered fibrinolysis on fibrin turnover, we characterized and quantified the fibrinogen-related molecules in pulmonary edema fluid.

METHODS

Patient enrollment. All patient protocols were approved by the Committee for Human Research of the University of California at San Francisco and the Institutional Review Board at the University of Alabama. This work was performed in accordance with the Declaration of Helsinki. Patients were eligible for inclusion in this study if they had pulmonary edema from either ALI or hydrostatic causes and required intubation for positive pressure ventilation (2). Pulmonary edema fluid was collected from patients within 12 h of intubation by gentle luminal suction with a 14-French catheter passed into the distal Airways as described (7, 46, 47). Plasma samples were collected simultaneously with the edema fluid samples by venipuncture. Samples were centrifuged at 3,000 g for 10 min at 4°C and frozen at −80°C until analysis. Patients were segregated into two groups on the basis of clinical criteria as outlined by the American European Consensus Conference and as published (2, 7, 47). The ALI group was defined by the presence of bilateral infiltrates on chest radiograph, PaO2/FiO2 of <300 and a pulmonary arterial wedge pressure of <18 mmHg, if measured, with clinical risk factors for the development of ALI (2, 7, 46). The hydrostatic group was defined by the presence of a wedge pressure of >18 mmHg or a two-dimensional echocardiogram demonstrating reduced left ventricular ejection fraction and a clinical history consistent with cardiac dysfunction (46). The measured edema fluid/plasma total protein ratio (of > or <0.65) was in concordance with the clinical classification in all patients.

Patients in the ALI group were further segregated according to the presence or absence of sepsis or pneumonia. Sepsis was defined as a temperature of >38.3°C or <35°C during the 48 h preceding the diagnosis of ALI, a systolic blood pressure of <100 mmHg or >30 mmHg below baseline for >1 h during the 48 h preceding the diagnosis of ALI, and a positive culture. Pneumonia was defined as the presence of infiltrates on the chest radiograph and positive respiratory tract microbiology. Patients with both pneumonia and sepsis were included in the sepsis group. The simplified acute physiology score (SAPS II) was calculated for each patient as described (28).

PAI-1 antigen assay. PAI-1 antigen levels were measured by sandwich ELISA (American Diagnostica, Greenwich, CT). This assay detects both active and latent forms of PAI-1 but is insensitive to PAI-2. The protocol was performed essentially as recommended by the manufacturer and as published (27, 34). Samples were incubated overnight in microplate wells precoated with capture antibody (murine monoclonal antibody to human PAI-1). The signals were detected with a secondary biotinylated antibody that recognizes the bound PAI-1 molecules using streptavidin-conjugated horseradish peroxidase (HRP)-dependent cleavage of the tetramethylbenzidine substrate in an end point format. PAI-1 levels were quantified by measuring the absorbance of the wells at 450 nm (MR 5000 Dynatech Microplate Reader) and comparing the values with those of a standard curve using known quantities of recombinant human PAI-1.

PAI-1 activity assay. PAI-1 antigen levels were measured by microplate tissue type plasminogen activators (t-PA) inhibition assay according to the manufacturer’s instructions and as published (Biopool, Ventura, CA) (27, 34). Briefly, samples or active PAI-1 standards were added to microplate wells precoated with immobilized active t-PA. The wells were then incubated (30 min at room temperature (RT)) with an HRP-linked monoclonal anti-PAI-1 antibody, MoAb-12A4, and washed. The HRP substrate reaction was stopped after 5 min at RT with 1.6 M sulfuric acid. PAI-1 activity levels were quantified by measuring the absorbance of the wells at 490 nm (MR 5000 Dynatech Microplate Reader) and comparing the values with those of a standard curve using known quantities of active human PAI-1.

Quantification and characterization of fibrinogen(ogen) fragments. The antigenic levels of soluble plasmin degradation product of cross-linked fibrin, D-dimer, were measured by sandwich ELISA according to the manufacturer’s instructions (Biopool). The samples and standards were added to microplate wells precoated with monoclonal antibody (MoAb-8D3). After HRP-linked anti-D-dimer Fab fragments were added, the plate was incubated (30 min at RT) and washed, and the HRP reaction was carried out (15 min at RT) with O-Phenylene-diamine substrate and stopped by the addition of 1.6 M sulfuric acid. D-dimer antigen levels were quantified by measuring the absorbance of the wells at 490 nm (MR 5000 Dynatech Microplate Reader) and comparing the values with those of a standard curve using known quantities of purified human D-dimer. Western blot analysis for fibrinogen or fragments was performed under reducing and nonreducing conditions as published (18, 35).

Outcome variables. Two outcome variables were assessed by chart review. Hospital mortality was determined for the period immediately after intubation and initiation of mechanical ventilation. Severity of lung injury was assessed by the number of days alive with unassisted ventilation over the 28-day period immediately after intubation and mechanical ventilation, a valuable end point in clinical lung injury (39).

Statistics. The continuous variables were compared by means of the unpaired Student’s t-test or the Mann-Whitney’s U-test if the variables were not normally distributed (50). All categorical values were compared by means of a Fisher’s exact test (50). Statistical significance was accepted at P ≤ 0.05.

RESULTS

Patient demographics. Patient characteristics from the ALI and HYDRO groups are summarized and compared in Table 1. The groups were demographically similar in age, gender, and smoking status. They also had similar initial physiological parameters and ventilator settings, including the SAPS II score, PaO2 – Pao2, PaO2/FiO2 ratio, the lung injury score, and tidal volume (in milliliters or per kilogram body wt). Thus there was no measurable difference in the severity of
the illness between the two groups. There was complete concordance of the clinically based group assignments and the plasma/edema fluid total protein ratio, which was significantly higher in the ALI group compared with the HYDRO group. The prevalence of sepsis and the mortality rate were higher in the ALI group compared with the HYDRO group. Furthermore, patients with ALI had significantly fewer days of unassisted ventilation compared with those who had HYDRO.

Plasma and pulmonary edema fluid PAI-1 antigen levels are increased in ALI. To determine whether PAI-1 antigen levels discriminate between ALI and HYDRO, we measured the PAI-1 antigen level in plasma and edema fluid by ELISA in both patient groups. Plasma PAI-1 antigen was more than threefold higher in the ALI group compared with the HYDRO group (Fig. 3), whereas the ratio of total protein concentration in edema fluid to plasma was equal to 1 (1 ± 0.2). Furthermore, there were approximately equal

in ALI, we calculated the ratio of PAI-1 antigen in the edema fluid to that of the simultaneously drawn plasma sample. There was eightfold more PAI-1 antigen in edema fluid compared with plasma in the ALI group (Fig. 3), whereas the ratio of total protein concentration in edema fluid to plasma was equal to 1 (1 ± 0.2). Furthermore, there were approximately equal

![Figure 1. Plasminogen activator inhibitor-1 (PAI-1) antigen levels in patients with acute lung injury (ALI) and hydrostatic pulmonary edema (HYDRO). PAI-1 antigen level was measured in plasma (open boxes) and edema fluid (filled boxes) from patients with either clinical ALI or HYDRO by ELISA as described in METHODS. Data are plotted in box plot format (median, 25–75%) and compared using the Mann-Whitney’s U-test. P values are as indicated.](http://ajplung.physiology.org/)

![Figure 2. Receiver operator curves (ROC) for PAI-1 antigen levels and etiology of pulmonary edema. ROCs were constructed to assess the sensitivity and specificity of PAI-1 antigen levels in plasma or edema fluid in identifying those with ALI. The inflection point of the edema fluid curve of PAI-1 antigen is 700 ng/ml.](http://ajplung.physiology.org/)
amounts of PAI-1 antigen in the edema fluid and plasma in the HYDRO group (means ± SD; ALI, 8 ± 7; HYDRO, 1.5 ± 2; \( P < 0.005 \); Fig. 3).

PAI-1 antigen has prognostic significance in ALI. To determine whether an elevated PAI-1 antigen level identifies patients with ALI with a poor outcome, we compared the PAI-1 antigen level in plasma and edema fluid with hospital mortality and with the number of days of unassisted ventilation. First, demographic and physiological parameters including age, smoking status, gender, lung injury score, edema fluid/plasma total protein ratio, \( \text{PaO}_2 - \text{PaO}_2 \), \( \text{PaO}_2/\text{FiO}_2 \) ratio, tidal volume, or tidal volume/kg body wt did not associate with mortality in either group. The presence of sepsis carries a higher risk for mortality (2.2-fold relative risk, \( P = 0.03 \)), and a higher SAPS II score trended with mortality (55, 1.6-fold relative risk, \( P = 0.08 \)) only in the ALI group.

Plasma PAI-1 antigen values were approximately fivefold higher in patients with ALI with a poor outcome (died or <5 days of unassisted ventilation) compared with those with a better outcome (survived or had >5 days of unassisted ventilation; Figs. 4 and 5). A plasma PAI-1 antigen value of 300 ng/ml was at the upper limit of two standard deviations above the mean (95% CI) in patients with ALI that do not survive. With the use of this threshold, the positive predictive value for an edema fluid PAI-1 antigen level of >6200 ng/ml was 100% for both mortality (\( P = 0.023 \), Fisher’s exact test) and for fewer than 5 days of unassisted ventilation (\( P = 0.023 \), Fisher’s exact test). These post hoc assessments of the data show that elevation of PAI-1 antigen in both plasma and edema fluid, within hours of intubation, carry significant negative prognostic implications in patients with clinical ALI. In contrast, a higher PAI-1 antigen level in plasma, edema fluid, or the edema fluid/plasma ratio of PAI-1 did not associate with mortality in the group with HYDRO.

PAI-1 activity levels in pulmonary edema fluid are higher in ALI. To determine the physiological relevance of our observations regarding PAI-1 antigen to alveolar fibrinolytic activity, we measured PAI-1 activity in plasma and edema fluid from patients in both groups by its ability to neutralize immobilized t-PA. There was a 13-fold greater PAI-1 activity in plasma in the patients with ALI compared with HYDRO (means ± SD; HYDRO, 8.9 ± 15 IU/ml; ALI, 120 ± 265 IU/ml; \( P = 0.04 \), Mann-Whitney’s U-test). Similarly, there was a 17-fold greater PAI-1 activity in the edema fluid of patients with ALI compared with those with hydrostatic edema (means ± SD; HYDRO, 8 ± 9 IU/ml; ALI, 265 ± 120 IU/ml).

Table 2. PAI-1 antigen level and mortality

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Survived</th>
<th>Expired</th>
<th>( P ) Value</th>
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<tr>
<td>Plasma PAI-1</td>
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<tr>
<td>&lt;640 ng/ml</td>
<td>7</td>
<td>6</td>
<td>0.005</td>
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<tr>
<td>&gt;640 ng/ml</td>
<td>0</td>
<td>12</td>
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<td>Edema fluid PAI-1</td>
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<tr>
<td>&lt;6,200 ng/ml</td>
<td>8</td>
<td>9</td>
<td>0.023</td>
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<tr>
<td>&gt;6,200 ng/ml</td>
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All \( P \) values were derived from the Fisher’s exact test. PAI-1, plasminogen activator inhibitor-1.
levels (e.g., fibrinolytic inhibition), the D-dimer levels in both plasma and edema fluid were surprisingly similar among the two patient groups (median, 25–75%; plasma = HYDRO, 1,757, 718 – 4,370 ng/ml; ALI, 1,653, 985 – 2,546 ng/ml; P = 0.53; edema fluid = HYDRO, 9,400, 4,800 – 24,650 ng/ml, ALI, 11,000, 4,100 – 36,575; P = 0.88). Furthermore, there was an ~17-fold greater concentration of D-dimer antigen in edema fluid compared with plasma in both groups (HYDRO = 21 and ALI = 14; P = 0.6), consistent with a compartmentalization of fibrin turnover in the alveoli in both groups. There was no discernable relationship between survival and D-dimer antigen (edema fluid, plasma, or edema fluid/plasma ratio) in either patient group.

Although the quantity of D-dimer antigen by ELISA was similar among the groups, analysis of the soluble fibrinogen-derived components by Western blot analysis revealed important differences between ALI and HYDRO fluid (Fig. 6). In both groups, the major products comigrated with plasmin-derived fragments of fibrinogen and confirmed the D-dimer ELISA data. However, there was a sevenfold greater proportion of D-dimer (80–100 kDa; fibrinogen/noncross-linked fibrin-derived fragments) relative to D-dimer (180 kDa; cross-linked fibrin-derived fragment) in ALI than in HYDRO (ALI, 1.4 ± 0.8; HYDRO, 0.2 ± 0.3; P = 0.001; Fig. 6). Thus a sevenfold greater proportion of the plasmin-cleaved fibrinogen fragments was derived from fibrinogen/noncross-linked fibrin in the ALI group.

Effect of sepsis on PAI-1 antigen levels in ALI. Previous reports have documented an increase in plasma
PAI-1 in populations of patients with sepsis (43). Among patients with ALI, the level of PAI-1 antigen in plasma was approximately fourfold greater in those who had sepsis as an underlying condition compared with those who did not have sepsis (ALI with sepsis, 1,479 ± 1,435 ng/ml; ALI without sepsis, 369 ± 165 ng/ml; P = 0.02, Mann-Whitney U-test; Fig. 7). In contrast, pulmonary edema fluid levels of PAI-1 antigen were clearly overlapping in septic and nonseptic patients with ALI (median, 25–75%; ALI with sepsis, 2,920, 1,474–10,025 ng/ml; ALI without sepsis, 3,916, 2,696–4,648 ng/ml; P = 0.7, Mann-Whitney U-test). These data indicate that PAI-1 levels in the plasma compartment, but not the pulmonary alveolar compartment, are affected by the septic state.

**DISCUSSION**

This analysis of the fibrinolytic system in patients with ALI and HYDRO demonstrates for the first time that elevated PAI-1 antigen levels can identify and predict the outcome of patients with clinical ALI. Together with the known alveolar fibrin deposition in hyaline membranes in early lung injury, our analysis of fibrin fragments suggests that fibrinolysis is partly inhibited, but not blocked, in patients with clinical ALI. Elevated PAI-1 antigen levels are higher in plasma and edema fluid in ALI compared with HYDRO. PAI-1 antigen levels in plasma and edema fluid discriminate between these etiological classes of hypoxic respiratory failure. For example, patients with a PAI-1 antigen level that is >700 ng/ml in edema fluid were 40-fold more likely to have ALI. A significant fraction of the alveolar compartment PAI-1 is produced within the lung itself, as demonstrated by our detection of eightfold more PAI-1 antigen in edema fluid than in plasma. Most important, elevated PAI-1 antigen levels in plasma and in edema fluid were predictors of a poor outcome as measured by both mortality and a reduction in the number of days of unassisted ventilation in this population of patients with clinical ALI. Levels of PAI-1 antigen outside the 95% CI for >640 ng/ml in plasma and >6,200 ng/ml in edema fluid have a positive predictive value of 100% for mortality and for fewer than 5 days of unassisted ventilation. These data clearly demonstrate the significance of a high PAI-1 antigen level measured early in the course of lung injury, with respect to both the etiology and prognosis of clinical ALI.

Whereas patients with sepsis-related ALI have higher levels of PAI-1 antigen in plasma, as previously reported (43), there was no difference in the edema fluid levels of PAI-1 antigen in patients with and without sepsis. This suggests that the elevation of PAI-1 in the alveolar compartment was a consequence of local, rather than systemic, factors. The correlation of PAI-1 in edema fluid with the clinically assessed degree of lung injury (mechanical ventilation requirement) and its increase in the alveolar compartment compared with plasma point toward the extent of intrapulmonary injury as the dominant factor in determining the level of PAI-1 in the alveolar compartment. In contrast, the edema fluid/plasma ratio of PAI-1 in HYDRO was close to 1, indicating little or no intrapulmonary PAI-1 production.

Although endothelial cells, epithelial cells, fibroblasts, and macrophages produce PAI-1 in a regulated manner in vitro, no observations regarding the cell of origin are available in humans with early lung injury. This is due to a lack of availability of appropriately timed biopsy material for study. However, prior work in patients later in the course of lung injury reveals high levels of PAI-1 mRNA in alveolar macrophages (6). In vitro studies have also identified several potent cytokine regulators of macrophage PAI-1 production with direct relevance to the pathobiology of ALI. These include IL-1, IL-10, TNF-α, LPS, and the matrix glycosaminoglycan hyaluronan (6, 20). Likewise, in a model of hemorrhagic shock, increased alveolar fibrin deposition was shown to be related to stimulation of macrophages by TNF-α (12). Experimental lung injury models also indicate a high level of PAI-1 expression in fibroblasts during the later phases of lung injury in which transforming growth factor (TGF)-β and fibrin fragments can upregulate PAI-1 expression in lung fibroblasts (18, 26, 31, 32, 36). Thus there are multiple cell type-specific PAI-1 mediators present and active in excess, which can, alone or in concert, mediate the induction of PAI-1 in the alveolar compartment in ALI.

One of the strengths of this study compared with previous studies is that we were able to measure a discrete PAI-1 antigen value in all patients and, therefore, make quantitative and predictive conclusions. A comparison with prior studies was performed by normalizing to the total protein to account for dilutional effects of the BAL procedure. Interestingly, we still found PAI-1 antigen levels in ALI edema fluid to be 3-fold and 30-fold higher than that previously reported in BAL performed within the first few days after intu-
bation (17, 24). This difference is likely time related, since the elevations in PAI-1 antigen in BAL in ALI normalize over the first 10–14 days after intubation (17, 24). In contrast, the edema fluid PAI-1 antigen/total protein ratio of hydrostatic control patients was similar to that seen in the BAL from healthy controls, suggesting that the alveolar fibrinolytic balance is not altered by hydrostatic edema (17).

Alveolar compartment fibrinogen-related molecules are generated as a consequence of extravasation of plasma-derived fibrinogen and the action of several proteases. D-dimer, a unique product of plasmin action on cross-linked fibrin, is several logs higher in BAL from patients with ALI compared with healthy controls (14). To our surprise, D-dimer levels, as measured by ELISA in edema fluid, were similar in ALI and hydrostatic edema. However, a much greater fraction of the total alveolar fibrinogen burden in ALI patients was due to D-monomer, a product of plasmin action on fibrinogen/noncross-linked fibrin. Furthermore, the histological hallmark of ALI, namely, insoluble fibrin-rich hyaline membranes, does not develop in hydrostatic edema. Together, these data suggest that the vast majority of fibrinogen in the alveolar compartment in hydrostatic edema is cross-linked by thrombin/transglutaminase capacity to generate cross-linked fibrin (i.e., incomplete coagulation), and only a minor fraction of the total fibrin is solubilized by plasmin (i.e., incomplete fibrinolysis). The incomplete fibrinolysis in ALI can be partly ascribed to the elevated, locally produced, alveolar compartment PAI-1 activity that slows the activation of the zymogen plasminogen to plasmin.

We have identified an early elevation in PAI-1 antigen as a clinical marker for both the underlying etiology and prognosis of early ALI. The question of its pathophysiological importance and the mechanism of its effect on lung injury and repair remain unresolved. Fortunately, animal models have contributed to our understanding of the pathophysiological importance of PAI-1 in lung injury and repair. Rodent models of lung injury due to hyperoxia and intratracheal bleomycin show a clear detrimental effect of PAI-1 in terms of mortality and subsequent fibrosis (1, 10). In a similar vein, manipulation of the alveolar fibrinolytic activity with an adenovirus encoding the human u-PA, PAI-1’s target proteinase, exhibited a small, protective effect toward bleomycin-induced lung injury (41). From these data, it seems that PAI-1 plays a pivotal role in the progression of ALI and the development of pulmonary fibrosis in this species.

Of less certainty is the mechanism of PAI-1’s detrimental effect on lung injury and repair. This is critically important because PAI-1 has both protease inhibitor-dependent and -independent effects on cell adhesion, migration, and intracellular signaling as well as effects on proteolysis that are directed pericellularly and on bulk proteolysis of the fibrinous alveolar provisional matrix (5, 35, 42, 49). It has been well established that alveolar fibrin is a prominent component of the surfactant dysfunction in early lung injury and may play a role in repair (16, 40). However, recent data suggest that PAI-1 exerts its effect on bleomycin lung injury independent of the presence of fibrin in the injured alveolus (19). Furthermore, through inhibition of target proteinases in a matrix-directed fashion, PAI-1 can affect the activation and matrix release of several growth factors, including basic fibroblast growth factor, TGF-β, and hepatocyte growth factor (5, 11). The mechanism of PAI-1’s effects in lung injury remains an area of active basic research.

This study has some limitations. Because no biopsy material was available from our patients, we were unable to investigate the cellular source(s) of PAI-1. Furthermore, the ALI patients were from a mixed population of sepsis-related and nonsepsis-related medical and surgical intensive care unit populations with a relatively high overall mortality rate. The 95% CI for mortality rate in our study overlaps that of some prospective outcome-based studies and prior studies of ALI patients with aspirable pulmonary edema fluid (50–70%) (9, 30, 45, 51). However, the mortality rate is higher than other, more recent, outcome-based or therapeutic trials (29, 44). We acknowledge the possibility that patients who have aspirable edema fluid may have more severe lung injury and a higher mortality rate than the general population with ALI. This possibility does not diminish the prognostic significance of high PAI-1 levels in this group. Clearly, our findings should be prospectively extended to a larger group of ALI patients, including those with milder forms of lung injury and/or those with nonaspirable edema fluid. Unexpectedly, the presence of sepsis did not influence the alveolar compartment PAI-1 or D-dimer findings, thus providing further support for the importance of intrapulmonary inflammation in governing alveolar compartment fibrinolytic alterations.

In conclusion, elevated levels of PAI-1 antigen in both plasma and pulmonary edema fluid are higher in ALI than in hydrostatic edema. Furthermore, these elevated levels of plasma and edema fluid PAI-1 antigen predict a higher mortality and fewer days of unassisted ventilation in patients with ALI. Together with the fibrin fragment results, the data indicate that fibrin turnover in early lung injury is a consequence of rapid fibrinogen influx and fractional fibrinolytic inhibition.

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REFERENCES


