EXPERIMENTAL

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Elevated PAI-1 is associated with poor clinical outcomes in pediatric patients with acute lung injury

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Abstract *Purpose:* Deposition of
fibrin in the alveolar space is a hall-
mark of acute lung injury (ALI).
Plasminogen activator inhibitor-1
(PAI-1) is an antifibrinolytic agent
that is activated during inflammation.
Increased plasma and pulmonary
edema fluid levels of PAI-1 are
associated with increased mortality in
adults with ALI. This relationship has
not been examined in children. The
objective of this study was to test
whether increased plasma PAI-1 lev-
els are associated with worse clinical
outcomes in pediatric patients with
ALI. *Design/methods:* We mea-
sured plasma PAI-1 levels on the first
day of ALI among 94 pediatric
patients enrolled in two separate
prospective, multicenter investiga-
tions and followed them for clinical
outcomes. All patients met American
European Consensus Conference cri-
teria for ALI. *Results:* A total of 94
patients were included. The median
age was 3.2 years (range 16 days–
18 years), the PaO_2/F_iO_2 was
141 ± 72 (mean $\pm$ SD), and overall
mortality was 14/94 (15%). PAI-1
levels were significantly higher in
nonsurvivors compared to survivors
($P < 0.01$). The adjusted odds of
mortality doubled for every log
increase in the level of plasma PAI-1
after adjustment for age and severity
of illness. *Conclusions:* Higher
PAI-1 levels are associated with
increased mortality and fewer venti-
lator-free days among pediatric
patients with ALI. These findings
suggest that impaired fibrinolysis may
play a role in the pathogenesis of ALI
in pediatric patients and suggest that
PAI-1 may serve as a useful bio-
marker of prognosis in patients with
ALI.

Keywords Accurate respiratory
distress syndrome · Acute lung
injury · Clinical studies · Pediatrics ·
Plasminogen activator inhibitor-1
(PAI-1)

Introduction

Acute lung injury (ALI) is a life-threatening disorder
characterized by severe inflammation in the lungs and
frequently by the occurrence of multiple organ failure [1].
ALI is a major cause of morbidity and mortality among
critically ill children and accounts for approximately 1–4%
of all pediatric intensive care unit (PICU) admissions; up to
8% of all mechanically ventilated children meet criteria for
ALI. There are both similarities and significant differences
in the predisposing causes and clinical course of ALI in
adults and children [2–7]. The reported mortality from ALI

in the pediatric age group overall ranges from 8 to 60% with higher mortality reported among immunocompromised children [2–8]. The only therapy that has been proven to reduce mortality in ALI in adults is the use of lung-protective ventilatory strategy [1]. Insight into pathophysiological derangements that characterize early clinical ALI in children may help in identifying patients at greatest risk and guide the development of new therapies for this devastating disorder.

The pathophysiology of ALI is characterized by disruption of the lung endothelial and alveolar epithelial barriers, translocation of protein-rich edema fluid into the alveoli, release of pro-inflammatory cytokines, and deposition of fibrin in the alveolar space [9]. The deposition of fibrin in the alveoli is the net result of an alteration in the balance of coagulation and fibrinolysis. Early in the course of ALI, there is elevation of PAI-1 activity in plasma and air spaces, which is accompanied by a decrease in alveolar fibrinolysis [10–14]. Excessive fibrin deposition enhances inflammatory responses by activating endothelial cells to produce pro-inflammatory mediators and increases lung vascular permeability [15]. Therefore, assessing the extent of fibrinolysis in ALI may be important from a pathogenetic and prognostic standpoint [9].

Previous studies in adults with ALI have reported that an early increase in plasma levels of PAI-1 is an independent risk factor for mortality and adverse clinical outcomes and therefore, measurement of PAI-1 may be useful to (a) identify those at highest risk of adverse clinical outcomes, and (b) lead to the development of new therapies [9, 13, 16]. There have been no studies to date examining the relationship of plasma PAI-1 levels to clinical outcomes in pediatric patients with ALI. It is not clear whether the association of elevated PAI-1 levels with increased mortality described in adults is true for pediatric patients as well because there are significant differences between adults and children in terms of (1) predisposing conditions leading to ALI, (2) mortality from ALI, and (3) response to therapy with activated protein C in patients with sepsis, which is a common predisposing cause of ALI. Therefore, in order to test the relationship between plasma levels of PAI-1 and mortality, we hypothesized that early elevation of plasma PAI-1 would be associated with increased mortality and fewer ventilator-free days in pediatric patients with ALI.

Methods

Human subjects

The study was carried out on pediatric patients with ALI enrolled in two clinical trials of ALI.

1. Prospective observational trial of ALI in pediatric patients with ALI. A total of 320 patients with ALI, age 1 month to 18 years, were enrolled at Children's Hospital and Research Center, Oakland, CA, USA (CHRCO) and University of California, San Francisco (UCSF) Children's Hospital, USA. The overall mortality in the cohort was 22% [4]. Plasma samples were obtained from 72 patients within 24 h of development of ALI.

2. Randomized clinical trial of prone positioning in the management of pediatric patients with ALI. A total of 102 patients with ALI, age 2 weeks to 18 years were enrolled from seven U.S. PICUs including CHRCO and UCSF Children's Hospital. Patients were randomized to the supine or prone position. The primary outcome was the number of ventilator-free days. There was no difference in the number of ventilator-free days in the two arms and overall. The overall mortality in this trial was 8% [2]. Plasma samples were obtained within 24 h of development of ALI from 22 patients in this trial.

Both of these studies prospectively identified and enrolled pediatric patients meeting the standard American European Consensus Conference (AECC) criteria for ALI [17]. The AECC criteria for definition of ALI are $PaO_2/F_iO_2 < 300$, bilateral opacities on the chest radiograph, and either a pulmonary wedge pressure of less than 18 mmHg or the absence of clinical evidence of left atrial hypertension.

The research featured here is based on a subset of ALI patients enrolled in the two studies. We could not include all of the enrolled patients in the parent studies because collection of blood samples was not originally a part of either of the primary studies. This study was added on as an ancillary study to test the pathogenetic and prognostic value of biological markers in ALI. Blood samples could be collected only in a subset of patients because (1) the ancillary study with collection of blood samples from enrolled patients started after the studies were under way, (2) collection of blood samples was limited to centers that participated in the ancillary study, and (3) collection of blood samples was possible only for patients in whom consent for blood collection could be obtained. We included all 94 patients from the two studies who had a plasma sample collected within 24 h of meeting criteria for the diagnosis of ALI.

There was no difference in ventilator strategies between the ALI patients who had blood samples collected for the biomarker studies and those who did not. Study guidelines based on current best practice were followed for ventilator management and extubation in the interventional trial of prone positioning. In the ALI observational trial, ventilator management was as per the physician's preference, but a protocol was followed to guide the timing of extubation. However, management

decisions were made by clinicians blinded to PAI-1 levels and therefore are unlikely to have led to any bias.

Data collection

Patient demographics, overall clinical status at onset of ALI, pediatric risk of mortality (PRISM) III score, diagnosis associated with the onset of ALI, PaO_2/F_iO_2, chest radiograph findings, duration of mechanical ventilation, and 28-day all cause in-hospital mortality were collected at enrollment and during the hospital stay. Patients were followed for 28 days or in-hospital death.

Measurements

Plasma PAI-1 levels were measured on samples drawn within 24 h after meeting criteria for ALI. Plasma PAI-1 was measured using a standard ELISA technique with plates and reagents supplied by American Diagnostics, Greenwich, CT, USA. The protocol was performed essentially as recommended by the manufacturers and as published [18].

Outcomes

The primary outcome was mortality at 28 days and the secondary outcome was ventilator-free days (VFD).

Data analysis

Normally distributed data are presented as mean and SD and non-normally distributed data are presented as median and ranges. We compared non-normally distributed continuous variables using the nonparametric Spearman's correlation test. We also compared VFD across ordered categories of plasma PAI-1 levels using the test for trend across ordered categories. We used regression models to control for the potential confounding effect of age and severity of illness on mortality and VFD. Due to their right skewed distribution, and as has been done in previous studies, we log-transformed PAI-1 levels prior to carrying out parametric tests to compare PAI-1 levels within groups and for regression analysis. The statistical analysis was carried out on Stata 9 software (Stata, College Station, TX, USA).

Results

A total of 94 patients were available to us for this study. The baseline characteristics and primary diagnoses predisposing to ALI among these patients are shown in Table 1.

Since the present study includes only those patients from whom blood samples were collected within the first 24 h of diagnosis of ALI, we compared the baseline characteristics among patients having blood samples with

Table 1 Baseline characteristics of 94 patients with ALI included in the current study and of patients enrolled in the parent studies

	(A) ALI observational study			(B) Prone positioning study			Current study	
	Overall cohort ($n = 320$)	Included here ($n = 72$)	P	Overall cohort ($n = 101$)	Included here ($n = 22$)	P	Included here (A + B) ($n = 94$)	P
Age (years)	3.4 (0–18)	3.2 (0.5–18)	0.7	2.1 (0.3–11)	4.1 (0.04–17)		3.2 (0.04–18)	0.5
Male	57%	51%	0.4	52%	54%	0.8	49 (52%)	0.8
Race								
Caucasian	38%	40%	0.5	55%	35%	0.08	35 (39%)	0.7
Hispanic	18%	23%		24%	20%		20 (22%)	
African American	22%	21%		11%	35%		22 (24%)	
Asian	8%	4%		4%	0%		3 (3%)	
Other/unknown	15%	11%		6%	10%		10 (11%)	
Primary diagnosis associated with ALI								
Pneumonia	20%	33%	0.1	56%	45%	0.6	34 (36%)	0.2
Viral/bronchitis	17%	18%		14%	18%		17 (18%)	
Aspiration	15%	14%		11%	18%		14 (15%)	
Sepsis and meningitis	14%	14%		15%	18%		14 (15%)	
Others	33%	21%		5%	0%		15 (16%)	
Physiological variables								
PaO_2/F_iO_2	161 ± 74	145 ± 74	0.09	100 ± 65	125 ± 64	0.1	141 ± 72	0.2
PRISM III (unadjusted)	10 ± 9	10 ± 6	1	11 ± 8	11 ± 8	0.96	10 ± 7	0.6
Oxygenation index	10 ± 10	12 ± 12	0.07	16 ± 11	15 ± 10	0.31	13 ± 12	0.4

Data are expressed as median (range), mean ± SD, or n (%)

patients in the entire cohort in each of the parent studies (Table 1). For the 72 patients drawn from the ALI observational study, the PaO_2/F_iO_2 was 145 ± 74, PRISM score was 10 ± 6, and the VFDs were 14 ± 10. These data are not statistically different from the values reported for the overall cohort in the parent study (Table 1) [4]. Among the 22 patients drawn from the prone positioning trial, the PaO_2/F_iO_2 was 125 ± 64, the PRISM score was 11 ± 9, and the VFDs were 15 ± 8. These data are not statistically different from the values reported for the overall cohort in the parent study (see Table 1) [2, 4].
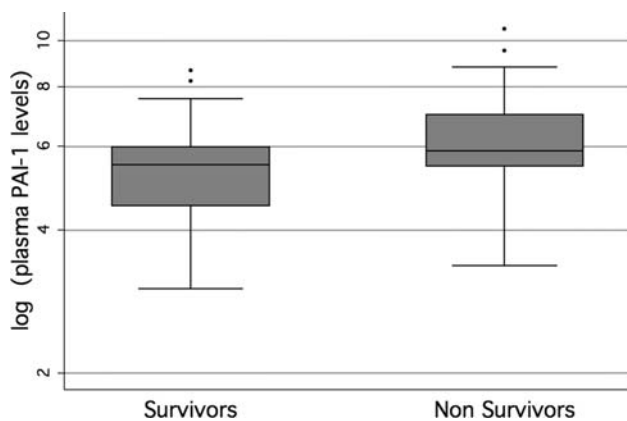

Fig. 1 Boxplots of log-transformed day 1 plasma levels of plasminogen activator inhibitor-1 (PAI-1) stratified by 28-day mortality among 94 pediatric patients with acute lung injury (ALI). There was a statistically significant difference in the levels between survivors and nonsurvivors ($P < 0.01$)

Primary outcome (mortality)

A total of 14/94 (15%) patients died prior to hospital discharge. The primary diagnosis, associated multiorgan failure and attributable causes of death are listed in Table 2. PAI-1 levels were higher among nonsurvivors than among survivors. Among nonsurvivors, the median PAI-1 level was 350 ng/mL with an interquartile range from 232 to 1,083 ng/mL, whereas among the survivors the median PAI-1 level was 239 ng/mL with the interquartile range from 90 to 393 ng/mL. Due to their nonnormal distribution, PAI-1 levels were log-transformed before applying parametric tests to compare the levels between survivors and nonsurvivors (Fig. 1). There was a statistically significant difference between PAI-1 levels among survivors and nonsurvivors ($P < 0.01$). Using multivariate logistic regression to adjust for age and the severity of illness, the odds of mortality increased by 2.0 for every log increase in the level of plasma PAI-1 (Table 3). There was no statistically significant difference in PAI-1 levels based on the primary diagnoses.

Secondary outcome (VFD)

Ventilator-free days were calculated as the number of days of unassisted breathing between enrollment on day 0 until day 28 when the patient was alive [19]. Increasing PAI-1 levels were correlated with the need for prolonged mechanical ventilation. Spearman's correlation was $\rho = -0.26$ ($P < 0.02$). The number of VFDs was lower among patients with increasing quartiles of plasma PAI-1 levels ($P < 0.02$) (Fig. 2). By multivariate linear regression that was adjusted for age and severity of illness, there

Table 2 Diagnosis, multiorgan failure, and cause of death among nonsurvivors

Primary diagnosis	Multi-organ failure	Final cause of death
Leukemia	ARDS, CV, heme, renal	Brain death
Lupus flare	ARDS, renal, hep, GI, heme,	Brain death
Pneumonia (pneumococcal)	ARDS, CV, heme, CNS	Cardiac arrest with clinical herniation
Vasoocclusive disease and graft-versus-host disease after BMT	ARDS, hepatic, renal, heme, CV	No escalation of care
Sepsis and candida superinfection	ARDS, CV, heme, hep, renal	No escalation of care
Sepsis (pneumococcal)	ARDS, CV, renal, hepatic, heme, CNS	Withdrawal of support
Pneumonia (influenza A and candida)	ARDS, heme, renal, liver	Withdrawal of support
Sepsis (strept viridans)	ARDS, CV, heme, GI	Withdrawal of support
Cardiac arrest, pulmonary HTN	ARDS, CV, hepatic	Withdrawal of nitric oxide
Adenoviral pneumonia	ARDs, bleeding on ECLS	Withdrawal of support
Aspiration pneumonia	ARDS, heme	Withdrawal of support
Meningococcemia with peripheral ischaemia	ARDS, renal, CV, heme, hep, CNS	Withdrawal of support
Head trauma	ARDS, CV, heme	No escalation of care
Sickle cell disease, stroke, desferoxmine toxicity	ARDS, CV, heme, CNS	Withdrawal of support

ARDS Acute respiratory distress syndrome, *CV* cardiovascular, *heme* hematologic failure, *hep* hepatologic failure, *ECLS* extracorporeal life support, *CNS* central nervous system, *GI* gastrointestinal

Table 3 Association of ventilator-free days and mortality and PAI-1

Predictor log PAI-1 levels	Linear regression outcome: VFD		Logistic regression outcome: mortality	
	Coefficient [95% CI]	P	Odds ratio [95% CI]	P
Unadjusted	−2.0 [−3.4 to 0.6]	<0.01	1.75 [1.1–2.6]	<0.01
Adjusted[a]	−1.6 [−2.9 to 0.3]	<0.02	2.0 [1.04–3.0]	<0.02

[a] Adjusted for age and PRISM III
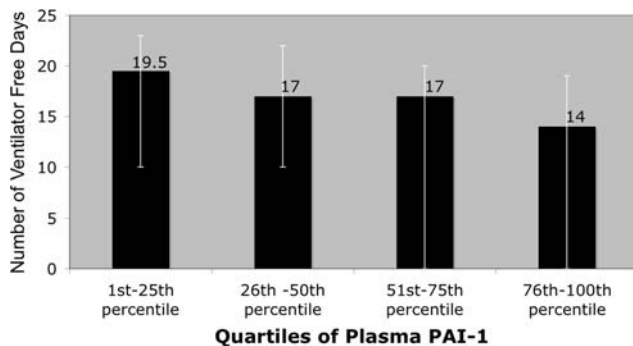
Fig. 2 Number of ventilator-free days (VFD) among patients stratified by quartiles of plasma levels of plasma plasminogen activator inhibitor-1 (PAI-1). The *height* of the *bar* represents the median and the *error bars* represent the interquartile range (25th–75th percentile) of the number of VFDs in each stratum of patients. There was a decrease in the number of VFDs with increasing levels of plasma PAI-1 (trend test $P < 0.02$)

was a decrease of 1.6 VFD for each log increase in PAI-1 levels (Table 3).

Discussion

The important findings from this prospective study of 94 pediatric patients with ALI are that elevated plasma levels of PAI-1 are associated with increased mortality and fewer VFDs in pediatric patients with ALI, independent of age and severity of illness. These results expand upon prior findings in adults from an earlier study that examined the relationship of plasma PAI-1 levels to clinical outcomes in a cohort of 779 patients with ALI [9]. The study by Ware and colleagues reported that among adults with ALI, the odds of mortality increased by 2.0 for every log increase in the level of PAI-1. In this study of children with ALI, we found that the adjusted odds of mortality increased by 2.0 for every log increase in the level of plasma PAI-1 after adjustment for age and severity of illness.

Early elevation of plasma PAI-1 could be a useful biomarker of prognosis in pediatric patients with ALI because the association of elevated plasma PAI-1 levels with clinical outcomes was independent of age and severity of illness at the time of presentation. Biomarkers of prognosis can have value because they may help identify patients who are at the greatest risk for poor clinical outcomes, allowing therapeutic and preventive interventions to be directed towards these more susceptible patients. In addition, if causality can be established in future studies, the biomarkers may provide targets for pharmacologic intervention.

Intra-alveolar fibrin deposition, which is a pathologic hallmark of ALI, may exert beneficial effects by sealing leakage sites when the capillary endothelium and alveolar epithelial barrier are compromised. However, severe and persistent fibrin deposition can have deleterious effects [10–14, 20–26]. Tissue-type and urokinase-type plasminogen activators (uPA) activate plasminogen to form plasmin, which degrades fibrin. PAI-1 is an endogenous inhibitor of the plasminogen activators and therefore elevation of PAI-1 may lead to an unfavorable balance of fibrin deposition and fibrinolysis [27, 28]. In addition, uPA has a role in migration of inflammatory cells [29]. PAI-1 also directly inhibits integrin-mediated cell migration and thereby has a direct effect on the host immune response as well [30, 31].

There are some limitations to this study. It was carried out by combining subsets of patients from two prospective studies of ALI in whom plasma samples were available. We combined patients from two studies because there were not enough patients with plasma samples collected in either of the studies individually. Both studies prospectively identified patients with ALI using the AECC criteria for definition of ALI. The distributions of predisposing conditions leading to ALI and baseline demographics of the enrolled patients were similar in the two studies (Table 1). There was a difference in the overall mortality in the two studies, which probably is a reflection of historic trends of improving mortality in ALI, most likely due to increasing use of lung-protective ventilation strategies, and of the different exclusion criteria in the two studies. The prone positioning study excluded patients with cardiovascular instability [2]. We believe that patients with plasma samples included in the present study are a true representation of each of the parent studies because the PaO_2/F_iO_2, PRISM scores, and VFDs among the subsets of patients for whom plasma samples were available (and thus included in this study) were not statistically different from the PaO_2/F_iO_2, PRISM scores, and VFDd for patients enrolled in each of the parent studies.

In conclusion, the results from this study indicate that early elevation of plasma PAI-1 is associated with higher mortality and fewer VFDs among pediatric patients with ALI, independent of age and severity of illness. These findings support a likely role of altered fibrinolysis in the pathogenesis of pediatric ALI and they also suggest that elevated plasma PAI-1 may be a useful biomarker of prognosis in patients with ALI. In addition, if causality

can be established in future studies, PAI-1 may be a target for future pharmacologic intervention.

Conflict of interest statement None.

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