Expression patterns of plasma von Willebrand factor and serum interleukin-8 in patients with early-stage severe pulmonary contusion

Jin-xian Qian, Shi-qi Lu, Yi-ming Zhao, Jun-hao Lu

ICU, the First Affiliated Hospital of Soochow University, Soochow 215006, China (Qian JX, Lu SQ); Blood Institute, Soochow University, Soochow, China (Zhao YM); Wujiang Affiliated Hospital, Nantong University, Wujiang 215200, China (Lu JH)

Corresponding Author: Shi-qi Lu, Email: lushiqi2004@126.com

BACKGROUND: von Willebrand factor (vWF) is only released from endothelial cells and platelets and is an in vivo and in vitro marker of endothelial injury in septic patients with acute lung injury (ALI). Interleukin-8 (IL-8), as a proinflammatory mediator causing recruitment of inflammatory cells, induces an increase in oxidant stress mediators and makes it as a key parameter for localized inflammation. However, it has not been well established whether the level of serum IL-8 is associated with the severity of lung injury and whether it is a prognosis marker for severe lung contusion. This study was to investigate the expression of plasma vWF and IL-8 and their association with the severity and outcomes of severe pulmonary contusion.

METHODS: A total of 63 patients were divided into a severe pulmonary contusion with acute respiratory distress syndrome (ARDS) group and a non-ARDS group, or a survivor group and a non-survivor group, or an injury severity score (ISS) <20 group and an ISS ≥20 group. Another 20 healthy volunteers served as controls. The levels of plasma vWF and serum IL-8 were measured by enzyme-linked immunosorbent assay (ELISA) at 1, 3, 5 and 7 days after injury. The expression patterns of the plasma vWF and serum IL-8 were compared between different groups.

RESULTS: The concentrations of plasma vWF and serum IL-8 were significantly increased in all severe pulmonary contusion patients at all time points in comparison with the control group. The concentrations of plasma vWF in patients with ARDS increased during the whole study period, but vWF in patients with non-ARDS increased gradually until day 5 and then decreased at day 7. The concentration of serum IL-8 showed a similar expression pattern in both groups, but the expression increased more significantly in the ARDS group than in the non-ARDS group. Interestingly, both plasma vWF and serum IL-8 levels steadily increased in the non-survivor group. Furthermore, the level of plasma vWF was higher in the ISS≥20 group than in the ISS<20 group. The level of serum IL-8 in the ISS≥20 group was consistently high, while that in the ISS<20 group peaked at day 3 and decreased at day 5. In addition, the level of plasma vWF was positively correlated with platelet count, but negatively correlated with oxygen index. The level of serum IL-8 was positively correlated with white blood cell count and ISS score, and inversely correlated with oxygen index.

CONCLUSION: The elevated levels of plasma vWF and serum IL-8 in severe pulmonary contusion patients reflect the severity of pulmonary injury and patients outcomes, suggesting that the plasma vWF and serum IL-8 are sensitive markers for clinical evaluation of the severity of pulmonary injury and predication of patient prognosis.

KEY WORDS: Von Willebrand factor; Interleukin-8; Pulmonary contusion

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INTRODUCTION
Traffic and industrial accidents have been increasing in China due to the rapid development of transportation and industrialization, and these accidents definitely cause multiple traumas each year such as chest wall injury. Severe chest wall trauma is often complicated with severe pulmonary contusion, which is a common cause of acute respiratory distress syndrome (ARDS) and is associated with a high mortality.\textsuperscript{[1,2]} Endothelial injury and inflammation contribute to lung injury.

Von Willebrand factor (vWF) is a blood glycoprotein involving in hemostasis. vWF, a large multimeric glycoprotein present in blood plasma, is produced constitutively in the endothelium (in the Weibel-Palade bodies), megakaryocytes (α-granules of platelets), and subendothelial connective tissue.\textsuperscript{[2]} vWF is only released from endothelial cells and platelets and is an \textit{in vivo} and \textit{in vitro} marker of endothelial injury in septic patients with acute lung injury (ALI).\textsuperscript{[3]} Interleukin-8 (IL-8), as a proinflammatory mediator causing recruitment of inflammatory cells, induces an increase in oxidant stress mediators and makes it as a key parameter for localized inflammation.\textsuperscript{[4]} However, it has not been well established whether the level of serum IL-8 is associated with the severity of lung injury and whether it is a prognosis marker for severe lung contusion. In the present study, we investigated the expression of vWF and IL-8 in the first 7 days after trauma and the association of their expressions with clinical data.

METHODS
Clinical data
Altogether 63 patients, 44 males and 19 females, were admitted to the intensive care unit (ICU), First Affiliated Hospital, Suzhou University between April 2008 and May 2010. All patients met the definition of severe pulmonary contusion.\textsuperscript{[5]} The data of the patients included Acute Physiology and Chronic Health Evaluation II (APCHE II), injury severity score (ISS), age, sex, white blood cell count, platelet count, and blood sugar level. Patients were excluded if they showed any evidence of blood diseases, disseminated intravascular coagulation (DIC), history of heart and lung diseases, recent infection and low innate immunity, and admission to hospital more than 24 hours after injury. Injuries included motor vehicle crash (46 patients), high fall (11 patients), pound injury (5 patients), and coup injury (1 patient). Thirty-eight patients had single side pulmonary contusion and 25 had bilateral pulmonary contusion. Forty patients suffered from single-sided multiple rib fracture and 23 suffered from bilateral multiple rib fracture. Among the 63 patients, 47 were complicated with hemopneumothorax, 28 with flail chest, 29 with head injury, 22 with limbs, pelvis or spine fracture, 15 with abdominal injury, and 29 with shock. Twenty-eight out of the 63 patients (19 males and 9 females) met the diagnostic criteria of ARDS \textsuperscript{[6]} with a mean age of 32±12.71 years and the rest 35 (25 males and 10 females) were non-ARDS patients with a mean age of 36.34±9.29 years.

The patients were also divided into a survivor (33 males and 12 females) group with a mean age of 35.28±10.12 years and a non-survivor (11 males and 7 females) group with a mean age of 38.02±7.14 years. The ISS scores of 21 patients (16 males and 5 females) with an average age of 29.86±12.17 years were less than 20, and those of 42 patients with an average age of 34.44±11.14 years were equal to or more than 20.

In 20 healthy volunteers who served as controls, there were 12 males and 8 females with a mean age of 30.23±8.21 years. There was no significant difference in the clinical treatment of all patients.

Methods
At 1, 3, 5, and 7 days after injury, 4 mL blood was taken from the patients, and 2 mL blood was added into a tube containing anti-coagulant EDTA. The blood sample was centrifuged at 3000 r/min for 10 minutes. Plasma was collected and stored at –70 °C. The rest 2 mL blood was added into a tube without anticoagulant, and left at room temperature for 30 minutes. The sample was centrifuged at 2000 r/min for 15 minutes. Serum was taken and stored at –70 °C. Samples from healthy volunteers were processed by the same procedures. The kit for measuring vWF was purchased from the Blood Institute of Suzhou University. The kit for testing IL-8 was purchased from Genzyme (USA). Plasma vWF and serum IL-8 were measured by ELISA according to the manufacturer's instruction.

Statistical analysis
Data of vWF and IL-8 were presented as mean ± standard deviation. The difference between the groups were analyzed by two-way ANOVA or the chi-square test where appropriate using SPSS13.0 software. The correlation study was analyzed by Spearman's rank-order correlation coefficient.
RESULTS

vWF

The mean vWF level of healthy volunteers was 92.36%±22.47%. The mean vWF level of different patient groups at all time points was 1.7 to 3 fold higher than that of the healthy volunteers (P<0.05). In the ARDS group, the vWF level increased gradually, peaked at the 5th day, and maintained at the same level at the 7th day; whereas the vWF level from the non-ARDS group gradually increased until the 5th day and declined at the 7th day (Table 1). Interestingly, the vWF level in the non-survivor group showed a steady increase, while the vWF level in the survivor group increased and peaked at day 3 and declined afterward (Table 1). The vWF level from patients of the ISS score≥20 group increased and peaked at the 5th day and declined at the 7th day, whereas the vWF level from the ISS score<20 group maintained at a low level throughout the 7 days (Table 1).

Serum IL-8 level

The mean level of IL-8 expression of healthy volunteers was 43.16±7.65 pg/mL. The level in all patient groups was 1.8 to 6.6 fold higher than that of the control group. In the ARDS group, the IL-8 level increased and peaked at the 5th day and declined at the 7th day. The IL-8 expression in the non-ARDS group showed a similar pattern but with a less increased level of expression (Table 2). The IL-8 expression in the non-survivor group again maintained a steady increase like vWF, whereas the expression in the survivor group was always lower than that in the non-survivor group and declined at the 3rd day (Table 2). The IL-8 expression in the ISS score≥20 group was higher than that in the ISS score<20 group and maintained a steady increase. The difference between the two groups was significant (P<0.05) (Table 2).

Correlation analysis

The plasma vWF expression was positively correlated with platelet count (r=0.618, P<0.05) and negatively correlated with oxygen index (r=−0.628, P<0.05). There was no correlation among vWF expression, white blood cell count, blood sugar level, ISS and age. IL-8 expression was positively correlated with white blood cell count (r=0.689, P<0.05) and ISS (r=0.604, P<0.05) and negatively correlated with oxygen index (r=0.721, P<0.05). There was no correlation among IL-8 expression, platelet count, blood sugar level, and age.

DISCUSSION

Pulmonary contusion is a common pulmonary injury resulted from blunt chest wall injury and often occurs after traffic accident, high fall injury, crush injury, coup injury etc, with a mortality of 10%-25%.[7] About 12% of cases of severe pulmonary contusion caused by chest wall injury have a high mortality from 14% to 40%.[8] Pulmonary contusion is an independent risk factor for ALI and ventilator-associated pneumonia[9,10] and is also a risk factor for ALI patients with poor prognosis.[11]

Endothelial injury promotes platelet aggregation and triggers the coagulation cascade leading to micro vessels thrombosis.[12] It has been reported that endothelial injury may be a major contributor to ALI/ARDS.[13] VWF has

Table 1. VWF expression (mean±SD, %)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 5</th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-ARDS</td>
<td>28</td>
<td>178.86±31.64</td>
<td>199.47±30.44</td>
<td>226.85±36.11</td>
<td>209.27±33.70</td>
</tr>
<tr>
<td>ARDS</td>
<td>35</td>
<td>170.51±40.52</td>
<td>210.94±46.57</td>
<td>255.84±52.74*</td>
<td>260.92±46.74*</td>
</tr>
<tr>
<td>Survivor</td>
<td>45</td>
<td>162.49±35.42</td>
<td>218.27±36.19</td>
<td>265.23±42.48*</td>
<td>168.34±42.33</td>
</tr>
<tr>
<td>Non-survivor</td>
<td>18</td>
<td>215.43±25.48*</td>
<td>243.15±31.27*</td>
<td>261.29±41.23*</td>
<td>271.17±29.16*</td>
</tr>
<tr>
<td>ISS&lt;20</td>
<td>21</td>
<td>168.49±23.14*</td>
<td>192.35±29.36*</td>
<td>183.43±35.52</td>
<td>177.83±27.18</td>
</tr>
<tr>
<td>ISS≥20</td>
<td>42</td>
<td>174.36±31.21</td>
<td>243.63±38.71*</td>
<td>262.81±41.82*</td>
<td>212.91±34.83*</td>
</tr>
</tbody>
</table>

Non-ARDS vs. ARDS; survivor vs. non-survivor; and ISS<20 vs. ISS≥20. *P<0.05

Table 2. IL-8 expression (mean ± SD, pg/mL)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 5</th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-ARDS</td>
<td>28</td>
<td>92.27±27.18</td>
<td>122.36±17.23*</td>
<td>114.77±31.28</td>
<td>78.27±11.19</td>
</tr>
<tr>
<td>ARDS</td>
<td>35</td>
<td>142.31±23.43*</td>
<td>164.72±14.26*</td>
<td>217.47±20.19*</td>
<td>159.33±21.73*</td>
</tr>
<tr>
<td>Survivor</td>
<td>45</td>
<td>112.27±21.43*</td>
<td>146.14±17.21*</td>
<td>117.33±21.35</td>
<td>81.29±19.34</td>
</tr>
<tr>
<td>Non-survivor</td>
<td>18</td>
<td>221.52±18.13*</td>
<td>246.37±14.31*</td>
<td>262.14±15.05*</td>
<td>284.17±26.25*</td>
</tr>
<tr>
<td>ISS&lt;20</td>
<td>21</td>
<td>83.57±19.02*</td>
<td>141.58±19.03</td>
<td>135.12±16.72</td>
<td>89.55±12.91</td>
</tr>
<tr>
<td>ISS≥20</td>
<td>42</td>
<td>207.43±17.05*</td>
<td>213.86±21.50*</td>
<td>223.60±27.42*</td>
<td>231.88±31.02*</td>
</tr>
</tbody>
</table>

Non-ARDS vs. ARDS; survivor vs. non-survivor; and ISS<20 vs. ISS≥20. *P<0.05
been recognized as a biological marker of endothelial injury in patients both at risk and with established ALI/ARDS.\textsuperscript{[2,14]} It was also reported that vWF level was associated with prolonged mechanical ventilation and increased mortality in pediatric patients.\textsuperscript{[15]} Our results demonstrated that vWF increased continuously in severe pulmonary contusion patients with ARDS and maintained at a high level at the 7th day, whereas the vWF expression in the non-ARDS patients decreased at the 7th day. This was consistent with the finding of Ware and colleagues\textsuperscript{[2]} and supported the theory that there was constant endothelial injury in ARDS patients. Other studies also reported an association between vWF and outcomes of ARDS patients. In our study, we found a steady increase in vWF level in the non-survivor patients and ARDS patients, and this indicated that plasma vWF level predicted the severity of pulmonary injury and poor outcomes of the patients. VWF level was not correlated with ISS, indicating that vWF can't predict the degree of multiple injuries. This finding was contradictory to the result of Siemiatkowski's et al\textsuperscript{[16]} that early vWF level was associated with the severity of multiple injury with ISS>35. The reason may be that the selection of patients from our study was different from theirs and we divided patients according to ISS≥20 and ISS<20. Our study revealed a negative correlation between vWF expression and oxygen index, indicating that vWF could predict the damage of lung function and the severity of lung injury.

Mechanical injury can induce serial inflammatory reactions, which may directly or indirectly cause pulmonary injury. Pulmonary contusion activates localized and systemic innate immune mechanisms and recruits neutrophils to the injured lung. Neutrophils are involved in the pathogenesis of ALI/ARDS.\textsuperscript{[17]} Chemokines like IL-8 recruit leukocytes to the injured areas. IL-8 has been identified as the main chemotactic factor for neutrophils in lung fluids of patients with ALI/ARDS.\textsuperscript{[18]} A study\textsuperscript{[19]} reported that IL-8 level increased in the serum and bronchoalveolar lavage fluid in patients with pulmonary contusion, and that the serum IL-8 level was correlated with the severity of injury, indicating that IL-8 was involved in inflammation of pulmonary contusion. Serum IL-8 was also an independent risk factor for prediction of prognosis in ALI.\textsuperscript{[20]} In our study, the elevated level of IL-8 was higher in the severe pulmonary contusion patients with ARDS, non-survivor and ISS≥20 groups compared with the non-ARDS group, survivor group and ISS<20 group, respectively. This finding was consistent with the result of the previous study. Interestingly we found that IL-8 in the non-ARDS, survivor and ISS<20 groups peaked at the 3rd day and decreased gradually, whereas IL-8 level was not decreased in the non-survivor and ISS≥20 groups but declined in the non-ARDS group after the 7th day. Our results suggested that IL-8 was an important inflammatory mediator in pulmonary lung injury induced inflammation. IL-8 expression was persistent and associated with the severity of the disease in our study. In addition, correlation analysis demonstrated that IL-8 was positively correlated with white blood cell count and ISS, and negatively correlated with oxygen index, suggesting that IL-8 can reflect the severity of multiple injury as well as lung injury.

In conclusion, plasma vWF and serum IL-8 are useful markers for predicting the severity of lung injury and prognosis in severe pulmonary contusion patients, particularly the expression patterns during the first week of the injury. However, the cut-off points for vWF and IL-8 level in severe pulmonary contusion with poor prognosis still require multi-center large-scale studies.

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**Contributors:** Qian JX proposed and wrote the main body of the article. All authors contributed to the design and interpretation of the study and to further drafts.

**REFERENCES**


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