Relationship between disseminated intravascular coagulation and levels of plasma thrombinogen segment 1+2, D-dimer, and thrombomodulin in patients with multiple injuries

ZHÚ Yú-jūn朱渝军* and HUÁNG Xian-kái黄显凯

Objective: To explore the relationship between disseminated intravascular coagulation (DIC) and levels of plasma thrombinogen segment 1+2 (F1+2), D-dimer (D-D), and thrombomodulin (TM) in patients with severe multiple injuries.

Methods: In this study, 66 patients (49 males and 17 females, aged 15-74 years, mean=38.4 years) with multiple injuries, who were admitted to our hospital within 24 hours after injury with no personal or family history of hematopathy or coagulopathy, were divided into a minor injury group (ISS <16, n=21) and a major injury group (ISS ≥16, n=45) according to the injury severity. The patients in the major injury group were divided into a subgroup complicated with DIC (DIC subgroup, n=12) and a subgroup complicated with no DIC (non-DIC subgroup, n=33). Ten healthy people (7 males and 3 females, aged 22-61 years, mean=36.5 years±9.0 years), who received somatoscopy and diagnosed as healthy, served as the control group. Venous blood samples were collected once in the control group and 1, 3 and 7 days after trauma in the injury groups. The F1+2 and TM concentrations were determined by enzyme linked immunosorbent assay (ELISA), and D-D concentrations were measured by automated latex enhanced immunoassay.

Results: F1+2, D-D and TM levels were higher in the minor and major injury groups than in the control group. They were markedly higher in the major injury group than in the minor injury group. In the non-DIC subgroup, F1+2 levels declined gradually while D-D and TM levels declined continuously. In the DIC subgroup, F1+2 and D-D levels remained elevated while TM levels exhibited an early rise and subsequent decrease. Plasma F1+2, D-D and TM levels were higher in the DIC patients than in the non-DIC patients. Injury-induced increases in F1+2, D-D and TM plasma levels had significant positive correlation with each other at each time point.

Conclusions: Besides being related to trauma severity, F1+2, D-D and TM levels correlate closely with the occurrence of posttraumatic DIC. Therefore, changes in plasma F1+2, D-D and TM levels may predict the occurrence of DIC.

Key words: Multiple injuries; Disseminated intravascular coagulation; Thrombomodulin

Disseminated intravascular coagulation (DIC) is characterized by a widespread activation of coagulation, which results in the intravascular formation of fibrin and ultimately thrombotic occlusion of small and mid-sized vessels.1-2 DIC is associated with a large variety of clinical disorders, among which severe trauma is one of the most frequent clinical precursors. Indeed, it is a common complication of severe trauma with 50%-70% of severely injured patients presenting with this problem.3

Findings from many years of basic researches and clinical practice have provided a strong basis for understanding the pathophysiology of DIC.4-5 A variety of therapeutic measures have been developed to decrease DIC-related mortalities. However, DIC-related mortalities remain unacceptably high for approximately 24.1%-80.8%.6

Coagulation involves the activation and interaction of numerous molecules, including thrombinogen segment 1+2 (F1+2), D-dimer (D-D), and thrombomodulin (TM). An upregulation in these factors may be a harbin-
ger of impending DIC. Reliable predictive biomarkers of DIC may enable it to be prevented or treated earlier in its pathogenic course, thus reducing DIC-related mortality. Therefore, we examined whether the changes in the plasma levels of F1+2, D-D, and/or TM correlated with the incidence of DIC to explore the clinical significance of these factors in patients with severe multiple injuries in this study.

METHODS

General data of patients

The study cohort included 66 patients (49 males and 17 females, aged 15-74 years, mean=38.4 years) with multiple injuries, who were admitted to our hospital within 24 hours after injury with no personal or family history of hematopathy or coagulopathy, were divided into a minor injury group (ISS<16, n=21) and a major injury group (ISS ≥16, n=45) according to the injury severity. The general clinical data of patients are summarized in Table 1. The patients in the major injury group were divided into a subgroup complicated with DIC (DIC subgroup, n=12) and a subgroup complicated with no DIC (non-DIC subgroup, n=33). Ten healthy people (7 males and 3 females, aged 22-61 years, mean=36.5 years ±9.0 years), who received somatoscopy and diagnosed as healthy, served as the control group.

Assaying methods

Peripheral venous blood samples were collected once from the control subjects and 1, 3 and 7 days after trauma from the injured patients. The blood specimens were centrifuged at 1500 ×g for 15 minutes and then immediately stored at -80°C. Plasma F1+2 and TM concentrations were measured by Sandwich enzyme linked immunosorbent assay (ELISA, Dade-Berring, Deerfield, IL, USA and Diaclone, Besancon Cedex, France). Plasma D-D concentrations were measured by automated latex enhanced immunoassay kit (Instrumentation Laboratory Corporation, Yaphank, NY, USA).

Statistical analysis

All data were expressed as means ± standard deviation (SD) and subjected to Student's t test. Spearman’s correlation coefficients were used for correlation analysis. A P value less than 0.05 was considered statistically significant in all cases.

RESULTS

Among the 66 patients with multiple injuries, 12 developed symptoms consistent with the diagnostic criteria of DIC on the 4th day after injury. Among them, 6 patients died of respiratory and circulatory failures or multiple organ failure (MOF), and the remaining 6 patients recovered without sequelae. All 54 patients in the non-DIC subgroup recovered from their injuries (Table 1).

Changes of plasma F1+2, D-D, and TM levels in patients with multiple injuries

The plasma levels of F1+2, D-D and TM 1, 3 and 7 days after injury are summarized in Table 2. Plasma F1+2 levels were elevated markedly in both the minor and the major injury groups on the 1st, 3rd and 7th days after injury (P<0.01 compared with the healthy controls). The plasma F1+2 levels decreased gradually during the post-injury period in both groups, and the decrease was more pronounced in the minor injury group (P<0.05 in day-to-day comparisons). Plasma F1+2 levels in the major injury group were all significantly higher than those in the minor injury group (P<0.01) at every time point. Plasma F1+2 levels did not differ significantly between time points in the major injury subgroups (P>0.05).

As shown in Table 2, plasma D-D levels in the minor and major injury groups were elevated at all time points (P<0.01, compared with the healthy controls). In the minor injury group, plasma D-D levels showed a modest (non-significant) increase between day 1 and day 3 after injury and then decreased markedly from day 3 to day 7 after injury (P<0.01). In the major injury group, plasma D-D levels were not significantly different from the healthy controls on day 1, but the increase became significant on days 3 and 7 after injury (P<0.05, compared with the healthy controls). The plasma D-D levels in the major injury group were all significantly higher than those in the minor injury group at every time point (P<0.01).

Plasma TM levels were significantly higher in the minor injury group than in the control group on day 1 after injury (P<0.05, Table 2). This effect dissipated over time. On the 3rd day after injury, TM levels were similar to those in the control group. Plasma TM levels were higher in the major injury group than in the minor injury and the control group at every time point (P<0.01). Plasma TM levels in the major injury group showed a
modest increase from day 1 to day 3 and decreased markedly from day 3 to day 7 \( (P<0.01) \).

**Relationship between DIC and changes in plasma F1+2, D-D, and TM levels**

The \( r \) and \( P \) values yielded by the statistical analysis of the hypothesized relationships between the molecular data and the DIC occurrence data are presented in Table 3.

Plasma F1+2 levels in the non-DIC subgroup gradually decreased, with levels becoming significantly different from the healthy controls on days 3 and 7 \( (P<0.01) \). Meanwhile, plasma F1+2 levels in the DIC subgroup persistently rose and manifested a clear difference from the levels in non-DIC subgroup at every time point \( (P<0.01) \). Plasma F1+2 levels on days 1, 3 and 7 after injury had a positive relationship with the occurrence of DIC \( (P<0.01) \).

As shown in Table 4, plasma D-D levels in the non-DIC subgroup were consistently decreased on days 1, 3 and 7 after injury \( (P<0.01, \) compared with the healthy controls), while plasma D-D levels in the DIC subgroup were consistently increased \( (P<0.01, \) compared with the healthy controls). Plasma D-D levels differed between the two subgroups on days 3 and 7 after injury \( (P<0.01) \). Furthermore, plasma D-D levels on days 1, 3 and 7 had a positive relationship with the occurrence of DIC \( (P<0.01) \).

Plasma TM levels in the non-DIC subgroup decreased from day 1 to day 3 as well as from day 3 to day 7 after injury \( (P<0.01, \) Table 4). Meanwhile, plasma TM levels in the DIC subgroup rose from the 1st day to the 3rd day after injury \( (P<0.01) \), and then decreased sharply on day 7 \( (P<0.01, \) compared with the levels on the 3rd day). Plasma TM levels in the DIC subgroup remained significantly higher than those in the non-DIC subgroup at all three time points \( (P<0.01) \). Plasma TM levels on days 1, 3 and 7 had a positive relationship with the occurrence of DIC \( (P<0.01) \).

**DISCUSSION**

Relationship between early changes in plasma F1+2 levels and traumatic DIC

F1+2 consists of 273 amino acids and its molecular weight is \( 5.8 \times 10^{-23} \) kg. The active polypeptide segment is generated when prothrombinase complex converts prothrombin into its bioactive derivative thrombin. Hence, increased F1+2 levels are often accompanied by thrombin formation and can be used to assess thrombotic risk and monitor the efficacy of anticoagulant therapy.

The present study revealed marked increases in plasma F1+2 levels in the minor and major injury groups on days 1, 3 and 7 after injury. Higher F1+2 levels in the major injury group than the minor injury group observed at every time point indicated that patients with more severe trauma were more likely to develop excess thrombosis, which may break the balance of coagulation and fibrinolysis. Indeed, more severely the trauma is sustained, more intensely the blood coagulation can be observed. Accordingly, higher F1+2 levels with more severe injury are consistent with the supposition that F1+2 can serve as a direct marker of thrombin formation.

Three mechanisms are thought to lead to traumatic DIC.\(^8\),\(^9\) The first mechanism is known as the extrinsic pathway, that is, severe tissue injury exposes endothelial cells and monocytes to bacteria and pro-inflammatory cytokines, such as tumor necrosis factor-\( \alpha \) (TNF-\( \alpha \)) and interleukin-1 \( \beta \) (IL-1 \( \beta \)), which induces them to release tissue factor (TF). The released TF forms the FVII-TF complex, which then stimulates the coagulation cascade. The second mechanism is known as the intrinsic pathway, that is, trauma-associated ischemia, hypoxia and acidosis damage vascular endothelial cells, exposing endomembranous collagen, which activates blood coagulation factor XII. Alternatively, DIC may result from infection-associated trauma, especially Gram-negative bacterial infection. Bacterial endotoxins induce mononuclear macrophages and neutrophils to release TNF-\( \alpha \), which results in downregulation of TM expression in endothelial cells and decreases the activity of activated protein C (APC), ultimately facilitating coagulation.
Table 1. General clinical data of patients with multiple injuries

<table>
<thead>
<tr>
<th>Sites of major injury</th>
<th>Number of patients</th>
<th>Causes of injury</th>
<th>Number of DIC patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spine &amp; limbs</td>
<td>22</td>
<td>Fall from height, traffic accident</td>
<td>4</td>
</tr>
<tr>
<td>Head</td>
<td>15</td>
<td>Traffic accident, fall from height</td>
<td>2</td>
</tr>
<tr>
<td>Abdomen</td>
<td>9</td>
<td>Traffic accident, penetration wound</td>
<td>2</td>
</tr>
<tr>
<td>Chest</td>
<td>4</td>
<td>Traffic accident, penetration wound</td>
<td>1</td>
</tr>
<tr>
<td>Multiple major sites</td>
<td>16</td>
<td>Traffic accident, fall from height</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 2. F1+2, D-D, and TM concentrations after injury

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>1 day after injury</th>
<th>3 days after injury</th>
<th>7 days after injury</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F1+2 (nmol/L)</td>
<td>D-D (ng/L)</td>
<td>TM (ng/ml)</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>0.73 ± 0.42</td>
<td>177.10 ± 43.9</td>
<td>4.13 ± 1.12</td>
</tr>
<tr>
<td>Minor injury</td>
<td>21</td>
<td>1.66 ± 0.64</td>
<td>678.88 ± 375.16</td>
<td>5.01 ± 1.24</td>
</tr>
<tr>
<td>Major injury</td>
<td>45</td>
<td>2.09 ± 0.84</td>
<td>1177.23 ± 268.61</td>
<td>7.15 ± 2.48</td>
</tr>
</tbody>
</table>

Table 3. Relationship between DIC occurrence and plasma F1+2, D-D and TM levels after injury

<table>
<thead>
<tr>
<th>Values</th>
<th>1 day after injury</th>
<th>3 days after injury</th>
<th>7 days after injury</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F1+2 (nmol/L)</td>
<td>D-D (ng/L)</td>
<td>TM (ng/ml)</td>
</tr>
<tr>
<td>r</td>
<td>0.458</td>
<td>0.400</td>
<td>0.738</td>
</tr>
<tr>
<td>P</td>
<td>0</td>
<td>0.001</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 4. Post-injury changes in plasma F1+2, D-D and TM levels in non-DIC group and DIC subgroups

<table>
<thead>
<tr>
<th>Subgroups</th>
<th>1 day after injury</th>
<th>3 days after injury</th>
<th>7 days after injury</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F1+2 (nmol/L)</td>
<td>D-D (ng/L)</td>
<td>TM (ng/ml)</td>
</tr>
<tr>
<td>Non-DIC</td>
<td>1.76 ± 0.53</td>
<td>1111.34 ± 231.98</td>
<td>5.96 ± 1.77</td>
</tr>
<tr>
<td>DIC</td>
<td>2.53 ± 0.93</td>
<td>1245.52 ± 286.99</td>
<td>8.92 ± 2.32</td>
</tr>
</tbody>
</table>

\( P<0.01 \), compared with the healthy controls; \( P<0.05 \), compared with the minor injury group; \( P<0.01 \), compared with the levels 1 day after injury; \( P<0.01 \), compared with the levels 3 days after injury.

\( P<0.01 \), compared with the non-DIC subgroup; \( P<0.01 \), compared with the values on the 1st day; \( P<0.01 \), compared with the values on the 3rd day.
Increased plasma F1+2 levels in the patients with severe multiple injuries indicate that the patients are in a hypercoagulable state. In the non-DIC subgroup, we observed that the plasma F1+2 levels were declined when the patients’ conditions were alleviated and their inflammation was controlled. Meanwhile in the DIC subgroup, plasma F1+2 levels rose when the patients’ conditions were aggravated, coagulation pathology progressed, and systemic inflammation became more severe. Hence, the incipient concentration of F1+2 was closely related with the incidence of DIC, and its dynamic tendency exactly reflected the change of coagulation in the body. These observations confirmed that F1+2 was a sensitive marker of coagulation.

Relationship between traumatic DIC and changes in plasma D-D levels during an early stage of severe multiple injuries

The pro-coagulant thrombin binds to the central domain of fibrinogen and thereby releases Fibrinopeptides A and B, resulting in fibrin monomer and polymer formation. The fibrin network is subsequently stabilized (cross-linked) through its interaction with activated coagulation factor XIII. Fibrin degradation products such as D-D are released when cross-linked fibrin networks are lysed by plasmin. Thus D-D levels may reflect the fibrin degradation and change in parallel with the production of thrombin and plasmin. Plasma D-D levels have been reported to be greatly elevated in hypercoagulable states and thrombosis. And D-D is generally considered to be an important marker of fibrin production and fibrinolysis activation. Furthermore, D-D is a sensitive and specific marker of secondary hyperfibrinolysis. Takagi et al discovered that D-D plasma concentrations were elevated several days before the onset of clinical DIC. In light of such findings, D-D is considered as one of the most valuable markers in the diagnosis of DIC.

In this study, plasma D-D levels in the minor injury and major injury groups remained elevated throughout the post-traumatic study period, and the levels in the major injury group were significantly higher than those of the minor injury group at every time point. These findings suggested that all kinds of trauma patients, especially the patients with severe multiple injuries, readily developed excess thrombosis and hyperfibrinolysis, which led to abnormal coagulation. Furthermore, higher D-D levels were observed with greater trauma severity and presumably greater coagulation and fibrinolysis. After injury, plasma D-D levels decreased over time in the non-DIC subgroup but remained increased in the DIC subgroup. The enduring elevation of D-D levels in the DIC patients indicated that the DIC patients had secondary fibrinolysis and persistently aggravated coagulation. The positive relationship between our plasma D-D data on days 1, 3 and 7 after injury and the occurrence of DIC indicates that elevated plasma D-D concentration has important clinical value in terms of its ability to predict the occurrence of DIC early.

Relationship between traumatic DIC and changes in plasma TM levels at an early stage of severe multiple injuries

TM is a glycoprotein on the surface of vascular endothelial cells. It is widely dispersed in the endothelial tissues of blood and lymphatic vessels, especially in the lungs, kidney and placenta. In addition to accelerating thrombin-mediated activation of activated protein C (APC) for anticoagulation and profibrinolysis, it also inhibits thrombin activities, thereby having a direct effect on anticoagulation. Because TM is specifically distributed in vascular endothelial cells, it has been regarded as a molecular marker of vascular endothelial injury in clinical studies of microangiopathy, cerebrovascular disorders, cardiovascular diseases, diabetic nephropathy, burn and tumor.

The thrombin-TM complex activates Protein C on membrane-associated phospholipids. Thrombin binding to TM reduces the efficiency of thrombin catalysis of procoagulant reactions. The mechanisms mediating the effects of TM include disruption of fibrin formation, inhibition of the activation of Factors V and VIII, and inhibition of platelet aggregation and release. TM can also degrade the plasminogen activator inhibitor to promote fibrinolysis. TM has also been reported to reduce the activation of prothrombin when it is compounded with Factor Xa. Moreover, TM can clear away the inactivated thrombin by receptor-mediated endocytosis. Hence, overall TM can decrease thrombin’s effect through repressing the formation and activity of thrombins.

In this study, all patients with elevated plasma TM levels suffered endothelial cell injury. Furthermore, plasma TM levels were higher in the patients with major injury than those with minor injury at every time point.
These findings demonstrate that the severity of trauma is closely related with the degree of endothelium damage.

The presence of the anticoagulant factor TM is closely related to the changes of coagulation/fibrinolysis. TM levels are elevated in DIC patients because DIC pathology involves vascular endothelium damage. The observation of higher TM levels in DIC patients relative to non-DIC patients is consistent with the supposition that DIC pathology involves substantial vascular endothelium damage. Widespread and sustained endothelial cell damage produces a homeostatic imbalance and is thus accompanied by disruption of blood coagulation, which results in DIC.18-20

Plasma TM levels on days 1, 3 and 7 after injury had a positive relationship with the occurrence of DIC. Importantly, TM levels were elevated before the clinical diagnosis of DIC, which were earlier than prothrombin time, activated partial thromboplastin time and platelet abnormalities.21 Therefore, TM changes are clinically important for predicting the occurrence of DIC at the early stage.

**Significance of changes in plasma F1+2, D-D and TM levels in patients with multiple injuries**

Fibrinolysis is a protective physiological reaction to counteract coagulation through which fibrin clots are dissolved and microcirculation is recovered. However, excess fibrinolysis may increase the possibility of DIC hemorrhage and can even cause recurrent bleeding.

Vascular endothelial cell is a multifunctional cell and plays an important role in systemic protection, material transportation and non-specific immunity. It also has various regulation effects on hemorheology, vasomotion, coagulation, anti-coagulation and fibrinolysis.22 When vascular endothelium is damaged, it releases TF triggering the extrinsic coagulation pathway.

The fibrinolytic system is essential for blood homeostasis. Endothelial cells synthesize tissue-type plasminogen activator, urokinase-like plasminogen activator and their respective receptors. Endothelial cells synthesize plasminogen receptors, enabling them to bind high concentrations of plasminogens secreted from other cells. This plasminogen binding with endothelial cells is very important for maintainance of blood flow through fibrinolysis.

Coagulation, fibrinolysis and endothelial physiology are three important inter-related factors in DIC. Plasma F1+2, D-D and TM levels are elevated following trauma, and higher levels following more severe trauma. Our findings indicate that they can serve as beneficial indices for predicting DIC at an early stage, especially when assessed as a group.

**REFERENCES**


