Plasma Level of Thrombomodulin is an Early Indication of Pancreatic Necrosis in Patients with Acute Pancreatitis

Xin-Liang Lu¹, Jian-Ting Cai¹, Xing-Guo Lu², Jian-Min Si³ and Ke-Da Qian¹

Abstract

**Background** The potential to predict pancreatic necrosis within the first 48 h by using plasma soluble thrombomodulin (sTM) in 104 patients with acute pancreatitis (AP) was analyzed in a prospective 5-year investigation performed at a single institution.

**Methods** According to Balthazar CT grade, pancreatitis was classified as no necrosis in 72 patients, one-third necrotic in 18 patients, one-half necrotic in 10 patients and more than one-half necrotic in 4 patients. Blood was collected at the first 48 hours after the onset of pain and analyzed for sTM.

**Results** In the healthy volunteers, plasma levels of TM were 16.49±5.24 μg/L. By comparison, the mean plasma levels of TM in each group of pancreatitis patients were as follows: CT grade A group, 34.21±10.73 μg/L; CT grade B group, 36.18±12.50 μg/L; CT grade C group, 49.39±18.38 μg/L; CT grade D group, 114.46±39.44 μg/L; CT grade E group, 100.22±15.97 μg/L (p<0.01). And for the patients, the Pearson correlation coefficient between the CT grade and TM values was 0.784 (p<0.01). No necrosis group, 39.22±13.75 μg/L; one-third necrotic group, 71.44±18.02 μg/L; one-half necrotic group, 123.50±28.57 μg/L; more than one-half necrotic group, 129.00±33.28 μg/L (p<0.01); And for the patients, the Pearson correlation coefficient between the degree of necrosis and TM values was 0.888 (p<0.01). ROC analysis indicated the area under the ROC curve (AUC ± SE) for sTM was 0.949±0.020, clearly supportive of the high accuracy of this index in predicting the necrosis of AP.

**Conclusion** Plasma soluble thrombomodulin (sTM) is a potential marker to predict pancreatic necrosis within the first 48 h, and further investigation in a multicentre study is necessary.

**Key words:** thrombomodulin, acute pancreatitis

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**Introduction**

The most important prognostic factor in acute pancreatitis (AP) is the development of pancreatic necrosis with subsequent risk of pancreatic infection, multi-organ failure and death. After the accurate diagnosis of AP, the next challenge is to identify early those patients with pancreatic necrosis to instigate proper management. Contrast-enhanced CT currently is the most accurate single imaging modality for diagnosis, staging the severity of the inflammatory process, and detecting complications of AP. However, in the UK it is not current practice to perform early CT for the detection and staging of severe cases of AP since anxieties persist over the potential for extension of necrosis and exacerbation of renal impairment following the use of intravenous contrast media (1). Furthermore, it is not clear how soon the full extent of the necrotic process will occur, but it is at least four days after the onset of symptoms and early CT may therefore underestimate the final severity of the disease (1). As an alternative, the sequential measurement in the serum or plasma of biochemical indicators of pancreatitis severity could be applied. Of all biochemical markers available today, C-reactive protein (CRP) is the ‘gold standard’ in predicting the severity of AP (2) and it has been recommended by several organizations (1, 3). Other parameters proposed to predict the severity of AP include trypsinogen activation peptide, phospholipase A₂, polymorphonuclear (PMN) elastase,
hepatocyte growth factor, interleukin-6, and others (4). However, there is no single laboratory indicator to predict a potentially pancreatic necrosis. Soluble thrombomodulin (sTM) has been identified as a marker of poor prognosis in the critically ill. TM is a vascular endothelial, cell-surface glycoprotein that promotes activation of the anticoagulant protein C and also inhibits the procoagulant properties of thrombin (5). These antithrombogenic properties are diminished in the presence of proteases, endotoxin, or various cytokines. Elevated serum or plasma concentrations of TM are caused by shedding of membrane-bound TM from endothelial cells into the circulating blood. Increased levels of sTM in several nonpancreatic conditions have been found to be associated with inflammatory disease activity (6), sepsis (7-9), adult respiratory distress syndrome (10), multiple organ dysfunction syndrome (8, 9, 11, 12), shock (12), and lethality (11, 13). These latter conditions are also of relevance in severe AP. It was the aim of the present study to evaluate the potential of plasma sTM to identify pancreatic necrosis within the first 48 h.

**Methods**

**Study population**

All patients with AP admitted to the Department of Gastroenterology, 2nd Affiliated Hospital, Zhejiang University School of Medicine, P.R. China, from January 2001 to November 2005, were included in the primary analysis. The diagnosis was established on the basis of clinical presentation (acute onset of epigastric pain, nausea, vomiting) and findings on physical examination (epigastric tenderness, decreased bowel sounds, tachycardia, hypotension), supported by laboratory determinations (at least 3-fold elevated levels of serum pancreatic-type isoamylase). Patients with an accompanying disease that might have influenced the data were excluded from the study. The excluded group consisted of persons with postoperative, post-traumatic pancreatitis and post-endoscopic retrograde cholangiopancreatography (ERCP) pancreatitis, and those who received immunosuppressive therapy after organ transplantation. Other exclusion criteria was an admission later than 24 h after the onset of pain. Other causes of acute abdominal pain were ruled out. Demographics and the cause of the pancreatitis (cholelithiasis, alcohol abuse, other) were noted. According to the Atlanta criteria (14), pancreatitis was classified as mild acute pancreatitis (MAP) and severe acute pancreatitis (SAP). APACHE II scoring parameters were also measured at 48 hours after the onset of pain.

**Radiologic studies**

A plain abdominal x-ray was obtained for every patient to identify a possible sentinel loop, vanished psoas lines, or glandular calcifications, and to rule out pneumoperitoneum caused by a perforated peptic ulcer. All patients underwent abdominal ultrasonography within 24 h to give support to the diagnosis of AP and identify the possible presence of gallstones. Spiral CT with intravenous contrast was performed in 72 hours after the onset of pain to assess the extent of inflammation and the amount of pancreas necrosis according to Balthazar’s classification (15, 16).

**Sample collection**

All blood samples were collected at 48 hours after disease onset by using 3.8% sodium citrate at a ratio of 1 part citrate: 9 parts blood for the TM assay. Plasma was separated by centrifugation at 3000 rpm for 10 min at 4°C and stored at -80°C until assayed. Forty-nine blood samples were obtained from healthy volunteers as controls.

**ELISA for TM**

Plasma TM was quantitated with commercially available enzyme-linked immunosorbent assay (ELISA) kits (Diagnostica Stago, Asnieres, France) on an MR-5000 microplate reader (Dynatech Deutschland, Denkendorf, Germany). Measurements were performed according to the instructions of the manufacturer.

**Ethics**

Informed consent was obtained from each patient on the day of admission. The study protocol conforms to the ethical guidelines of the World Medical Association, Declaration of Helsinki-Ethical Principles for Medical Research Involving Human Subjects adopted by the 18th WMAG General Assembly, Helsinki, Finland, June 1964, as revised in Tokyo 2004, as reflected in a prior approval by the appropriate institutional review committee.

**Presentation of Data and Statistical Analysis**

Values were expressed as the mean ± SEM. Student’s t test and one-way ANOVA test were used to examine differences between patient groups when indicated. Pearson’s correlation coefficients were used for correlation analysis when indicated. We considered values of p<0.05 to be statistically significant.

**Results**

**Patients and controls**

We studied 104 patients, after obtaining the approval of our institutional review board for the study. There were 61 men and 43 women, aged 19-79 years, with a mean age of 45 years. The cause of pancreatitis was cholelithiasis in 71 patients and alcohol abuse in 16; it was classified as miscellaneous or unknown in 17. The population of healthy volunteers consisted of 29 men (age, 38±15 years; range, 20 to 77 years) and 20 women (age, 48±15 years; range, 20 to 71 years) (Table 1).

**Plasma levels of TM**

In the healthy volunteers, plasma levels of TM averaged...
16.49±5.24 μg/L, and ranged from 2.00 to 24.00 μg/L. By comparison, the plasma levels of TM in patients with AP ranged from 17.00 to 171.00 μg/L, and the mean (56.36±34.28 μg/L) was significantly higher than that of controls (p <0.01) (Fig. 1A). The mean plasma levels of TM in each group of pancreatitis patients were as follows: MAP group, 34.22±10.94 μg/L, range: 17.00 to 62.00 μg/L; SAP group, 76.85±35.82 μg/L, range: 35.00 to 171.00 μg/L (p<0.01) (Fig. 1B). CT grade A group, 34.21±10.73 μg/L, range: 22.00 to 62.00 μg/L; CT grade B group, 36.18±12.50 μg/L, range: 17.00 to 58.00 μg/L; CT grade C group, 49.39±18.38 μg/L, range: 25.00 to 103.00 μg/L; CT grade D group, 114.46±39.44 μg/L, range: 58.00 to 171.00 μg/L; CT grade E group, 100.22±15.97 μg/L, range: 83.00 to 133.00 μg/L (Fig. 1C). The mean difference of sTM in all CT grade groups was significant at the 0.01 level, and the mean difference of sTM between any two CT grade groups (except between groups A and B, between groups D and E) was significant at the 0.05 level; And for patients, the Pearson correlation coefficient between the CT grade and TM values was 0.784 (p<0.01). No necrosis group, 39.22±13.75 μg/L, range: 17.00 to 78.00 μg/L; one-third necrotic group, 71.44±18.02 μg/L, range: 46.00 to 94.00 μg/L; one-half necrotic group, 123.50±28.57 μg/L, range: 95.00 to 171.00 μg/L; more than one-half necrotic group, 129.00±33.28 μg/L, range: 98.00 to 163.00 μg/L (Fig. 1D); The mean difference of sTM in all necrotic groups was significant at the 0.01 level, and the mean difference of sTM between any two groups (except between more than one-half necrotic group and any others) was significant at the 0.01 level; And for patients, the Pearson correlation coefficient between the degree of necrosis and TM values was 0.888 (p<0.01) and between the APACHE II score and TM values was 0.634 (p<0.01). Plasma TM level overlapped to some extent between any two groups of patients examined, but an extremely high level of plasma TM (>94 μg/L) was observed only in the more than one-third necrotic groups (including one-half group and more than one-half necrotic group).

Table 1. Characteristics of Patients with Acute Pancreatitis and Controls

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Mild (MAP)</th>
<th>Severe (SAP)</th>
</tr>
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<tbody>
<tr>
<td>No. of patients</td>
<td>49</td>
<td>50</td>
<td>54</td>
</tr>
<tr>
<td>Age, yr, mean ± SD</td>
<td>42±15</td>
<td>46±13</td>
<td>45±12</td>
</tr>
<tr>
<td>Gender, male/female</td>
<td>29/20</td>
<td>33/17</td>
<td>29/26</td>
</tr>
<tr>
<td>Causes, cholestroliasis/alcohol abuse/other</td>
<td>30/12/8</td>
<td>41/4/9</td>
<td></td>
</tr>
<tr>
<td>Serum pancreatic-type isomylase, mean ± SD</td>
<td>51±23</td>
<td>1280±1416</td>
<td>1496±1396</td>
</tr>
<tr>
<td>APACHE II score at 48 hours</td>
<td>4.86±1.43</td>
<td>9.67±2.04</td>
<td></td>
</tr>
<tr>
<td>No. of patients with CT grade A/B/C/D/E</td>
<td>19/19/12/0/0</td>
<td>0/3/29/13/9</td>
<td></td>
</tr>
<tr>
<td>No. of patients with degree of necrosis (No&lt;30%/30%-50%/&gt;50%)</td>
<td>50/0/0</td>
<td>22/18/10/4</td>
<td></td>
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</tbody>
</table>

APACHE=acute physiology and chronic health evaluation.

Receiver-operating characteristics analysis

With our results of sTM levels at 48 h we performed an analysis of receiver-operating characteristics (ROC). The area under the ROC curve (AUC ± SE) for sTM was 0.949±0.020, clearly supportive of the high accuracy of this index in predicting the necrosis of AP. We observed a sensitivity of 100% and a specificity of 71% of sTM levels to predict a pancreatic necrosis at a cutoff level of 45.5 μg/L on 48h (positive predictive value, 60%; negative predictive value, 100%), and a sensitivity of 72% and a specificity of 100% of sTM levels to predict a pancreatic necrosis at a cutoff level of 78.5 μg/L at 48h (positive predictive value, 100%; negative predictive value, 89%). A sensitivity of 75% and a specificity of 99% of sTM levels to predict a pancreatic necrosis at a cutoff level of 71.5 μg/L was the best Youden’s index (Fig. 2).

Discussion

In the present study, there was a positive relationship between the sTM and pancreatic necrosis, and the higher sTM, the more pancreatic necrosis. There was also a positive relationship between the plasma levels of TM and pancreatic CT grade, and the higher sTM, the worse CT grade.

The endothelium contributes to regulation of hemostasis mainly by producing local antithrombotic substances such as TM and heparan sulfate. Inflammatory cytokines have been reported to shift the hemostatic balance of endothelial cell surfaces (7). Endotoxin and cytokines act on the vascular endothelial cells in particular under conditions of severe inflammation, giving a rationale for sTM measurement in AP too. It has been reported that patients with sepsis who developed multiorgan failure had significantly higher plasma levels of sTM than those who did not. In addition, a positive correlation between interleukin-6, PMN elastase, and plasma sTM levels has been reported (8). Plasma sTM has also been described as a marker of poor prognosis and of a severe course of disease in several nonpancreatic conditions.
Figure 1. Plasma levels of TM in each group of acute pancreatitis patients and normal controls. (A) Plasma levels of TM in patients with AP were significantly higher than those of normal controls (p < 0.01). (B) The plasma levels of TM in normal controls, in mild acute pancreatitis (MAP) and in the severe acute pancreatitis (SAP) group increased gradually (p < 0.01). (C) The mean difference of sTM in all CT grade groups was significant at the 0.01 level, and Post Hoc Multiple Comparisons analysis indicated that the mean difference of sTM between any two CT grade groups (except between groups A and B, between groups D and E) was significant at the 0.05 level. (D) The mean difference of sTM in all necrotic groups was significant at the 0.01 level, and Post Hoc Multiple Comparisons analysis indicated that the mean difference of sTM between any two groups (except between more than one-half necrotic group and any others) was significant at the 0.01 level. The Pearson correlation coefficient between the degree of necrosis and TM values was 0.888 (p<0.01).

(7, 8, 11, 12). Everything should be done to avoid stopping treatment in patients with AP and high sTM levels, because each case is individual and it is well established that early and adequate intensive care therapy is able to save lives in severe cases of this disease.

The increase in plasma sTM in patients with AP potentially reflects endothelial cell damage and dysfunction in the pancreas itself as well as in secondarily damaged organs such as the lungs, kidneys, or liver. Factors that may contribute to endothelial cell damage are hypoxia or microcirculatory abnormalities, release of proteinases from activated granulocytes, activation of mediator systems and activation of platelets (7). The increased plasma levels of sTM may also be caused by changes to TM expression on endothelial surfaces and increased shedding of membrane-bound TM. Activated proteolytic enzymes of the coagulation/fibrinolysis system may induce the release of TM as shown in patients with disseminated intravascular coagulation (7). In the case of adult respiratory distress syndrome, it is suggested that leukocyte proteases split TM from the endothelial surface (10). In addition, endotoxin and a variety of cytokines are documented stimulants for shedding of TM. Of the latter, interleukin-1 and tumor necrosis factor-α are known to damage endothelial cells through the activation of leukocytes that attack the endothelium by elastase and oxygen free radicals, resulting in the liberation of TM into the bloodstream (8).

Lack of diagnosis and emergency therapy in AP has been
Figure 2. Receiver-operating characteristics analysis (ROC) of plasma levels of TM in acute pancreatitis patients. The area under the ROC curve (AUC ± SE) for sTM was 0.949 ± 0.020.

shown in Great Britain (17). Only 19% of patients with AP underwent sufficient severity stratification within 48 hours after hospital admission. Only 67% of severe cases were treated in a high dependency unit or intensive care unit. Only 41% of patients with severe gallstone pancreatitis underwent urgent ERCP, and only 33% of the severe cases underwent a dynamic CT scan by days 3 to 10 (17). In our opinion, these data show the usefulness of a single laboratory parameter such as sTM for identifying patients with a high risk of pancreatic necrosis who could most benefit from an urgent and suitable therapy in a specialized department with prophylactic antibiotics, in cases of gallstone-linked disease from emergency endoscopic sphincterotomy, or in the future with an intervention in the complex media-

tor pathways that induce the multiorgan dysfunction. However, the use of an ELISA still limits the routine application of sTM analysis at present. One consequence of our data for the management of patients with AP and sTM levels greater than 71.5 μg/L might be the early initiation of hemofiltration irrespective of renal function. The clinical value of our data and our proposals has to be proved in more prospective multicentre studies.

References