

Elevated Plasma Tissue-type Plasminogen Activator (t-PA) and Soluble Thrombomodulin in Patients Suffering From Severe Acute Respiratory Syndrome (SARS) as a Possible Index for Prognosis and Treatment Strategy¹

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Objective To detect the presence of endothelial injury in patients with severe acute respiratory syndrome (SARS) via enhanced levels of tissue-type plasminogen activator (t-PA) and soluble thrombomodulin (sTM). **Methods** Case patients were from Xuanwu Hospital (Capital University of Medical Sciences, Beijing, China), and all of them met clinical criteria for SARS. Healthy controls were some of the hospital employees. Endothelial injury bio-markers tPA and sTM were detected by commercial ELISA-methods. **Results** Classic plasma markers of endothelial injury, tPA and sTM significantly elevated in SARS patients in comparison to controls [t-PA: 1.48±0.16 nmol/L versus 0.25±0.03 nmol/L ($P<0.0001$), and sTM: 0.26±0.06 nmol/L versus 0.14±0.02 nmol/L ($P<0.05$)]. The only patient who died had extremely high levels of these endothelial injury markers (t-PA: 2.77 nmol/L and sTM: 1.01 nmol/L). The likelihood ratio analysis indicated the excellent discriminating power for SARS at the optimal cut-point of 0.49 nmol/L for tPA and 0.20 nmol/L for sTM, respectively. Significant numerical correlations were found among these endothelial injury markers in SARS patients. The numerical coefficient of correlation Pearson r between t-PA and sTM was 0.5867 ($P<0.05$). **Conclusion** Increased plasma concentrations of tPA and sTM in patients with SARS suggest the possibility of endothelial injury. SARS patients might need anticoagulant therapy or fibrinolytic therapy in order to reverse intraalveolar coagulation, microthrombi formation, alveolar and interstitial fibrin deposition. It may not only provide a useful treatment and prognostic index but also allow a further understanding of the pathological condition of the disease.

Key words: Severe acute respiratory syndrome (SARS); Tissue-type plasminogen activator (t-PA); Soluble thrombomodulin (sTM); SARS-coronavirus; Bio-markers; Endothelial injury

INTRODUCTION

Severe acute respiratory syndrome (SARS) is a life-threatening infectious disease associated with endothelial injury, coagulation activation, and intravascular fibrin deposition. A recent study of 138 patients in Hong Kong has shown that 44.8% of the patients have thrombocytopenia, 45.0% have elevated level of D-dimers, and 42.8% have a prolonged

activated partial-thromboplastin time^[1], suggesting that SARS infection is associated with disseminated intravascular coagulation (DIC)^[2-4].

SARS patients who have elevated D-dimer levels might need anticoagulant or fibrinolytic therapy with plasminogen activators, activated protein C, soluble thrombomodulin, antithrombin, tissue factor-pathway inhibitor, activated factor VII-pathway inhibitor, heparin, or low-molecular-weight heparin in order to

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reverse intraalveolar coagulation, microthrombi formation, and alveolar and interstitial fibrin deposition^[2]. Such reversals might improve survival.

At present it is unknown why SARS-coronavirus induces activated coagulation and fibrinolysis in the infected persons. One of the possibilities is that a severe vessel wall injury is the cause of accelerated hemostasis^[5]. This study was to evaluate the possible presence of endothelial injury by determining the levels of tissue-type plasminogen activator (t-PA) and soluble-thrombomodulin (sTM) in plasma of 16 SARS patients from Beijing area of China.

Circulating t-PA is an endothelial marker released from stimulated or damaged cells^[6]. Endothelial dysfunction is defined as the loss of endothelium properties, such as alteration of protein synthesis, increased vascular tone and permeability, and acquisition of prothrombotic and antifibrinolytic properties^[6]. Thrombomodulin is a novel endothelial marker comparable with tPA, which may also reflect endothelial damage. Increased circulating soluble TM (sTM) is a marker of the prothrombotic and antifibrinolytic state associated with poor outcomes in patients with acute lung injury (ALI)/ acute respiratory distress syndrome (ARDS) and sepsis. Level of plasma sTM in ALI/ARDS patients is 2-fold higher than that of normal plasma and has not been measured in SARS patients^[7].

PATIENTS AND METHODS

Patients and Blood Samples

Sixteen patients (7 males, 9 females, the mean age was 40.5 years) from the major hospital in Beijing who met the modified WHO case definition of SARS were included in this study^[8], 20 healthy volunteer subjects (10 males, 10 females, the mean age was 43.7 years) were included in this study as controls. One of the SARS patient died.

Blood samples were collected by clean venipuncture and added into plastic tubes containing 110 mmol/L trisodium citrate (ratio 1:10). After centrifugation at 500 g for 10 minutes, platelet-poor plasma was immediately deactivated and stored at -70°C . The plasma levels of anti-SARS-associated coronavirus (SARS-CoV) specific IgG antibody were measured by kits from the Chinese Center for Disease Control and Prevention (27 Nanwei Road, Beijing 100050, China) and the Centers for Disease Control and Prevention (1600 Clifton Rd, Atlanta, GA 30333, USA).

Detection of tPA and sTM

Plasma levels of tPA and sTM were evaluated by

solid phase sandwich enzyme linked immunosorbent assay (ELISA) with from Sunbiotec, Shanghai, China and AMS Biotechnology (Europe) UK. These kits were monoclonal antibody specific for tPA or sTM and coated onto the wells of the microtitre strips provided. Samples including standards of known and unknown tPA or sTM concentrations were pipetted into these wells. During the final incubation, sTM antigen and a biotinylated monoclonal antibody specific for tPA or sTM were simultaneously incubated. After being washed, the enzyme (streptavidin-peroxidase) was added. After incubation and washing to remove the unbound enzyme, a substrate solution acting on the bound enzyme was added to induce a colored reaction product. The intensity of this coloured product was directly proportional to the concentration of tPA or sTM present in plasma samples.

Statistics

Statistical evaluation included calculation of mean values, standard error of the mean, medians, minimum and maximum values, nonparametric test for comparison of two groups and correlation analysis between experimental parameters, using Pearson's method for numerical correlation coefficient r (GraphPad, Prism and Instat, San Diego, USA).

As a final step in our study, we evaluated the prognostic power of tPA and sTM to predict evolution of SARS^[9-11]. In order to distinguish SARS patients from healthy controls, optimal cut-off points were determined by the misclassification cost term curves (MCT). The area under the curve of the receiver-operating characteristic curve (AUC), sensitivity (Se) and specificity (Sp), positive likelihood ratio (LR+), negative likelihood ratio (LR-), as well as likelihood ratios, were calculated and showed 95% confidence interval (CMDT, Freie Universität, Berlin, Germany, SPSS).

RESULTS

Classic plasma markers of endothelial injury such as tPA and sTM significantly elevated in SARS patients compared with controls [t-PA: 1.48 ± 0.16 nmol/L versus 0.25 ± 0.03 nmol/L ($P < 0.0001$) and sTM: 0.26 ± 0.06 nmol/L versus 0.14 ± 0.02 nmol/L ($P < 0.05$)] (Fig. 1). The 16 SARS patients had elevated levels of at least one of these markers associated with the activation of endothelial cells. The only patient who died had extremely high levels of these endothelial injury markers (t-PA: 2.77 nmol/L and sTM: 1.01 nmol/L).

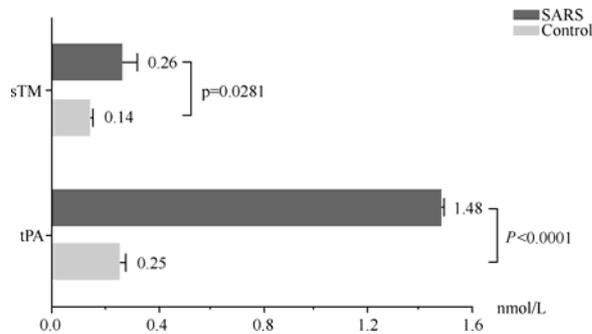


FIG. 1. Comparison of plasma sTM and t-PA in SARS patients and controls. Markers of endothelial injury, sTM and t-PA, significantly elevated in SARS patients compared with controls. The plasma concentration of sTM and t-PA in SARS patients (violet bar) and control (green bar) was expressed as $\bar{x} \pm s$.

The likelihood ratio analysis indicated the excellent discriminating power for SARS at the optimal cut-point of 0.49 nmol/L for tPA. In this cut-point, the sensitivity (Se) was 100% (95% confidence interval: 80.64% to 100%), the specificity (Sp) was 100% (95% confidence interval: 83.59% to 100%), the positive likelihood ratio (LR+) was ∞ , the negative likelihood ratio (LR-) was 0 and the area under the curve of the receiver-operating characteristic curve (AUC) was 1 (95% confidence interval: 1 to 1), indicating its excellent discriminating powers.

The optimal cut-point was 0.20 nmol/L for sTM to distinguish SARS patients from healthy controls. In this cut-point, the sensitivity (Se) was 68.75% (95% confidence interval: 44.4% to 85.84%), the specificity (Sp) was 85% (95% confidence interval: 63.96% to 94.76%), the positive likelihood ratio (LR+) was 4.583 (95% confidence interval: 1.534 to 13.691), the negative likelihood ratio (LR-) was 0.368 (95% confidence interval: 0.174 to 0.778) and the area under the curve of the receiver-operating characteristic curve (AUC) was 0.717 (95% confidence interval: 0.533 to 0.902), indicating that sTM was also able to accurately help predict evolution of SARS.

In SARS patients, a significant numerical correlation was found among these endothelial injury markers. The numerical coefficient of correlation Pearson r between t-PA and sTM was 0.5867 ($P < 0.05$) (Fig. 2).

DISCUSSION

This prospective study was conducted to determine the plasma levels of markers of endothelial injury in patients suffering from infections with SARS

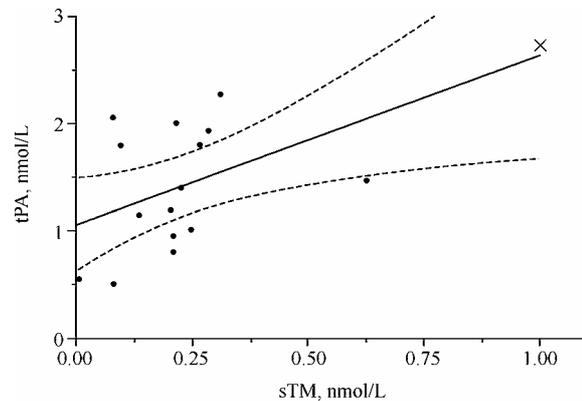


FIG. 2. Linear regression analysis for plasma t-PA and sTM concentrations (nmol/L) in SARS patients. Linear regression analysis for plasma sTM and tPA concentration (nmol/L) in SARS is shown by the solid line. The 95% confidence intervals of the regression line were calculated and shown by the dotted line. The Pearson r p -value was significant ($P < 0.05$). The patient who died had excessively elevated levels of these endothelial injury markers, and here the cross 'x' in these linear regression curves represents the experimental data from this patient.

coronaviruses. Pathological studies strongly suggest that local fibrin deposition contributes to lung or multiple organ failure and death in SARS patients with severe systemic disorders^[1,2,5,12-13]. Studies on markers of activation of coagulation and fibrinolysis strongly suggest activation of coagulation in SARS patients. Mixed thrombi present in small veins and hyaline thrombi appear in microvessels^[14]. SARS seems to be an example of DIC with intra-alveolar fibrin deposits in lungs^[15]. An important question is why coagulation and fibrinolysis are activated in these patients. A possible candidate is massive vessel wall injury. Endothelial dysfunction is an early pre-clinical manifestation of disease and is associated with increased plasma levels of tPA, thrombomodulin and von Willebrand factor (vWF), markers of endothelial cell damage/activation^[16]. They are major hemostatic regulatory molecules synthesized by endothelium. There is a relation between plasma levels of these molecules and the development of coronary heart disease as an independent risk factor^[17]. In our study, tPA and sTM levels increased and were extremely high in the patient who died, suggesting that increased tPA and sTM level can result in injury of endothelial cells.

It has been reported that an elevated t-PA plasma level is a specific marker of endothelial cell function related to the degree of pulmonary disturbance^[5]. Vasoprotective function of endothelial cells is associated with biosynthesis and release of t-PA, nitric oxide (NO), prostacyclin (PGI₂), prostaglandin

E2 (PGE₂) and carbon monoxide (CO) in patients with lung injury. These endothelial mediators calm down activated platelets and leukocytes, prevent occurrence of parietal thrombotic events, promote thrombolysis, maintain tissue perfusion and protect vascular wall against acute damage and chronic remodelling^[18]. Excessive t-PA release can lead to excessive activation of fibrinolysis, causing bleeding episodes in patients. In our experiments, t-PA was significantly elevated in patients with SARS (Fig. 1), the maximum t-PA plasma concentration was 2.77 nmol/L (patient who died).

TM as an endothelial cell surface glycoprotein can form a 1:1 complex with thrombin. In this complex, thrombin can activate protein C approximately 1000-fold more efficient than thrombin alone and does not activate coagulation factors, V and VIII, or platelets^[16,19]. Thus TM converts thrombin from a procoagulant protease to an anticoagulant and TM also allows thrombin to activate thrombin-activatable fibrinolysis inhibitor (TAFI). Activated protein C and TAFI inhibit coagulation and fibrinolysis, respectively. So TM is a linker of endogenous control of coagulation and fibrinolysis^[7].

TM is cleaved to its soluble form by neutrophil elastase and other substances produced during acute and chronic inflammatory responses, immunologic reactions and complement activation^[20]. Plasma soluble TM, cleaved products of cellular TM, also have anticoagulant and antifibrinolytic properties. The high level of sTM present in human plasma, urine and pulmonary oedema fluid appears to represent a truncated form that lacks the transmembrane and cytoplasmic domains of tissue thrombomodulin^[16]. TM plays an important role in thromboresistance. In our experiments, sTM was significantly elevated in patients with SARS, while the maximum sTM concentration was 1.01 nmol/L (patient who died). So the significant elevation of plasma sTM level in SARS patients, may reflect a vascular response in the control of both coagulation and fibrinolysis. TM is a negative-feed-back loop product to prevent thrombus formation in SARS patients. SARS patients might need anticoagulant or fibrinolytic therapy in order to reverse intraalveolar coagulation, microthrombi formation, alveolar and interstitial fibrin deposition^[2].

Plasma levels of these sensitive bio-markers of endothelial injury in SARS patients may be useful for evaluating the endothelial injury in different disease states. Firstly, this marker may provide a useful prognostic and treatment index for corticosteroid administration in serious SARS cases^[1]. Furthermore, it may facilitate research into the pathological conditions underlying SARS and provide targets for

therapy.

CONCLUSIONS

Plasma levels of tPA and sTM in SARS patients represent a useful treatment and prognostic index, possible therapy targets^[21]. Our data suggest that the elevation of circulating t-PA and sTM in plasma of SARS patients may be related to damage of endothelial tissue. Although our experimental population was smaller, increase of t-PA may reflect active attempts of regeneration and repair, indicating that endothelial cell viability rather than damage is a matter of speculation.

TM binds to thrombin, changes thrombin conformation, and allows thrombin to activate protein C and thrombin-activatable fibrinolysis inhibitor (TAFI). Activated protein C and TAFI can inhibit coagulation and fibrinolysis, respectively. Therefore the significant elevation of the level of plasma TM, may play an important role in endogenous control of both coagulation and fibrinolysis.

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