Dear Sir,

Severe acute respiratory syndrome (SARS) disease has been shown to be associated with changes in coagulation related to both pulmonary and extra-pulmonary manifestations (1). We previously investigated inflammation-dependent coagulation responses in SARS-patients in Hong Kong (2). In particular, 44.8% of these patients presented with thrombocytopenia, 45.0% had elevated levels of D-dimers, and 42.8% had a prolonged activated partial-thromboplastin time. This combination of pathological parameters suggests the presence of a form of disseminated intravascular coagulation (DIC) or unusually disseminated small vessel thrombosis in the lungs with consumption of platelets and clotting factors (2). Post-mortem examinations indicated the presence of vascular fibrin thrombi, pulmonary alveoli capillary microthromboses or thromboembolic bronchial arterioles (3).

In this study, we examined the profile of the inflammation-dependent coagulation response and measured the levels of circulating markers of coagulation, including tissue factor (TF), von Willebrand factor (vWF), plasminogen activator inhibitor-1 (PAI-1) and vitronectin (VN), which serves as a PAI-1 cofactor for rapid inhibition of activated protein C (4).

All sixteen consecutive patients with SARS (seven male; nine female; mean age 40.5 years), who were newly hospitalized and diagnosed with the modified WHO definition of SARS (5) in the medical intensive care unit of the Xuanwu Hospital of the Capital University of Medical Sciences, China between April and June 2003, were enrolled in the study. The samples were collected immediately after diagnosis of the disease. One SARS-infected patient died. The clinical findings and laboratory data were documented.

Nineteen patients with other etiologies of community-acquired pneumonia (Streptococcus pneumonia) (15 male; four female; mean age 50.5 years) presented with new and persistent initial radiographic manifestations (initial abnormal chest radiograph and abnormal CT scan, bilateral involvement, unilateral involvement or lower-zone involvement were mentioned) also associated with at least one of the following: purulent tracheal secretions, a body temperature of at least 38.3°C, leukocytosis (> 10,000 leukocytes/µl) or leukopenia (< 4000 leukocytes/µl). These patients had no history of contact with SARS patients.

Presence or absence of SARS-coronavirus infection was confirmed by testing for anti-SARS-coronavirus specific IgG. Nasopharyngeal-aspirate, or mini-bronchoalveolar lavage, sampling and processing of microbiologic specimens were performed to confirm the diagnosis of S. pneumonia. The clinical presentation and the severity of the lung injury of SARS resemble that of the other etiologies of community-acquired pneumonia, as reported (6). Demographic factors such as age, sex, nursing home residence, coexisting illnesses, findings on physical examination, altered mental status, respiratory rate, systolic blood pressure, temperature pulse and laboratory and radiographic findings were recorded. Here, 6.25% of SARS patients developed acute respiratory distress syndrome (ARDS) as compared to 5.3% in patients with infectious pneumonias of other etiologies.

Twenty healthy volunteer controls (10 male; 10 female; mean age 43.7 years) recruited from hospital employees or their family members were included in this study. Study participants did not have major chronic medical illnesses and were not taking any medication known to influence coagulation. No clinically significant abnormalities were found during physical examination; participants were not anaemic and had normal liver and kidney functions. They did not have a history of contact with SARS patients. The absence of SARS-coronavirus infection was confirmed by test of anti-SARS-coronavirus specific IgG.

Plasma levels of the procoagulants TF, vWF, PAI-1 and VN were determined using commercially available enzyme-linked immunosorbent assay (ELISA) kits from American Diagnostica (USA), DAKO (UK), Sunbiotec (China) and Technoclone (Australia). Non-parametric ANOVA (Kruskal-Wallis test) followed by Dunn’s Multiple Comparisons Test were performed to compare statistically significant differences. After ANOVA, receiver-operating characteristic curve analysis was used to calculate the test characteristics for distinguishing the SARS patients from patients with the infectious pneumonias of other etiologies. Optimal cut-off points were identified and analyzed for predictive power by the misclassifications costs term curves (7, 8). The area under the curve (AUC) of the receiver-operating characteristic, sensitivity and specificity, positive likelihood ratio (LR+),

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**Analysis of thrombotic factors in severe acute respiratory syndrome (SARS) patients**

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negative likelihood ratio (LR-), as well as their ratios, were calculated, and showed 95% confidence interval (CI) (CMDT, Freie Universität, Berlin, Germany; MedCalc, http://www.medcalc. be; and SPSS, Chicago, IL, USA).

The blood plasma levels of vWF and VN in the SARS patients and patients with infectious pneumonias of other etiologies were both significantly elevated compared to those found in healthy controls. The blood plasma levels of TF did not differ significantly (Fig. 1). The elevation in PAI-1 levels in patients with SARS was significant not only compared to the controls but also to patients with other pneumonias, and the estimated AUC of the receiver-operating characteristic value was 0.987 (95% CI: 0.875 – 0.990) at the optimal cut-off point of 222 ng/ml, indicative for high-test accuracy (9). The PAI-1 levels did not differ significantly between the patients with infectious pneumonias of other etiologies and the controls.

Our findings were consistent with the PAI-1 gene transcription experiments by microarray analysis (10). The PAI-1 mRNA and protein levels in the human hepatoma cell line HuH7 infected with SARS-coronavirus are much higher as compared to infection with human Coronavirus 229E (which is associated with the common cold), as evidenced by transcriptome experiments, qRT-PCR and ELISA. The plasma levels of PAI-1 were significantly higher in patients who showed less activation of secondary fibrinolysis, which might explain the occurrence of organ failure and poor outcome in DIC patients (11).

Further investigations are needed to understand the exorbitant increase of VN in the infectious pneumonia patients; to our knowledge, these are the highest VN values observed in blood plasma of patients ever. In the presence of VN, PAI-1 displays a 200-fold accelerated thrombin inhibition and may compete with urokinase-receptor dependent or integrin-dependent binding of cells to the extracellular matrix (12). Multimeric VN (derived from platelets) also supports thrombus formation and stabilization after vascular injury (13–15). It remains to be established whether these dramatically elevated VN levels originate from expression in the liver (the main organ of VN production) or from lung damage, and whether they correlate with thrombotic complications in SARS patients. Our results should stimulate further attempts to include coagulation parameter testing into research and diagnosis on SARS. Besides antiviral treatment, modifiers of coagulation should be considered (2, 10).

References

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Figure 1: Blood plasma levels of thrombotic factors in SARS patients. Individual values of the indicated parameters are shown for the healthy controls ( ), the infectious pneumonias of other etiologies ( ), or the pneumonias of the SARS patients ( ). Bars represent the means of each group; note the differences in scale for the individual factors. A non-parametric ANOVA ( Kruskal-Wallis test) and Dunn’s post test was performed to compare the differences. All reported P values are calculated based on two-sided tests. The analyses were performed with Graph Pad Instat software, version 4.00 (San Diego, CA, USA). Blood plasma levels of vWF and VN in SARS patients were significantly elevated compared with those found in healthy controls (157 ± 31 nM vs. 50 ± 5 nM; P<0.001 and 1,538 ± 364 mg/l vs. 310 ± 35 mg/l; P<0.0001). Blood plasma levels of vWF and VN in infectious pneumonias of patients with other etiologies were also significantly elevated compared with those found in healthy controls (107 ± 16 nM vs. 50 ± 5 nM; P<0.01 and 1,544 ± 301 mg/l vs. 310 ± 35 mg/l; P<0.0001). Blood plasma levels of TF did not differ significantly among SARS, infectious pneumonia of other etiologies and controls (221 ± 31 mg/ml vs. 181 ± 15 mg/ml vs. 145 ± 15 mg/ml). Blood plasma levels of PAI-1 in SARS patients were significantly higher (355 ± 16 mg/ml) as compared to both patients with infectious pneumonias of other etiologies (88 ± 17 mg/ml; P<0.001) or healthy controls (61 ± 2 mg/ml; P<0.001).