Association of the Serum Angiotensin II Level with Disease Severity in Severe Fever with Thrombocytopenia Syndrome Patients

Jiamei Cheng¹, Huiyu Li² and Shenghua Jie¹

Abstract

Objective  Severe fever with thrombocytopenia syndrome (SFTS) is an emerging infectious disease caused by a novel Bunyavirus. Recent data suggest that the physiological balance of multiple proinflammatory cytokines is substantially changed in cases of severe fever with thrombocytopenia syndrome virus (SFTSV) infection, and the inflammatory response probably plays an important role in disease progression. Angiotensin II is an important active substance of the renin-angiotensin system, and studies have demonstrated that angiotensin II is involved in key events in the inflammatory process and can regulate inflammatory cell responses.

Methods  In order to elucidate the role of angiotensin II in the pathogenesis of SFTS, we collected serum samples from SFTS patients in the acute or convalescent phase and tested the angiotensin II levels using an enzyme-linked immunosorbent assay as well as SFTSV viral RNA with real-time reverse-transcriptase polymerase chain reaction. Furthermore, we explored possible correlations between the angiotensin II levels and clinical parameters in SFTS patients.

Results  Our data showed that the serum level of angiotensin II was significantly increased in the acute phase compared with that seen in the convalescent phase and the healthy controls, while there were no significant differences between the convalescent cases and healthy controls (p>0.05). A correlation analysis demonstrated that the level of angiotensin II positively correlated with the SFTS viral RNA load. The angiotensin II levels were also found to be correlated with clinical parameters indicating impairments in organ functions. Moreover, we also found that the angiotensin II levels were significantly increased in the severe cases versus the non-severe cases (p<0.001).

Conclusion  The serum angiotensin II levels in SFTS patients may be used to stratify the disease severity and are possibly predictive of disease outcomes.

Key words: angiotensin II, SFTS

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Introduction

Severe fever with thrombocytopenia syndrome (SFTS) is a hemorrhagic fever-like illnesses caused by the severe fever with thrombocytopenia syndrome virus (SFTSV), a member of the Bunyaviridae family and newly identified pathogen in central and northeast China (1). SFTS is an acute illness with a clinical presentation consisting of an abrupt fever, thrombocytopenia, leukocytopenia, gastrointestinal symptoms, neural disorders, proteinuria, hematuria and a bleeding tendency, with a reported fatality rate varying between 12% and 30%. In critically ill SFTS patients, the clinical condition may progress quite rapidly and end in multiorgan dysfunction and disseminated intravascular coagulation (2). The exact mechanisms underlying the pathogenesis of SFTSV

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remain unclear; however, it is generally suspected that immunopathology plays a key role (2, 3). As with other viral infections, immune activation and exaggerated cytokine production in the form of cytokine storm can potentially drive the SFTS disease process. Several studies have reported that SFTSV infection may lead to elevated levels of serum cytokines, which might contribute to disease severity and clinical outcomes (4).

Angiotensin II is an important active substance of the renin-angiotensin system (RAS). Angiotensin II combined with angiotensin receptor (ATR) plays various biological roles, including promoting the contraction of blood vessels, increasing blood pressure, stimulating the release of catecholamines at nerve endings, promoting cell growth, controlling hormone regulation and so on (5, 6). In monocytes, macrophages, vascular smooth muscle cells and endothelial cells, angiotensin II activates nuclear factor κB (NF-κB), which induces the production of chemokines, such as monocyte chemotactic protein-1 (MCP-1), IP-10, interleukin 6 and interleukin 8 (7, 8). These chemokines stimulate leukocytes to migrate into the vessel wall by activating signaling pathways through membrane receptors (9), as well as regulating the recruitment and activation of inflammatory cells, including monocytes/macrophages and T lymphocytes. It has also been reported that angiotensin II has significant proinflammatory functions in the vascular wall, including the production of reactive oxygen species, inflammatory cytokines and adhesion molecules and the activation of redox-sensitive inflammatory genes (10, 11). Furthermore, angiotensin II enhances the adhesion of monocytes and neutrophils to endothelial cells and mesangial cells, as well as increases leukocyte rolling flux, adhesion and migration without a vasoconstrictor activity (12).

Clinical studies have indicated that cytokine storm, characterized by the production of certain cytokines at high concentrations, is associated with the severe forms of several viral infections, including viral hemorrhagic fevers (13-16). Angiotensin II stimulation can arouse responses from circulating leukocytes, lymphocytes and macrophages by producing cytokines, chemokines and other proinflammatory mediators. These proinflammatory responses are associated with activation of the adaptive immune system. Studies of avian influenza virus infections have shown that the angiotensin II levels in human blood are associated with disease severity and may potentially predict patient mortality (17, 18). In the current study, we investigated the levels of serum angiotensin II and explored their correlations with clinical parameters in SFTS patients.

Materials and Methods

Patients and clinical samples

The present study involved 47 admitted SFTS patients (age: 18-73 years) treated at the Department of Infectious Diseases of Union Hospital, Tongji Medical College, Huazhong University of Science and Technology between May 2014 and September 2014. All patients were confirmed to be positive for SFTSV infection using reverse-transcriptase polymerase chain reaction (RT-PCR) on admission. The 47 confirmed SFTS cases included complete medical records of laboratory tests, including data for white blood cells (WBC), neutrophil percentage (NE%), lymphocyte percentage (LY%), platelets (PLTs), albumin (ALB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine kinase (CK) and lactate dehydrogenase (LDH). In addition, serum samples from 10 healthy volunteers (who did not suffer from any diseases or took any medications in recent weeks) were analyzed as healthy controls, and the SFTS patients were divided into different groups based on the presence of consistent clinical features. Serum samples were collected in the acute phase at around 6-9 days after the onset of illness and in the convalescent phase ranging from 14 to 60 days after illness onset.

Real-time RT-PCR for the SFTS viral RNA assay

Serum samples were collected from all patients on admission as well as in the acute and convalescent phases. SFTS viral RNA was extracted from the collected serum samples using the QIAamp Viral RNA kit (Qiagen, Hilden, Germany) following the manufacturer’s instructions. Viral detection was further performed using a certified clinical SFTS real-time PCR diagnosis kit from the Center for Disease Control of Wuhan (SFDA Registration No. 340166, China) based on the detection of the SFTS viral S genomic segment with specific primers and probes. The cut-off cycle threshold value for a positive sample was set at 35 cycles (19). The SFTSV-specific immunoglobulin M (IgM) and immunoglobulin G (IgG) levels in the sera were detected using an enzyme-linked immunosorbent assay (ELISA), as previously described (1).

Enzyme-linked immunosorbent assay (ELISA) for serum angiotensin II

The angiotensin II level was measured using a commercially available kit (Angiotensin II Human ELISA kit, ab108796, Abcam, USA) according to the manufacturer’s protocol. Each sample was assayed in duplicate along with a standard provided in the kit to generate a standard curve used to determine the unknown amount of targets. The standard curve was calculated using four-parameter polynomial regression employing the standard provided in the ELISA kits, and the concentrations of angiotensin II in the experimental samples were calculated based on the curve.

Statistical analysis

All analyses were performed using the SPSS 16.0 statistical software package. The Mann-Whitney U nonparametric test or Student’s t-test, where appropriate, was used for assessments of numerical data, and the results were two-tailed. The correlations between clinical parameters and the angiotensin II levels were assessed using the Pearson test. A p
Table. Comparison of Demographic and Clinical Characteristics in Different Stages of SFTSV Infection.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Acute phase (n = 33)</th>
<th>Convalescent phase (n = 14)</th>
<th>All patients (n=47)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographic features</strong></td>
<td></td>
<td></td>
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<tr>
<td>Male gender/No. (%)</td>
<td>12 (36.4)</td>
<td>8 (57.1)</td>
<td>20 (42.6)</td>
</tr>
<tr>
<td>Age/y, mean ± SD</td>
<td>53.2 ± 13.4</td>
<td>49.7 ± 15.1</td>
<td>51.9 ± 14</td>
</tr>
<tr>
<td><strong>Clinical parameters</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Viral load (copies/mL)</td>
<td>2.54×10⁶</td>
<td>3.06×10⁴</td>
<td>9.37×10⁵</td>
</tr>
<tr>
<td>WBC count (× 10⁹/L)</td>
<td>4.3 ± 2.9</td>
<td>6.3 ± 3.5</td>
<td>5.0 ± 3.3</td>
</tr>
<tr>
<td>PLT count (× 10⁹/L)</td>
<td>55.8 ± 47.3</td>
<td>243.9 ± 107.9</td>
<td>127.9 ± 119.2</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>68.2 ± 13.2</td>
<td>57.2 ± 12.2</td>
<td>63.7 ± 13.8</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>22.8 ± 10.9</td>
<td>30.3 ± 9.9</td>
<td>25.8 ± 11.0</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>258 ± 208.3</td>
<td>65.1 ± 53.5</td>
<td>173.3 ± 185.7</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>101.7 ± 63.3</td>
<td>78.9 ± 44.6</td>
<td>91.7 ± 56.4</td>
</tr>
<tr>
<td>LDH (U/L)</td>
<td>849.6 ± 587.4</td>
<td>359.4 ± 136.9</td>
<td>664.1 ± 525.1</td>
</tr>
<tr>
<td>CK (U/L)</td>
<td>762.8 ± 1080.4</td>
<td>109 ± 93.2</td>
<td>520 ± 909.3</td>
</tr>
<tr>
<td>ALB (g/L)</td>
<td>31.3 ± 5.2</td>
<td>32.6 ± 4.0</td>
<td>32 ± 3.8</td>
</tr>
</tbody>
</table>

Data are No. (%) of patients, mean value ± standard deviation, or median (range).

ALB: albumin (Normal range, 35-50 g/L), ALT: alanine transaminase (Normal range, 0-40 U/L), AST: aspartate aminotransferase (Normal range, 0-40 U/L), CK: creatine kinase (Normal range, 25-200 U/L), LDH: lactate dehydrogenase (Normal range, 109-245 U/L), Lymphocytes (%) (Normal range, 20-40), Neutrophils (%) (Normal range, 50-70), PLT: platelet (Normal range, 100-300 × 10⁹/L), WBC: white blood cell (Normal range, 4-10 × 10⁹/L).

A value of <0.05 was considered to be statistically significant.

**Results**

**Clinical laboratory parameters in the SFTS patients**

We recorded the clinical course and laboratory data for the SFTS patients prospectively. Forty-seven SFTS patients were admitted from hilly or mountainous areas of Hubei and Henan province, who described a history of field exposure within two weeks of illness onset. The mean age was 51.9 years (range, 18-73 years), and 20 (42.6%) subjects were men. The incidence was slightly higher in women than in men; however, there were no differences in sex. Among the 47 SFTS patients, 33 cases (70%) were in the acute phase, including 12 men and 21 women, with an average age of 53.2 years. Upon hospital admission, abnormal laboratory parameters included decreased WBC and PLT values and increased ALT, AST, LDH and CK values, as observed in the acute cases (Table). In comparison with that observed in the SFTS patients in the convalescent phase, the levels of WBC and PLT were significantly lower in the acute phase (p<0.05) and the ALT, AST, CK and LDH levels were significantly higher in the acute phase (p<0.05), whereas the levels of NE, LY and ALB did not show any statistically significant differences (p>0.05).

**Serum concentrations of angiotensin II in the SFTS patients**

The mean serum level of angiotensin II was 250.30±71.45 pg/mL in the acute phase, 181.52±106.30 pg/mL in the convalescent phase and 181.01±33.81 pg/mL in the control group. Compared with that seen in the convalescent phase and healthy controls, the levels of angiotensin II were significantly increased in the acute phase (p<0.01), although the there were no significant differences between the convalescent phase and the healthy controls (p>0.05) (Fig. 1a). Moreover, we analyzed the serum levels of angiotensin II in severe cases and non-severe cases, and the results showed that the angiotensin II levels were significantly increased in the severe cases versus the non-severe cases (p<0.001): the mean serum levels of angiotensin II in the severe and non-severe cases were 294.76±58.51 pg/mL and 173.53±40.97 pg/mL, respectively (Fig. 1b).

**Correlations between the angiotensin II levels and laboratory parameters in the patients with SFTS**

We assayed the SFTSV viral RNA load in the circulating blood of the SFTS patients using real-time RT-PCR. The SFTSV viral RNA load in the circulating blood of SFTS patients ranged from 0.51×10² to 7.7×10⁶ copies/mL, and the viral load was significantly higher in the acute phase (average RNA viral load: 2.54×10⁶ copies/mL) than in the convalescent phase (average RNA viral load: 3.06×10³ copies/mL) (p<0.0001, Table, Fig. 3). We next analyzed the potential
Figure 1. Comparison of the serum concentrations of angiotensin between the severe fever with thrombocytopenia syndrome (SFTS) cases and healthy controls. a. Comparison of the serum concentrations of angiotensin among the acute phase cases, convalescent phase cases and healthy controls. b. Comparison of the serum concentrations of angiotensin among the severe cases, non-severe cases and healthy controls. Data are expressed as the mean with SEM. *p<0.05, **p<0.005.

Figure 2. Relationships between the serum concentrations of angiotensin II and laboratory parameters in the SFTS patients. (a) Relationship between the serum concentration of angiotensin II and peripheral blood WBC count. (b) Relationship between the serum concentration of angiotensin II and platelet (PLT) count.

Figure 3. Viral loads in the acute and convalescent phases of severe fever with thrombocytopenia syndrome (SFTS). Statistical significance (p<0.001) for the viral load between the cases of acute SFTS and convalescent SFTS is indicated.

Figure 4. Relationship between the serum angiotensin II level and SFTS viral load.

correlation between the serum angiotensin II levels and the viral load. The Pearson correlation analysis showed that the serum angiotensin II levels were positively correlated with the SFTSV viral RNA load during the acute phase (r=0.77, p<0.001) (Fig. 4), but not the convalescent phase.
Association of the angiotensin II levels with disease outcomes

To investigate whether changes in the angiotensin II levels among SFTSV-infected patients are associated with disease progression, we analyzed the angiotensin II levels in 14 SFTS patients from whom serum samples were collected serially in both the acute and convalescent phases. Our results showed that the serum angiotensin II levels were decreased in the convalescent phase compared with that seen in the acute phase (Fig. 5). Moreover, the higher the serum angiotensin II levels, the more severe the clinical symptoms, and two patients with very high serum angiotensin II levels died of MODS.

Discussion

The inflammatory response is involved in the progression of SFTS. Angiotensin II recruits inflammatory cells into tissues through the regulation of adhesion molecules and chemokines by resident cells (20) and possibly influences the balance of multiple inflammatory cytokines in cases of SFTS infection. In the current study, we found that the levels of angiotensin II were increased in the acute phase, which may lead to changes in the balance of multiple inflammatory cytokines in patients with acute SFTS infection. Angiotensin II may induce an immune response to viral infection by activating many inflammatory cytokines. However, when the disease progressed to the convalescent phase, the levels of angiotensin II decreased to near normal levels, which may be due to the inflammation subsiding.

On the other hand, inflammatory cells express all components of the RAS and may produce angiotensin II. One of the early responses to inflammation is upregulation of the angiotensinogen levels. During the differentiation process from monocytes to macrophages, there is activation of the RAS and re-expression of the angiotensin II type 2 receptor (AT2). Moreover, the monocytes/macrophages present at inflammatory sites have a high angiotensin-converting enzyme (ACE) activity. These data show that angiotensin II activates inflammatory cells, which in turn can activate the RAS and increase local angiotensin II generation, therefore contributing to the progression and perpetuation of inflammation (20).

The onset of cytokine storm in the acute phase of the disease has been widely hypothesized to be the main cause of morbidity and mortality for several viral infections (21). The physiological balance of multiple proinflammatory cytokines is substantially changed in SFTS patients during the acute phase of viral infection. However, most of these cytokines return to the physiological range during the recovery phase (4). In several clinical studies of hemorrhagic fever diseases, cytokine storm has been shown to be associated with disease severity. In cases of acute viral infection, a panel of cytokines, including IL-6, IL-8, IL-10, MCP-1, MIP-1α, MIP-1β, IP-10, RANTES, IFN-γ, and TNF-α, were reported to correlate with severe disease in patients with dengue fever (22). Angiotensin II may elicit cellular responses by activating many cytokines, while our results showed that the levels of angiotensin II are increased in the acute phase, which may indicate that angiotensin II promotes the immune response in order to resist SFTS viral infection.

The serum viral load is known to be highly correlated with the production of angiotensin II in SFTS patients, and the angiotensin II levels have been further found to be correlated with clinical parameters indicating impairments in organ functions. These observations suggest that the abnormal production of angiotensin II during the acute phase of SFTSV infection may be induced by active viral replication, although further studies are needed to verify this speculation. When the initial immune response is unable to restrain viral replication, the virus may induce the release of overwhelming amounts of angiotensin II, thus leading to the response of inflammatory cells and cytokines and development of pathological lesions.

It has been demonstrated that the SFTS viral load is correlated with the clinical outcome of SFTS patients (23), and, in this study, we found that the viral load correlates with the production of angiotensin II. Hence, angiotensin II may be a marker for the outcome of SFTS. Moreover, previous studies have shown that RAS has important roles in the onset of cardiovascular disease, neurodegenerative disorders and acute lung injury (24, 25). Angiotensin II involved in the RAS has various functions in multiple organs and is indeed a likely biomarker that can be used to predict fatal outcomes in lethal diseases. In critically ill SFTS patients, the clinical symptoms usually become exacerbated during the acute phase of the disease and quickly proceed to multiple organ dysfunction syndrome (MODS) and disseminated intravascular coagulation (DIC), ending in death around 1-2 weeks after the onset of the disease (26).

Written informed consent was obtained from all participating subjects. This study was performed according to the guidelines of Huazhong University of Science and Technology, which abide
by the Helsinki Declaration on ethical principles for medical research involving human subjects.

The authors state that they have no Conflict of Interest (COI).

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