High Serum Procalcitonin Concentrations in Patients With Hemorrhagic Fever With Renal Syndrome Caused by Hantaan Virus

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Objective: This study analyzed the significance of procalcitonin (PCT) in patients with hemorrhagic fever with renal syndrome (HFRS) caused by Hantaan virus.

Methods: The demographics and clinical and laboratory data including PCT at hospital admission in 146 adults with HFRS were retrospectively analyzed.

Results: PCT level was significantly higher in severe patients ($n = 72$) than in mild patients ($n = 74$, $p < 0.001$) and independently associated with disease severity (OR 2.544, 95% CI 1.330–4.868, $p = 0.005$). PCT had an area under the receiver operating characteristic curve (AUC) value of 0.738 (95% CI 0.657–0.820, $p < 0.001$) for predicting severity. PCT level was significantly increased in patients with bacterial infection ($n = 87$) compared with those without ($n=59$, $p = 0.037$) and associated with bacterial infection (OR 1.685, 95% CI 1.026–2.768, $p = 0.039$). The AUC value of PCT for predicting bacterial infection was 0.618 (95% CI 0.524–0.711, $p = 0.016$). PCT level was significantly elevated in non-survivors ($n = 13$) compared with survivors ($n = 133$, $p < 0.001$) and independently associated with mortality (OR 1.075, 95% CI 1.003–1.152, $p = 0.041$). The AUC value of PCT for predicting mortality was 0.819 (95% CI 0.724–0.914, $p < 0.001$).

Conclusion: PCT concentrations at admission would be predictive of disease severity, secondary bacterial infection and mortality in patients with HFRS caused by Hantaan virus.

Keywords: hemorrhagic fever with renal syndrome, Hantaan virus, procalcitonin, disease severity, prognosis

INTRODUCTION

Hantaviruses, a group of enveloped single-stranded negative RNA viruses of the genus orthohantavirus in Hantaviridae family under Bunyavirales Order (Schmaljohn and Dalrymple, 1983; Schmaljohn et al., 1985; Adams et al., 2017), can cause hemorrhagic fever with renal syndrome (HFRS) primarily in Eurasia, and hantavirus cardiopulmonary syndrome in the Americas (Lee et al., 1978; Peters et al., 1999; Avsic-Zupanc et al., 2015). There are at least four HFRS-causing hantaviruses: Hantaan, Seoul, Puumala, and Dobrava viruses (Schmaljohn and Hjelle, 1997; Hart and Bennett, 1999). Clinically, typical HFRS occurs in five consecutive phases: febrile, hypotensive, oliguric, polyuric and convalescent (Peters et al., 1999; Vaheri et al., 2013). Pathophysiologically, endothelial dysfunction and increased vascular permeability are the
Procalcitonin (PCT), a precursor hormone of calcitonin, may be secreted by different cells in multiple organs when the body is stimulated by an inflammatory response, especially bacterial infection (Nishikura, 1999; Linscheid et al., 2003). Many studies have shown that PCT has an excellent predictive ability for sepsis (Arora et al., 2017; Nishikawa et al., 2017) and increased PCT is indicative of a risk of bacteremia in patients with acute fever (Kim et al., 2011). Notably, in hantavirus infections, an increased serum PCT level has been found in patients with acute nephropathia epidemica (NE) caused by Puumala virus (Jereb et al., 2011) although no association between PCT levels and severity of disease was observed (Latus et al., 2015a). PCT determined on admission to the hospital was also not shown to be able to predict the severity of acute kidney injury (AKI) in NE (Bunz et al., 2015). Differentially, patients with HFRS caused by Dobrava virus appeared to have a higher PCT level than those with Puumala virus infections (Jereb et al., 2011). However, few studies have investigated the usefulness of PCT for predicting the disease severity and prognosis in patients with the severe form HFRS caused by Hantaan virus. Therefore, the aim of this study was to analyse PCT levels in HFRS patients from Shaanxi, one of the high-endemic areas of the severe form HFSR caused by Hantaan virus in northwest China. (Huang et al., 2012; Ma et al., 2014; Tian et al., 2017).

**METHODS**

**Study Population**

Data from HFRS patients admitted from January, 2011 to December, 2016 in the First Affiliated Hospital of Xi’an Jiaotong University, a large tertiary-care hospital located in Xi’an, Shaanxi, northwest China, were retrospectively collected. Patients who are pregnant or younger than 18 years, diagnosed with AKI other than HFRS, chronic kidney disease, liver diseases, cancer or hemopathy and used anticoagulants prior to admission to hospital were all excluded from the study. The data from 146 adult HFRS patients were included in the analysis of the study. The disease severity in the patients was classified into mild, medium, severe and gravis clinical types according to the diagnostic criteria from the Prevention and Treatment Strategy of HFRS by the Ministry of Health, People’s Republic of China as described elsewhere (Liu et al., 2008). Clinically, 35 patients were classified as mild type, 37 medium type, 22 severe type, and 52 gravis type.

This retrospective study was conducted in accordance with the Declaration of Helsinki and the protocol was approved by the Ethics Committee of the First Affiliated Hospital of Xi’an Jiaotong University. Informed consent was not obtained from the patients as the patient records and information were anonymized and de-identified prior to the study.

**Determination of Procalcitonin, WBC and Platelet**

PCT was measured using VIDAS® Boehringer Mannheim® PCT™ assay (bioMérieux, S.A., Marcy l’Etoile, France). The low detection limit of this method is 0.05 ng/ml and the reference value of normal range is <0.5 ng/ml. WBC and platelet were determined by sysmex XE-2100 fully automatic hematology analyzer (TOA Medical Electronics, Kobe, Japan). The normal reference ranges of WBC and platelet are 3.5–9.5×10^9 cells/L and 125–350×10^9 cells/L, respectively. The tests were performed at the central lab of the hospital.

**Data Management and Laboratory Parameters**

Demographic and clinical data collected from the HFRS patients included the patient’s age, sex, max temperature, admission day after fever onset, blood pressure at the time of the assessment, cigarette and alcohol consumption, hospital stay, blood transfusion, continuous renal replacement therapy (CRRT), comorbidities (hypertension, diabetes mellitus, and coronary heart disease), and HFRS-related complications including hemorrhage, secondary bacterial infection, hepatic injury, sepsis, multiple organ dysfunction syndrome (MODS), and arrhythmia. Hemorrhage was defined as presentation of signs of hemorrhage (pulmonary hemorrhage, melena, hematemesis, hematuria, diffuse ecchymosis, and hematoma) during hospitalization. The diagnosis of bacterial infection was confirmed by clinical manifestation combined with serological and radiologic evidence, the presence of pathogenic bacteria by bacteriology and findings of infection features by imaginology examination. Sepsis and MODS were defined according to the criteria determined by Vincent et al. (2006) and the American-College of Chest Physicians Society of Critical Care Medicine Consensus Conference definition (Bone et al., 1992), respectively. Variables collected at hospital admission included serum PCT, white blood cell counts (WBC), platelet (PLT), neutrophil percentage, and lymphocyte percentage.

For the analysis of factors associated with disease severity, the patients were classified into two groups, with the patients with mild and medium types being designated as the mild group and the patients with severe and gravis types being included in the severe group. The factors potentially associated with secondary bacterial infection or the survival of the patients were analyzed according to the development of bacterial infection.
in the patients during hospitalization and the prognosis of the patients.

Statistical Analysis

Statistical analysis was performed using SPSS 20.0 software (SPSS Inc., Chicago, IL, USA). Log transformation was used to make data conform to normality and reduce the variability of data, especially in data sets that include outlying observations. Categorical data were presented as numbers and percentages and were analyzed using the Fisher's Exact or Pearson's Chi-square test where appropriate. Continuous, normally distributed data were described using mean and standard deviation and analyzed using Student's t-test. Non-parametric data were described using median and interquartile range (IQR) and analyzed using Mann-Whitney U test as appropriate. Results with a p-value of <0.05 were deemed as statistically significant. Variables found to be significantly associated with disease severity or death were tested in a logistic regression model for their potential to predict the corresponding outcome. Considering the effects that multicollinearity may have on regression analyses and subsequent results, variance inflation factor (VIF) was used to detect multicollinearity among variables. Variables with statistically significant results in the univariate analyses and without multicollinearity were included in multivariate logistic regression analyses for independent variables. Predictive values of variables for disease severity, bacterial infection or prognosis were tested with receiver operating characteristic (ROC) curves and quantified by calculating the area under the ROC curve (AUC) and the 95% confidence interval (CI).

RESULTS

PCT Association With Disease Severity

Of the 146 patients (109 males and 37 females; mean age 44.98 ± 16.08 years), 72 patients were included in the mild group, and 74 patients were included in the severe group. The mortality rate in the severe patient group was significantly higher than in the mild patient group (p = 0.002). With regard to HFRS-related complications, patients in the severe group had higher frequencies of hemorrhage, hepatic injury, sepsis, and MODS than those in the mild group (p = 0.001, p = 0.032, p = 0.040, and p = 0.012, respectively). The hospital stay was significantly longer in the severe group patients than in the mild group (p < 0.001). Patients in the severe group had more frequent blood transfusion and CRRT than those in the mild group (both p < 0.001). Patients in the severe group had significantly higher WBC and lower PLT levels (p < 0.001 and p = 0.007, respectively). The median serum PCT level in the 146 HFRS patients was 1.53 ng/ml (range 0.03–62.91 ng/ml), which is higher than the reference value of normal range (<0.5 ng/ml). The PCT levels in the severe group patients were significantly higher than those in the mild group patients (p < 0.001, Table 1).

In the 59 HFRS patients without secondary bacterial infection (30 patients in mild group and 29 patients in severe group), the median serum PCT level in the severe group patients [1.20 ng/ml (range 0.08–25.00 ng/ml)] was higher than that in the mild group patients [0.79 ng/ml (range 0.05–23.39 ng/ml)] although the difference was not statistically significant (p = 0.085). In the 140 HFRS patients without sepsis (72 patients in mild group and 68 patients in severe group), the median serum PCT levels in the severe group patients [2.49 ng/ml (range 0.08–62.91 ng/ml)] were statistically higher than those in the mild group patients [0.81 ng/ml (range 0.03–23.39 ng/ml), p < 0.001]. Alternatively, the mean (± standard deviation, SD) serum PCT levels in the severe group patients (5.87 ± 9.36 ng/ml) were also statistically higher than those in the mild group patients (2.40 ± 4.56 ng/ml, p = 0.006). In the 87 HFRS patients with bacterial infection (42 patients in mild group and 45 patients in severe group), the median serum PCT level in the severe group patients [3.73 ng/ml (range 0.28–62.91 ng/ml)] was higher than that in the mild group patients [0.96 ng/ml (range 0.03–15.44 ng/ml), p < 0.001].

Multivariate analysis for the entire cohort revealed that PCT, WBC and hemorrhage were independent factors associated with the severity of HFRS (OR 2.544, 95% CI 1.330–4.868, p = 0.005;
Of the 146 HFRS patients, 13 patients died of the disease, with a mortality rate of 8.9%. Non-survivors of the patients had older age and lower diastolic blood pressure (DBP) at hospital admission ($p = 0.010$ and $p = 0.030$, respectively, Table 6). The presence of hemorrhage, MODS and sepsis was significantly higher in non-survivors compared with survivors ($p = 0.007$, $p < 0.001$ and $p = 0.001$, respectively, Table 6).

Blood transfusion and CRRT were more frequently applied to non-survivors compared with survivors ($p = 0.001$ and $p < 0.001$, respectively, Table 6) although the non-survivors had a shorter hospital stay because of deaths during hospitalization ($p = 0.042$). Non-survivors had a significantly higher neutrophil percentage and lower lymphocyte percentage compared with survivors ($p = 0.012$ and $p = 0.031$, respectively, Table 6). The PCT levels in non-survivors were significantly elevated compared with survivors ($p < 0.001$, Table 6).

In multivariate analysis after adjusting for other clinical parameters identified in univariate analysis, PCT levels, neutrophil percentage and MODS were shown to be independent factors associated with mortality in HFRS patients (OR 1.075, 95% CI 1.003–1.152, $p = 0.041$; OR 32.151, 95% CI 4.499–229.749, $p = 0.001$ and OR 7.302, 95% CI 1.661–32.105, $p = 0.009$; respectively, Table 7).

The AUC values of PCT, neutrophil percentage and MODS for predicting mortality were 0.819 (95% CI 0.724–0.914, $p < 0.001$), 0.716 (95% CI 0.586–0.847, $p = 0.010$) and 0.723 (95% CI 0.544–0.903, $p = 0.008$), respectively (Table 8, Figure 3). By combining
these 3 factors, the AUC value rose to 0.907 (95% CI 0.821–0.993, p < 0.001) with a sensitivity of 92.0% and a specificity of 79.0%, respectively (Table 8, Figure 3).

### DISCUSSION

This study, for the first time to our knowledge, analyzed the changes of PCT levels in patients with HFRS caused by Hantaan virus. The results showed that serum PCT levels were elevated and associated with the disease severity, secondary bacterial infection and mortality in the patients.

This study, consistent with the findings in Puumala virus and Dobrava virus infection (Jereb et al., 2011; Bunz et al., 2015; Latus et al., 2015a), showed significantly elevated PCT levels in Hantaan virus-associated HFRS. It is well known that endotoxin may induce PCT in human (Dandona et al., 1994). Moreover, tumor necrosis factor (TNF)-α and interleukin (IL)-6 can induce PCT production in the absence of endotoxin (Nijsten et al., 2000). Increased concentrations of TNF-α and IL-6 have been demonstrated in HFRS patients (Linderholm et al., 1996). Therefore, the increased PCT levels documented in HFRS patients may be resulted from the stimuli by elevated TNF-α and IL-6 even in the absence of bacterial infection.

Although it was not applicable for the present study to compare PCT levels between individuals with Hantaan virus infection and those with other hantaviruses such as Puumala virus and Dobrava virus, PCT levels in patients with Hantaan virus-associated HFRS appeared to be higher than those in Puumala virus. The median serum PCT level in HFRS patients without bacterial infection was 0.94 ng/ml (range 0.05–25 ng/ml) in the present study. A previous study showed that patients with HFRS caused by Dobrava virus seemed to have a higher PCT level [0.74 μg/L (range 0.09–2.83 μg/L)] than those with NE caused by Puumala virus [0.50 μg/L (range 0.10–11.7 μg/L)] (Kim et al., 2011). Because Dobrava virus and Hantaan virus cause more severe clinical diseases compared with Puumala virus, it is suggested that different hantaviruses cause diseases of different clinical severity, resulting in the variations of PCT levels. A study showed that there were notable differences in viral load and antibody and cytokine response dynamics between Dobrava and Puumala infections, and these may be reflected in differing disease severities and clinical outcomes (Korva et al., 2013). Another study showed that patient with Dobrava-Belgrade virus infection had a prolonged clinical course and a late and enhanced mobilization of cytokines compared with patient with Puumala virus infection and these differences in

### TABLE 3 | Predictive values of parameters for the severity of HFRS.

| Variables          | AUC   | p value | Cut-off value | Sensitivity | Specificity | 95% CI for AUC         |
|--------------------|-------|---------|---------------|-------------|-------------|------------------------|
|                    |       |         |               |             |             | Lower | Upper |
| Lg PCT at admission| 0.738 | <0.001  | 0.26          | 0.65        | 0.76        | 0.657 | 0.820 |
| WBC at admission   | 0.706 | <0.001  | 9.30          | 0.68        | 0.63        | 0.623 | 0.789 |
| Hemorrhage         | 0.640 | 0.004   | –             | –           | –           | 0.549 | 0.731 |
| Combinationα       | 0.785 | <0.001  | 0.49β         | 0.74        | 0.78        | 0.710 | 0.861 |

AUC, area under the receiver operating characteristic (ROC) curve; p-value for calculated AUC in predicting severity; CI, confidence interval; Sensitivity, specificity and 95% CI are all presented as percentages; WBC, white blood counts; LgPCT, procalcitonin after log10 transformation.

αLgPCT, WBC, and the occurrence of hemorrhage in combination.

βProbability value of the combination was analyzed by logistic regression. The regression coefficients of these three parameters were used to set up a logit model for the progression of HFRS as follows: Logit(P|yαβγ = 0.50) = -2.223 + 0.078LgPCT + 0.934WBC + 0.765Hemorrhage.

### TABLE 4 | Demographics, clinical data and laboratory parameters at admission in HFRS patients with and without bacterial infection.

| Variables                        | Non-bacterial infection (n = 59) | Bacterial infection (n = 87) | P-value |
|----------------------------------|----------------------------------|-------------------------------|---------|
| Male, n (%)                      | 46 (42.2)                       | 63 (57.8)                     | 0.449   |
| Age, years                       | 41.93 ± 14.53                   | 47.05 ± 16.82                 | 0.059   |
| Max temperature, °C              | 38.86 ± 0.75                    | 39.07 ± 0.74                  | 0.097   |
| Admitted days after fever        | 5.80 ± 2.66                     | 6.18 ± 4.04                   | 0.518   |
| SBP, mmHg                        | 121.80 ± 20.24                  | 119.78 ± 17.10                | 0.518   |
| DBP, mmHg                        | 78.03 ± 13.48                   | 78.00 ± 14.87                 | 0.989   |
| Smoking, n (%)                   | 32 (54.2)                       | 39 (44.8)                     | 0.264   |
| Alcohol consumption, n (%)       | 30 (50.8)                       | 36 (41.4)                     | 0.259   |
| COMORBIDITY                      |                                  |                               |         |
| Hypertension, n (%)              | 10 (16.9)                       | 13 (14.9)                     | 0.744   |
| Diabetes mellitus, n (%)         | 2 (3.4)                         | 5 (5.7)                       | 0.513   |
| Coronary heart disease, n (%)    | 1 (1.7)                         | 4 (4.6)                       | 0.344   |
| HFRS-RELATED COMPLICATION        |                                  |                               |         |
| Hemorrhage, n (%)                | 33 (55.9)                       | 50 (57.5)                     | 0.854   |
| Hepatic injury, n (%)            | 30 (50.8)                       | 48 (55.2)                     | 0.607   |
| Sepsis, n (%)                    | 1 (1.7)                         | 5 (5.7)                       | 0.226   |
| MODS, n (%)                      | 2 (3.4)                         | 6 (6.9)                       | 0.361   |
| Arthritis, n (%)                 | 2 (3.4)                         | 7 (8.3)                       | 0.251   |
| Blood transfusion, n (%)         | 18 (30.5)                       | 37 (42.5)                     | 0.141   |
| CRRT, n (%)                      | 13 (22)                         | 26 (29.9)                     | 0.293   |
| Hospital stay, days              | 12.32 ± 7.26                    | 13.30 ± 7.06                  | 0.419   |
| Number of deaths, n (%)          | 2 (3.4)                         | 11 (12.6)                     | 0.054   |
| PARAMETERS                       |                                  |                               |         |
| WBC, ×10⁹ cells/L                | 11.79 ± 8.32                    | 12.06 ± 7.08                  | 0.831   |
| PLT, ×10⁹ cells/L                | 82.19 ± 74.46                   | 84.28 ± 79.17                 | 0.873   |
| Neutrophils percentage (%)       | 64.05 ± 13.01                   | 69.56 ± 16.07                 | 0.031   |
| Lymphocytes percentage (%)       | 25.62 ± 11.37                   | 20.20 ± 12.24                 | 0.008   |
| PCT,(ng/ml)                      | 0.940 (0.05-25.00)              | 2.00 (0.03-62.91)             | 0.016   |
| Lg PCT                           | 0.02 ± 0.68                     | 0.27 ± 0.69                   | 0.037   |

SBP, systolic blood pressure; DBP, diastolic blood pressure; MODS, multiple organ dysfunction syndrome; CRRT, continuous renal replacement therapy; WBC, white blood cell counts; PLT, platelet; PCT, procalcitonin; Lg PCT, procalcitonin after log10 transformation.
TABLE 5 | Predictive values of parameters for bacterial infection in HFRS patients.

| Variables at admission | AUC   | P-value | Cut-off value | Sensitivity | Specificity | 95% CI for AUC |
|------------------------|-------|---------|---------------|-------------|-------------|----------------|
| Lg PCT at admission    | 0.618 | 0.016   | 0.00          | 0.71        | 0.54        | 0.524 – 0.711  |
| Neutrophils percentage at admission | 0.639 | 0.005   | 67.47         | 0.68        | 0.59        | 0.550 – 0.729  |
| Lymphocytes percentage at admission | 0.651 | 0.002   | 18.51         | 0.67        | 0.59        | 0.563 – 0.740  |

AUC, area under the receiver operating characteristic (ROC) curve; CI, confidence interval; Lg PCT, procalcitonin after log10 transformation.

FIGURE 2 | Procalcitonin (PCT) and the percentages of neutrophils and lymphocytes in predicting secondary bacterial infection in hemorrhagic fever with renal syndrome (HFRS) patients by receiver operating characteristic curve (ROC) analysis.

TABLE 6 | Demographics, clinical data and laboratory parameter at admission in survivors and non-survivors of patients with HFRS.

| Variables                      | Survivors (n = 133) | Non-survivors (n = 13) | P-value |
|--------------------------------|---------------------|------------------------|---------|
| Male, n (%)                    | 100 (75.2)          | 9 (69.2)               | 0.891   |
| Age, years                     | 43.92 ± 16.18       | 55.76 ± 10.28          | 0.010   |
| Max temperature, °C            | 38.99 ± 0.75        | 38.91 ± 0.77           | 0.723   |
| Admitted days after fever      | 5.99 ± 3.59         | 6.46 ± 3.07            | 0.645   |
| SBP, mmHg                      | 121.17 ± 17.94      | 114.69 ± 22.50         | 0.226   |
| DBP, mmHg                      | 78.81 ± 13.09       | 69.85 ± 22.36          | 0.030   |
| Smoking, n (%)                 | 63 (47.4)           | 8 (61.5)               | 0.329   |
| Alcohol consumption, n (%)     | 59 (44.4)           | 7 (53.8)               | 0.512   |

COMORBIDITY

| Hypertension, n (%)           | 20 (15)             | 3 (23.1)               | 0.718   |
| Diabetes mellitus, n (%)      | 5 (3.8)             | 2 (15.4)               | 0.119   |
| Coronary heart disease, n (%) | 4 (3)               | 1 (7.7)                | 0.377   |

HFIRS-RELATED COMPLICATION

| Hemorrhage, n (%)             | 71 (53.4)           | 12 (92.3)              | 0.007   |
| Bacterial infection, n (%)    | 76 (57.1)           | 11 (84.6)              | 0.054   |
| Hepatic injury, n (%)         | 68 (51.1)           | 10 (76.9)              | 0.075   |
| Sepsis, n (%)                 | 2 (1.5)             | 4 (30.8)               | <0.001  |
| MODS, n (%)                   | 2 (1.5)             | 6 (46.2)               | <0.001  |
| Arhythmia, n (%)              | 9 (6.8)             | 0                      | 1.000   |
| Blood transfusion, n (%)      | 44 (33.1)           | 11 (84.6)              | 0.001   |
| CRRT, n (%)                   | 28 (21.1)           | 11 (84.6)              | <0.001  |
| Hospital stay, days           | 13.28 ± 7.11        | 9.08 ± 6.47            | 0.042   |

LABORATORYPARAMETERS

| WBC, x10^9 cells/L            | 11.78 ± 7.66        | 13.66 ± 6.79           | 0.395   |
| PLT, x10^9 cells/L            | 86.23 ± 79.07       | 54.92 ± 44.68          | 0.163   |
| Neutrophils percentage, (%)   | 66.38 ± 15.20       | 77.34 ± 10.16          | 0.012   |
| Lymphocytes percentage, (%)   | 1.72 (2.86)         | 1.69 (1.34)            | 0.031   |
| PCT, (ng/ml)                  | 1.22 (0.03-62.91)   | 7.65 (1.06-58.53)      | <0.001  |
| Lg PCT                        | 0.10 ± 0.68         | 0.87 ± 0.48            | <0.001  |

SBP, systolic blood pressure; DBP, diastolic blood pressure; MODS, multiple organ dysfunction syndrome; CRRT, continuous renal replacement therapy; WBC, white blood cell count; PLT, platelet; Lg PCT, procalcitonin after log10 transformation.

cytokine deregulation may contribute to the variations in the clinical course (Krautkrämer et al., 2016). It is possible that the higher PCT levels in patients with Hantaan virus infection may be at least partly associated with the enhanced responses to the virus, especially the higher magnitude of cytokine responses in the patients.

Elevated PCT level is documented to be especially indicative of bacterial infection (Nishikawa, 1999; Linscheid et al., 2003; Kim et al., 2011; Arora et al., 2017; Nishikawa et al., 2017) and PCT has been increasingly acknowledged to be a major biomarker of bacterial infection. Serum concentration of the PCT is also indicated to be a good marker in differentiating infectious from noninfectious causes of inflammation (Assicot et al., 1993). However, this study and other studies (Jereb et al., 2011; Bunz et al., 2015; Latus et al., 2015a) demonstrated the common presence of higher PCT level in infection of hantaviruses. It is suggested that PCT level may be of less clinically differential quality between infections of hantaviruses and bacteria and caution should be taken in the interpretation of PCT levels when hantavirus infection is suspected. Moreover, a study showed that PCT failed to predict bacteremia in systemic inflammatory response syndrome (SIRS) patients (Hoennigl et al., 2014) although it can accurately differentiate culture-negative sepsis from noninfectious SIRS (Anand et al., 2015). A study in trauma patients showed that PCT was related to inflammation caused by injury but not to infection (Mimoz et al., 1998). PCT is also indicated to be unable to predict between bacterial and viral meningitis (Sanaei Dashti et al., 2017).
With respect to the disease severity, this study showed that elevated PCT level, along with WBC and hemorrhage, was significantly associated with the disease severity in HFRS patients with Hantaan virus infection. It is acceptable that WBC count and hemorrhage are suggestive of the disease severity in that WBC count is a commonly used parameter of inflammation and hemorrhage is a prominent feature of the severe form HFRS. The leukocyte at admission was predictive of the subsequent development of oliguric acute renal failure in HFRS (Kim et al., 2007). In intracerebral hemorrhage patients, elevated PCT levels at admission were shown to be independently associated with unfavorable clinical outcome (He et al., 2017). This study demonstrated that PCT level is not only an independent factor associated with the severity of HFRS but also a parameter with slightly higher AUC value than WBC and hemorrhage for predicting the severity of HFRS. In patients with NE caused by Puumala virus and HFRS caused by Dobrava virus, no association between PCT levels and severity of disease was observed (Jereb et al., 2011; Bunz et al., 2015; Latus et al., 2015a). However, these studies were performed in relatively small number of patients and this may affect the results of the analysis. The comparison of PCT levels between patients with HFRS caused by Dobrava virus and those with Puumala virus infections (Jereb et al., 2011) had a smaller patient number but it indicated a higher PCT level in HFRS caused by Dobrava virus. The present study analyzed PCT levels in a relatively larger number of patients with severe form HFRS which may manifest severe inflammation. PCT concentrations may be proportional to the inflammatory stimulus. Elevated PCT levels represent more pronounced systemic inflammation, which might be associated with a more severe disease course. The association of PCT level with disease severity in diseases caused by different hantaviruses may be virus type and disease severity specific. Of course, further studies are needed to clarify the relationship between PCT levels and disease severity in various hantavirus infections.

Regarding secondary bacterial infection in HFRS patients, this study showed that PCT level, in addition to higher percentage of neutrophils, and lower percentage of lymphocytes, was a significant factor associated with bacterial infection. The significance of neutrophil and lymphocyte percentages has been widely accepted as indicators of bacterial infection. Consistent with the result in this study, a previous study in hantavirus infections showed that increased serum PCT level was found to exhibit overlapping results between viral and severe bacterial infections (Jereb et al., 2011). Notably, the value of PCT for predicting bacterial infection in HFRS patients appeared to be low in the current study. This is possibly related to the intrinsically increased PCT caused by the primary disease since HFRS patients without bacterial infection had a higher serum PCT level [0.94 ng/ml (range 0.05–25 ng/ml)] than the normal reference value (<0.5 ng/ml) and this may compromise the performance of PCT in identifying bacterial infections in this specific disease condition. Therefore, different thresholds of PCT should be applied in HFRS patients when secondary bacterial infection is suspected during the disease course.

With respect to the mortality of HFRS patients, this study showed that PCT, together with the percentage of neutrophils and MODS, was an independent factor and had a higher AUC value (0.819) than the percentage of neutrophils (0.716) and MODS (0.723) for predicting mortality of the patients.
Severe systemic inflammation is a common characteristic of severe HFRS. Neutrophils, in addition to their contribution to chronic inflammatory conditions and adaptive immune responses, are major effectors of acute inflammatory reaction (Kolaczkowska and Kubes, 2013). Increased PCT level and neutrophil percentage represent severe systemic inflammation, which may potentially contribute to a more severe disease course and increased mortality. MODS may occur in severe HFRS patients and contribute to the increased mortality of the patients (Zhao et al., 2009). PCT has been indicated to represent a sensitive and predictive indicator of severe MODS in injured patients (Wanner et al., 2000). Therefore, MODS is not only an independent factor of the mortality in HFRS patients but also a significant contributor of increased PCT levels in the patients. AKI is another prominent feature of HFRS. A study in patients after vascular surgery showed that renal function was a major determinant of PCT levels (Amour et al., 2008). Another study in critically ill patients showed a significant association of an elevated PCT on admission with the development of AKI in the non-septic patient (Jeeha et al., 2017). It is suggested that kidney injury in HFRS may be another contributor of the elevated PCT levels. Hemodynamical instability such as hypotension and even shock may develop in severe HFRS patients. A study in out-of-hospital cardiac arrest patients showed that elevated PCT was associated with hemodynamical instability and worsened long-term outcome (Pekkarinen et al., 2017). Therefore, the potential hemodynamical instability in HFRS patients may also relate to the elevation of PCT in HFRS patients. Taken together, multiple events such as severe SIRS, MODS, AKI and hemodynamical instability potentially presented in severe HFRS patients might promote the increase of PCT concentration associated with the increased mortality.

In conclusion, this study highlights the existence of a frequent association between HFRS and elevation of PCT in the absence of bacterial infection and the existence of overlapping results between Hantaan virus infection with and without bacterial infection. Importantly, higher PCT concentrations at hospital admission may be associated with disease severity, secondary bacterial infection and mortality in patients with HFRS caused by Hantaan virus. PCT level at admission in HFRS patients seems to aid early recognition of the disease severity and mortality with good performance and the secondary bacterial infection with moderate performance. These findings add novel information for the value of PCT application in clinical settings.

This study has some limitations. First, the retrospective nature of the study may affect the potency of the results. Second, the study is limited by the relatively small number of patients in a single medical center. Third, some parameters of inflammation such as elevated C-reactive protein levels, which have been shown to be associated with the severity in hantavirus-induced NE (Latus et al., 2015b), were not included in the study because of the non-routine detection in HFRS patients in our hospital. Fourth, the normal range of PCT from reference was used for the comparison of an elevated PCT levels in HFRS patients and no normal health control group was included for the comparison in the study. This may also potentially cause deviation to the results of the comparison of PCT levels between HFRS patients and normal individuals. Therefore, prospective studies with a large population of patients and normal health controls in multiple centers are needed to clarify the value of PCT in predicting the disease severity, bacterial infection and mortality of HFRS patients.

**AUTHOR CONTRIBUTIONS**

XF and ZL contributed to conception and design of the research. XF, HD, JS, NL, QH, and XZ acquired data in the study. XF, NL, HD, JS, XZ, and QH analyzed data. XF and ZL drafted the paper, and all authors revised it critically, read and approved the final manuscript.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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