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Severity Predictors in Eschar-Positive Scrub Typhus and Role of Serum Osteopontin

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Abstract. We prospectively evaluated severity predictors in terms of host, microorganism, and treatment factors in 153 eschar-positive scrub typhus patients. Severity was assessed with the Acute Physiology and Chronic Health Evaluation (APACHE) II score (< 10 versus ≥ 10) and predefined criteria of severe complications. Genotypes of *Orientia tsutsugamushi* were determined. Independent risk factors for severity (APACHE II score ≥ 10) were old age, diabetes mellitus, serum osteopontin > 100 ng/mL, and a group of underlying diseases (congestive heart failure, cerebrovascular disease, chronic liver disease, bronchial asthma, and chronic obstructive lung diseases). Anemia (≤ 10 g/dL) and C-reactive protein > 10 mg/dL were indicators of current severity. Neither the delay in antibiotics administration nor strain types (Boryong, Taguchi, or Kanda/Kawasaki) contributed to the severity. The risk factors for severe complications were similar. Serum osteopontin > 100 ng/mL had a negative predictive value of 96% for severe complications. This marker can be used to rule out severe disease status.

INTRODUCTION

Scrub typhus caused by *Orientia tsutsugamushi* is an endemic disease occurring throughout the Far East, northern Australia, and the Indian subcontinent.[1] Eschar and rash are key clues for early diagnosis. Indirect fluorescent antibody assay is a standard diagnostic method, but because of its low sensitivity, polymerase chain reaction assay may be an alternative.[2] Severe complications such as pneumonia, meningitis/encephalitis, septic shock, myocarditis, and acute renal failure can lead to death.[3–5] The case-fatality rate for untreated cases varies widely from 3% to 60%.[1] Several factors have been suggested to be associated with severe complications. *Orientia tsutsugamushi* has more than 20 antigenically distinct regionally distributed serotypes.[6] Some strains seem to have higher virulence.[1,7,8] Plasma rickettsial load is associated with disease severity in adults.[9] Clinical characteristics such as older age, underlying diseases, and delay of appropriate antibiotics administration seem to contributes to the severity.[10,11] Laboratory markers such as increased transaminases, hypoalbuminemia, leukocytosis, and elevated serum creatinine have been reported to be associated with the disease severity.[12–15] Although these factors can be used to identify the risk group, many clinicians want to know the specific parameters that indicate severity of this infectious disease.

Osteopontin (OPN) is a phosphorylated acidic glycoprotein that is involved in various physiological and pathological processes. It is expressed by bone, kidney, epithelial tissue, T cells, macrophages, and endothelial cells.[16] Osteopontin is known to regulate inflammation, tissue remodeling, and cell survival.[16] The OPN acts as a pro-inflammatory cytokine by chemoattracting macrophages and regulation of Th1/Th2 balance, but also has anti-inflammatory effects because it can block nitric oxide production by macrophages *in vitro* and help tissue repair at sites of inflammation.[17,18] Its tissue and serum levels are increased in several infectious or non-infectious diseases like tuberculosis and systemic lupus erythematosus.[19,20] The OPN enhances resistance to tsutsugamushi infection by affecting macrophages and Th1-mediated immune responses.[16] In mice studies, the osteopontin gene (*OPN*) was associated with disease severity in experimental scrub typhus.[21–23] However, little is known about the role of OPN in human tsutsugamushi disease.

Because OPN seems to be a key player in macrophage function and inflammation, we sought to determine the potential role of OPN as a marker of the disease severity in scrub typhus. Furthermore, with a prospective design we re-evaluated several factors that had been reported to be associated with the disease severity in previous studies, and combined genotypes of *O. tsutsugamushi* that most of the previous studies did not include in their analyses. Therefore, we tried to incorporate microorganism, host- and treatment-related factors as a whole in the severity analysis.

MATERIALS AND METHODS

Study design and clinical data. The study was conducted prospectively from September to December in 2006. Patients were recruited from eight community-based hospitals (Sanggye Paik Hospital, Ilsan Paik Hospital, Pusan Paik Hospital, Dongguk University Ilsan Hospital, Dankook University Hospital, Namwon Medical Center, Chonbuk National University Hospital, and Sunlin Hospital) located at the major endemic areas in South Korea. We enrolled clinically suspected scrub typhus patients > 16 years of age who had eschars and at least two of the following manifestations: fever, maculopapular skin rash, regional lymphadenopathy, headache, myalgia, cough, or abdominal discomfort. Although there was no doubt about the diagnosis because experienced infectious diseases specialists screened all the patients, the definite diagnosis of scrub typhus was based on the confirmation of nucleotide sequence of 56-kDa antigen gene of *O. tsutsugamushi* from each patient. The purpose of this sequencing is for both the definite diagnosis and determination of genotypes. To rule out other combined infectious diseases, blood culture was performed.

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Serologic tests were performed to rule out hemorrhagic fever with renal syndrome and leptospirosis (Genedia Hantadia and Genedia Lepto PHA, Greencross, South Korea), which are prevalent febrile diseases during the autumn season in South Korea. Only patients with genotypic confirmation and without other combined infectious diseases were included in the final analysis.

Demographic data, underlying diseases, type of patient referral, and distribution of eschar on the body were recorded. The extent of delay in appropriate antibiotics administration was estimated at the time between the appearance of scrub typhus-related clinical manifestations and the administration of appropriate antibiotics. General diagnostic and therapeutic measures not specified in the study were left to the physician's discretion. Informed consents were obtained from all patients and the study protocol was approved by the institutional review board at each participating hospital.

Definitions. The clinical severity of scrub typhus was assessed both by pre-defined severe complications and by the Acute Physiology and Chronic Health Evaluation (APACHE) II score.[24] Severe complication was defined as a new onset of problems and conditions as below. The central nervous system complication was defined as the presence of altered mental state, seizure, intracranial hemorrhage and/or infarct. Respiratory complication was defined as the presence of both lung infiltration and at least one of the followings: arterial $PaO_2/FiO_2 \leq 250$, respiratory rate ≥ 30/min, or requirement for mechanical ventilation.[25] Cardiac complication was defined as any presence of myocarditis, arrhythmia, or ischemic heart diseases. Renal complication was defined as serum creatinine level ≥ 2 mg/dL or the aggravation of pre-existing renal injury resulting in acute renal replacement therapy. Septic shock was defined as the need for parenteral vasopressor to maintain systolic blood pressure above 90 mm of Hg for more than 1 hour. Patients with an APACHE II score of ≥ 10, which was measured within 24 hours of initial admission, were regarded as severe cases. APACHE II score of 10 theoretically reflects overall risk of hospital mortality of about 10% in non-operative patients.[24] Underlying diseases with known or newly discovered below specific conditions were included: diabetes mellitus (DM) requiring oral or parenteral glucose lowering agents therapy; hypertension (HTN) requiring antihypertensive therapy; cerebrovascular diseases (CVD) with residual sequelae; symptomatic congestive heart failure (CHF) requiring medication; chronic liver diseases (CLD) including liver cirrhosis and chronic active hepatitis; bronchial asthma and chronic obstructive lung disease (COPD) requiring maintenance medication. All patients were treated with any of doxycycline, azithromycin, and rifampin, which were regarded as appropriate antibiotics.

Laboratory methods. Genotypes of *O. tsutsugamushi* were determined using eschar from each patient. In conjunction with this study, we performed another concurrent study regarding the genotypic distribution of *O. tsutsugamushi* in South Korea using the same patients. Because that study had a different objective and contents, its result was described and published separately.[26] The genotyping methods are detailed there, so we describe briefly the methodology here. The template DNA of *O. tsutsugamushi* was extracted from eschar. A gene fragment covering variable domains I and II of a 56-kDa antigen gene was amplified. The primers tsu-A (forward, 5′-TTT CGA ACG TGT CTT TAA GC-3′; nucleotide position –285 to –266 from the start codon of the 56-kDa gene based on the Gilliam strain) and tsu-B (reverse, 5′-ACA GAT GCA CTA TTA GGC AA-3′; 847 to 865) were used for the amplification. Direct sequencing of the polymerase chain reaction product was performed. Nucleotide sequences with maximum pairwise identity scores with the 56-kDa antigen gene were identified using GenBank. Serum OPN was measured with a commercial kit (Quantikine, R&D Systems, Minneapolis, MN). Serum was stored at –80°C until batch measurement of OPN. The average value of duplicate assays was recorded and expressed in ng/mL. Every assay plate was accompanied by a standard control.

Statistical analysis. Univariate comparisons were performed using the χ^2 test or Fisher's exact test for categorical variables. Continuous variables were compared by the Mann–Whitney U test or Student's t test as appropriate. Logistic regression analysis was carried out to determine the risk factors for severe scrub typhus. Variables with $P < 0.2$ in univariate analysis were included in the multivariate analysis. Multivariable analysis was performed as a stepwise multiple logistic regression analysis. Multivariable analyses were performed for both the risk factors leading to severe scrub typhus and the laboratory markers indicating current severity. Odds ratios (ORs) and corresponding 95% confidence intervals (CIs) were calculated. All statistical tests were two-tailed and P values < 0.05 were considered to be significant. The data were analyzed using SPSS for Windows (version 12.0; SPSS Inc., Chicago, IL).

RESULTS

Clinical characteristics. Among 192 patients who were enrolled, 153 had sufficient genotypic data to be confirmed as scrub typhus and, thus, were included in the final analysis. Median age of the patients was 63 years (interquartile range, IQR, 49–71). 41.2% were male. Fifty-five (35.9%) patients had one or more underlying chronic disease(s). These included HTN (21.6%), DM (8.5%), and other less frequent diseases (15.0%) such as CHF (six cases), CVD (six cases), CLD (six cases), asthma (three cases), and COPD (two cases). Median time from any kind of initial clinical manifestation to appropriate antibiotic administration was 7 days (IQR, 4–9). 61.4% of eschars were found on the trunk portion of the patients. One hundred ten patients (71.9%) visited hospitals by themselves or were referred by primary hospitals without diagnostic suspicion of scrub typhus. The other 43 patients were referred even though they already were suspected to have scrub typhus. Other variables are detailed in Table 1. All 153 patients responded well to antibiotics therapy and survived at least 30 days. Only one of the initially enrolled 192 patients died of rapidly progressive mental change, severe pneumonia requiring mechanical ventilation, and shock in 4 days after admission. However, this case was not included in the final analysis because the case did not meet inclusion criteria.

Regarding the clinical severity of scrub typhus, there were 16 cases of severe complications (10.5%). Respiratory complications (7.8%), CNS complications (5.9%), cardiac complications (2%), and septic shock (1.3%) were observed. The average APACHE II score of all the 153 patients was 6.6 (range, 0–22), and 32 patients (20.9%) had an APACHE II score ≥ 10. Genotypes of *O. tsutsugamushi* from the 153 patients were

TABLE 1
Characteristics of 153 scrub typhus patients according to the APACHE II score*

Variable	Total (*N* = 153)	APACHE II < 10 (*N* = 121)	APACHE II ≥ 10 (*N* = 32)	*P*
Age, median (IQR), year	63 (49–71)	59 (46–69)	73 (65–83)	< 0.001
Male sex	63/153 (41.2)	52/121 (43.0)	11/32 (34.4)	0.381
Underlying diseases	55/153 (35.9)	31/121 (28.1)	21/32 (65.6)	< 0.001
Diabetes mellitus	13/153 (8.5)	4/121 (3.3)	9/32 (28.1)	< 0.001
Hypertension	33/153 (21.6)	18/121 (14.9)	15/32 (46.9)	< 0.001
Others†	23/153 (15.0)	11/121 (9.1)	12/32 (37.5)	< 0.001
Antibiotic delay, days‡				
Any Sx§ to antibiotics	7 (4–9)	7 (5–9)	6 (3–10)	0.362
Fever from antibiotics	6 (4–8)	6 (4–8)	5 (3–10)	0.733
Rash from antibiotics	3 (1–5)	3 (1–5)	3 (1–4)	0.337
Location of eschar, trunk	94/151 (61.4)	70/119 (58.8)	24/32 (75.0)	0.449
Visit without diagnosis¶	110/153 (71.9)	93/121 (76.9)	17/32 (53.1)	0.01
Genotypes				
Boryong	105/153 (68.6)	82/121 (67.8)	23/32 (71.9)	0.656
Taguchi + Kanda/Kawasaki	44/153 (28.6)	37/121 (30.6)	7/32 (21.9)	0.336
Serum OPN > 100 ng/mL	55/146 (35.9)	34/114 (29.8)	21/32 (65.6)	< 0.001
Laboratory values				
WBC, > 10K or < 4 K/mm^3	57/153 (37.3)	43/121 (35.5)	14/32 (43.8)	0.394
Hemoglobin ≤ 10 g/dL	8/153 (5.2)	1/121 (0.8)	7/32 (21.9)	0.001
Platelet ≤ 100 K/mm^3	37/153 (24.2)	24/121 (19.8)	13/32 (40.6)	0.017
AST > 40 IU/L	127/153 (83.0)	97/121 (80.2)	30/32 (93.8)	0.087
ALT > 40 IU/L	114/153 (74.5)	87/121 (71.9)	27/32 (84.4)	0.157
Serum albumin ≤ 3.0 g/dL	34/153 (22.2)	19/121 (15.7)	15/32 (46.9)	< 0.001
CR*P* > 10 mg/dL	39/140 (27.9)	23/109 (21.1)	16/31 (51.6)	0.001
Potassium ≤ 3.5 mmol/L	35/153 (22.9)	24/121 (19.8)	11/32 (34.4)	0.086
LDH > 800 IU/L	36/140 (25.7)	26/108 (24.1)	10/32 (31.3)	0.416
Serum Cr > 2.0 mg/dL	3/153 (2.0)	1/121 (0.8)	2/32 (6.3)	0.095
ESR > 20 mm/hr	55/128 (43.0)	42/99 (42.4)	13/29 (44.8)	0.818

* IQR = interquartile range; OPN = serum osteopontin; WBC = white blood cell; AST = aspartate aminotransferase; ALT = alanine aminotransferase; CRP = C-reactive protein; LDH = lactate dehydrogenase; ESR = erythrocyte sedimentation rate.
† "Others" indicates a group of less frequent chronic diseases including congestive heart failure, cerebrovascular disease, chronic liver disease, asthma, and chronic obstructive pulmonary disease.
‡ Indicates the time between the appearance of scrub typhus-related clinical manifestations and the administration of appropriate antibiotics.
§ "Any Sx" indicates any kind of scrub typhus-related clinical manifestation.
¶ Patients who visited the hospitals by themselves or who were referred from primary hospitals without initial clinical suspicion of scrub typhus.

determined. There were five genotypes: 105 Boryong strains (68.6%), 30 Taguchi strains (19.6%), 14 Kanda/Kawasaki strains (9.2%), 2 Jecheon strains (1.3%), and 2 UAP7 strains (1.3%). Two patients with UAP7 strains all had severe complications, including altered mentality, pneumonia, and shock. Fourteen of the nucleotides sequences showed 100% homology to both Kanda and Kawasaki strains, therefore these strains were termed as "Kanda/Kawasaki strains." Serum OPN could be measured in 146 patients. The average value of serum OPN was 97.89 ± 72.7 ng/mL. When serum OPN levels were dotted according to the severity categories, the distribution showed a pattern dividing the upper and lower values at around 100 ng/mL (Figure 1).

Factors associated with APACHE II score ≥ 10. By univariate analysis (Table 1), patients with an APACHE II score ≥ 10 were more likely to have the following characteristics than patients with an APACHE II score < 10: old age ($P < 0.001$), underlying chronic diseases (65.6% versus 28.1%, $P < 0.001$), DM (28.1% versus 3.3%, $P < 0.001$), HTN (46.9% versus 14.9%, $P < 0.001$), a group of less frequent underlying diseases (37.5% versus 9.1%, $P < 0.001$), serum OPN > 100 ng/mL (65.6% versus 29.8%, $P < 0.001$), anemia (≤ 10 g/dL; 21.9% versus 0.8%, $P = 0.001$), thrombocytopenia (≤ 100,000/mm^3; 40.6% versus 19.8%, $P = 0.017$), hypoalbuminemia (≤ 3.0 g/dL; 46.9% versus 15.7%, $P < 0.001$), and C-reactive protein (CRP) > 10 mg/dL (51.6% versus 21.1%, $P = 0.001$). None of the gender, the time from onset of clinical manifestations to administration of appropriate antibiotics, the location of eschar on the body surface, and genotypes had a significant impact on the APACHE II score ≥ 10. The genotypes were compared both on an individual basis (Boryong, Taguchi, and Kanda/Kawasaki) and as phylogenetically similar groups (Taguchi + Kanda/Kawasaki and Boryong + Jecheon +

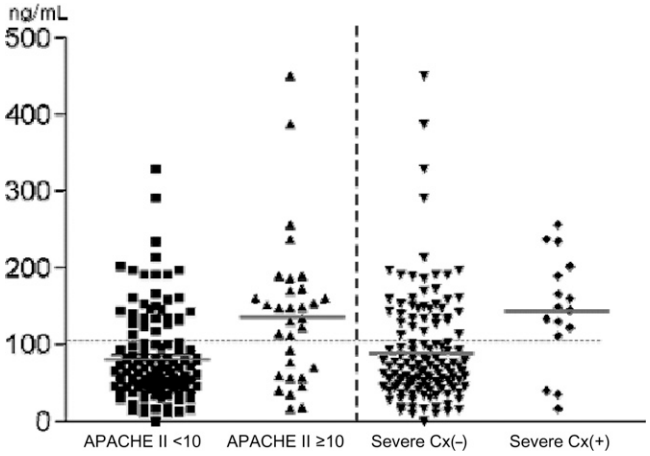

FIGURE 1. Distribution of serum osteopontin (OPN) levels according to the severity categories, "APACHE II score" and "severe complications." Short horizontal bars within each column represent the mean serum OPN level of each group. The dot distribution shows that there is a pattern dividing the upper and lower values at ~100 ng/mL in each column. Cx = complications.

TABLE 2

Multivariable logistic regression analysis of factors associated with the clinical severity of scrub typhus according to the APACHE II score ≥ 10*

Variable	OR (95% CI)	*P*
Factors predisposing for severity		
Age	1.07 (1.02–1.12)	0.003
Diabetes mellitus	5.55 (1.12–27.48)	0.036
Hypertension	2.08 (0.66–6.56)	0.211
Other less frequent chronic diseases†	3.65 (1.05–12.71)	0.042
Visit without diagnosis‡	0.32 (0.11–0.93)	0.036
Serum OPN (> 100 ng/mL)	3.35 (1.16–9.69)	0.026
Laboratory markers indicating current severity		
Serum OPN (> 100 ng/mL)	2.96 (1.09–8.04)	0.034
Hemoglobin (≤ 10 g/dL)	32.08 (2.61–393.87)	0.007
Platelets (≤ 100,000/mm^3)	1.45 (0.49–4.26)	0.499
Serum albumin (≤ 3.0 g/dL)	1.61 (0.49–5.32)	0.435
CRP (> 10 mg/dL)	3.47 (1.18–10.16)	0.023
Serum Cr > 2.0 mg/dL	6.98 (0.28–174.71)	0.237
AST > 40 IU/L	2.01 (0.23–17.62)	0.528
ALT > 40 IU/L	1.07 (0.24–4.66)	0.934
Potassium ≤ 3.5 mmol/L	1.51 (0.50–4.57)	0.468

* OR = odds ratio; CI = confidence interval; OPN = serum osteopontin; CRP = C-reactive protein; AST = aspartate aminotransferase; ALT = alanine aminotransferase.
† "Other less frequent chronic diseases" include congestive heart failure, cerebrovascular disease, chronic liver disease, asthma, and chronic obstructive pulmonary disease.
‡ Patients who visited the hospital by themselves or who were referred from primary hospitals without initial clinical suspicion of scrub typhus.

UAP7).[26] Visits without prior presumptive diagnosis of scrub typhus (53.1% versus 76.9%, $P = 0.01$) were less frequent among patients with an APACHE II score ≥ 10 than among patients with an APACHE II score < 10. Multivariable logistic regression analysis for risk factors leading to severe scrub typhus showed that old age (OR, 1.07; 95% CI, 1.02–1.12), DM (OR, 5.55; 95% CI, 1.12–27.48), a group of less frequent underlying diseases (OR, 3.65; 95% CI, 1.05–12.71), and serum OPN > 100 ng/mL (OR 3.35; 95% CI, 1.16–9.69) were independently associated. In a separate multivariable logistic regression for laboratory markers indicating current severity, serum OPN > 100 ng/mL (OR, 2.96; 95% CI, 1.09–8.04), anemia (OR, 32.08; 95% CI, 2.61–393.87), and CRP > 10 mg/dL (OR, 3.47; 95% CI, 1.18–10.16) were independently associated (Table 2).

Factors associated with severe complications. By univariate analysis, patients with severe complications were more likely to have the following characteristics than patients without severe complications: old age ($P = 0.003$), underlying chronic diseases (62.5% versus 32.8%, $P = 0.013$), HTN (50.0% versus 18.2%, $P = 0.006$), a group of less frequent underlying diseases (37.5% versus 12.4%, $P = 0.012$), serum OPN > 100 ng/mL (81.3% versus 32.3%, $P = 0.001$), anemia (25% versus 2.9%, $P = 0.002$), thrombocytopenia (56.3% versus 20.4%, $P = 0.003$), hypoalbuminemia (50.0% versus 19.0%, $P = 0.008$), CRP > 10 mg/dL (62.5% versus 23.4%, $P = 0.002$), and hypokalemia (≤ 3.5 mmol/L; 50.0% versus 19.7%, $P = 0.01$). Visits without prior presumptive diagnosis of scrub typhus (37.5% versus 75.9%, $P = 0.003$) were less frequent among patients with severe complications than among patients without severe complications (Table 3). Multivariable analysis with regard to severe complications was not done because of

TABLE 3

Characteristics of 153 scrub typhus patients associated with pre-defined severe complications*

Variable	Total (*N* = 153)	Not severe Cx (*N* = 137)	Severe Cx (*N* = 16)	*P*
Age, median (IQR), year	63 (49–71)	60 (47–70)	74 (61–82)	0.003
Male sex	63/153 (41.2)	56/137 (40.9)	7/16 (43.8)	0.825
Underlying diseases	55/153 (35.9)	45/137 (32.8)	10/16 (62.5)	0.013
Diabetes mellitus	13/153 (8.5)	10/137 (7.3)	3/16 (18.8)	0.135
Hypertension	33/153 (21.6)	25/137 (18.2)	8/16 (50.0)	0.006
Others†	23/153 (15.0)	17/137 (12.4)	6/16 (37.5)	0.012
Antibiotic delay, days‡				
Any Sx§ from antibiotics	7 (4–9)	7 (5–9)	6 (4–10)	0.933
Fever from antibiotics	6 (4–8)	6 (4–8)	6 (2–10)	0.985
Rash from antibiotics	3 (1–5)	3 (1–5)	2 (1–5)	0.444
Location of eschar, trunk	94/151 (61.4)	82/135 (60.7)	12/16 (75.0)	0.604
Visit without diagnosis¶	110/153 (71.9)	104/137 (75.9)	6/16 (37.5)	0.003
Genotypes				
Boryong	105/153 (68.6)	92/137 (67.2)	13/16 (81.3)	0.259
Taguch + Kanda/Kawasaki	44/153 (28.6)	43/137 (31.4)	1/16 (6.3)	0.066
Serum OPN > 100 ng/mL	55/146 (35.9)	42/130 (32.3)	13/16 (81.3)	0.001
Laboratory values				
WBC, > 10K or < 4K /mm^3	57/153 (37.3)	51/137 (37.2)	6/16 (37.5)	0.983
Hemoglobin ≤ 10 g/dL	8/153 (5.2)	4/137 (2.9)	4/16 (25.0)	0.002
Platelets ≤ 100,000/mm^3	37/153 (24.2)	28/137 (20.4)	9/16 (56.3)	0.003
AST > 40 IU/L	127/153 (83.0)	111/137 (81.0)	16/16 (100)	0.71
ALT > 40 IU/L	114/153 (74.5)	100/137 (73.0)	14/16 (87.5)	0.223
Serum albumin ≤ 3.0 g/dL	34/153 (22.2)	26/137 (19.0)	8/16 (50.0)	0.008
CRP > 10 mg/dL	39/140 (27.9)	29/124 (23.4)	10/16 (62.5)	0.002
Potassium ≤ 3.5 mmol/L	35/153 (22.9)	27/137 (19.7)	8/16 (50.0)	0.01
LDH > 800 IU/L	36/140 (25.7)	30/124 (24.2)	6/16 (37.5)	0.257
Serum Cr > 2.0 mg/dL	3/153 (2.0)	2/137 (1.5)	1/16 (6.3)	0.233
ESR > 20 mm/hr	55/128 (43.0)	49/112 (43.8)	6/16 (37.5)	0.637

* IQR = interquartile range; OPN = serum osteopontin; WBC = white blood cell; AST = aspartate aminotransferase; ALT = alanine aminotransferase; CRP = C-reactive protein; LDH = lactate dehydrogenase; ESR = erythrocyte sedimentation rate.
† "Others" indicates a group of less frequent chronic diseases including congestive heart failure, cerebrovascular disease, chronic liver disease, asthma and chronic obstructive pulmonary disease.
‡ Indicates the time between the appearance of scrub typhus-related clinical manifestations and the administration of appropriate antibiotics.
§ "Any Sx" indicates any kind of scrub typhus-related clinical manifestation.
¶ Patients who visited the hospital by themselves or who were referred from primary hospitals without initial clinical suspicion of scrub typhus.

the small number of patients and thus, multivariable analysis would have been less effective.

DISCUSSION

We assessed risk factors predicting the severe clinical presentation of scrub typhus and laboratory markers reflecting its current severity. Multiple studies have reported on risk factors related to the severity of this disease.[7–15] Some of them, however, had drawbacks such as retrospective design, obscure criteria for severity, and no consideration of serotypes. Therefore, we prospectively collected data according to the severity criteria of both pre-defined severe complications and the APACHE II score and included genotypes. We wanted to separately describe pre-infection and post-infection factors predicting the severity.

Scrub typhus is a communicable disease with a heavy disease burden in South Korea. We see a large surge of scrub typhus epidemic every autumn season.[27] Boryong strain has included ~70–80% of the causative *O. tsutsugamushi*.[28] Although the overall fatality rate is very low, initial severe clinical presentations are not rare. Thus, long-term assumption has been that a more virulent minor strain is responsible for the severe cases. Our study showed that genotypes of *O. tsutsugamushi* did not contribute to the disease severity. Among the 153 isolates identified, 68.6% were Boryong strains, and Kawasaki group (Taguchi and Kanda/Kawasaki strains) comprised the other 28.8%. Two UAP7 strains were identified, and both cases had severe complications. Serotypes of *O. tsutsugamushi* have been reported to differ in their virulence in experimental animals.[29,30] Gilliam, Karp, and Kato are such virulent strains that they can cause severe and potentially life-threatening disease.[8,31,32] The UAP7 strain found in our study is one of the Karp-antigen reacting groups.[33] Although our study failed to show the contribution of genotypes, it is highly likely that the types of strains including UAP7 should differently contribute to the severity of disease. Actually, our result shows that the cause of severity is multifactorial. Even with the majority of Boryong strains there were severe cases in terms of both the APACHE II score and severe complications.

The independent risk factors were old age and underlying chronic diseases like DM and other less frequent diseases. This corroborates our clinical impression that elderly patients from rural areas with underlying chronic diseases had more severe clinical presentations and prolonged morbidity. The less frequent underlying diseases included CHF, CVD, CLD, bronchial asthma, and COPD. We could not analyze these diseases separately because of their small number. One retrospective study showed that liver cirrhosis was associated with higher fatality.[10] Patients who visited hospitals by themselves or who were referred from primary hospitals without initial clinical suspicion of scrub typhus showed significantly lower severity. This may be caused by patient selection bias. Scrub typhus usually responds well to antibiotic therapy. With appropriate clinical diagnosis, doctors would not refer patients to upper level hospitals. However, severe or critical patients would be referred even under definite diagnosis. Regarding antibiotic therapy, the time from initial clinical manifestations including fever and/or rash to appropriate antibiotic administration was not associated with the severity. The fever and systemic symptoms of scrub typhus are so agonizing and the accessibility to primary medical care in South Korea is so high that patients should not be exposed to inappropriate delays of antibiotic therapy. The median delay was 7 (IQR, 4–9) days. This degree of treatment delay did not affect the prognosis in our study population.

Physicians need objective markers reflecting or predicting severe complications to efficiently identify the risky patients in time and concentrate appropriate cares. The pathogenesis of scrub typhus involves immune and inflammatory mediators.[16] Fulminant cases of scrub typhus have been found to show hypercytokinemia.[34,35] Inflammatory cytokines were increased in patients during the acute phase, and these cytokines were reduced after doxycycline treatment.[36,37] The tumor necrosis factor α (TNF-α) levels in the acute phase could predict the severity of scrub typhus.[36] The OPN is also closely associated with immune/inflammatory responses. Serum OPN can stimulate Th1 responses by inducing interleukin-12 (IL-12), a cytokine that drives Th1 responses, whereas OPN prevents production of T-helper 2 cytokine IL-10.[38] We measured serum OPN levels. No prior studies have related serum OPN levels to scrub typhus in humans. Our study showed that increased serum OPN was an independent risk factor for the severity. In mice studies, osteopontin gene (*OPN*) was associated with natural resistance in experimental scrub typhus.[21–23] The immune response to bacterial infections in *OPN* knockout mice was defective, because of the deficiency of IL-12 and interferon-gamma (IFN-γ) dependent T helper cells responses.[38,39] The messenger RNA (mRNA) level of *OPN* was associated with T helper 1 cells-mediated immune responses.[40] Therefore, if severe presentation is caused by the lack of OPN-related immune responses, serum OPN should be low. Otherwise, higher OPN levels might be related to the augmented immune/inflammatory responses. The interpretation is limited because we did not measure concurrent serum cytokine profiles related to the Th1 and Th2 pathways in our study. It might also result from endothelial cells infected by *O. tsutsugamushi*. The basic pathogenesis of scrub typhus is vasculitis affecting endothelial cells that express OPN. *Orientia tsutsugamushi is* located in endothelial cells in all the involved organs.[41] The OPN level can correlate with the degree of vasculitis. Our study showed that the distribution of OPN values was divided at 100 ng/mL (Figure 1). In the severe complications group, serum OPN > 100 ng/mL had a sensitivity of 81%, a specificity of 67%, a positive predictive value of 23%, and a negative predictive value of 96% for severe presentation. Therefore, this marker can be used to rule out severe disease status.

Most studies have used mortality as an index of severity. However, mortality is not a practical index in scrub typhus because the disease responds well to antibiotics. Although we used the APACHE II score as one of the indices assessing severity, there are no specific data on how the APACHE II score works in scrub typhus. In this study, the presence of severe complications was significantly associated with the APACHE II score (13.38 ± 4.72 versus 5.80 ± 3.42, $P < 0.001$). A cross-table analysis between severe complications and the APACHE II score ≥ 10 also showed a significant association ($P < 0.001$).

We only included eschar-positive patients. There are several reasons for us to select only eschar-positive patients. Practically, it was very hard to screen out enough eschar-negative patients to have sufficient genotypic data. Eschar is a good source to obtain genetic materials of *O. tsutsugamushi*. About 5–10% of scrub typhus patients in South Korea have been

known to be eschar negative.[15,42] Therefore, eschar-positive patients could reflect a majority of patients in South Korea, and even within this limited group there is a problem of severity and the causes of severity need to be elucidated. There is uncertainty whether both eschar-positive and eschar-negative patients are a homogenous group with same pathogenesis. In another sense we could avoid a bias by the absence of eschar. Our results may not be applicable to other endemic areas with higher rates of eschar-negative patients if the presence of eschar means different pathogenesis.

The number of patients included in this study was large compared with the other studies, but the number was not sufficient to characterize the minor strains, such as UAP7 that resulted in severe complications. We showed that none of the Boryong, Taguchi, and Kanda/Kawasaki strains, which comprise the majority of *O. tsutsugamushi* in South Korea contributed to the severity. There may be strains that do affect the severity, because our study did not include all strain types, but our study showed that there were also other important factors. Age and underlying diseases such as DM and other less common diseases were significantly associated with the disease severity. Laboratory parameters such as serum OPN > 100 ng/mL, anemia (≤ 10 g/dL), and CRP > 10 mg/dL were indicators of the current severity of scrub typhus. In particular, we first evaluated the significance of serum OPN in human scrub typhus and this marker can be used to rule out severe disease status. These results should be integrated into the clinical practice to predict risk groups and to assess the current severity of scrub typhus.

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