【 ORIGINAL ARTICLE 】

Prediction of Disseminated Intravascular Coagulation by Liver Function Tests in Patients with Japanese Spotted Fever

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Abstract:

Objective   Cases of Japanese spotted fever (JSF) are sometimes complicated by disseminated intravascular coagulation (DIC) with an abnormal liver function, resulting in unfavorable outcomes. The aim of the present study was to clarify the correlation between liver function test results and DIC scores.

Methods   Twenty patients diagnosed with JSF between April 2010 and April 2014 were enrolled. Age, gender, disturbance of consciousness, body temperature, pulse rate, presence of diffuse erythema, eschar and swelling of lymph nodes, laboratory test results at the time of initial presentation such as blood cell count, C-reactive protein, liver function, renal function and blood coagulation and fibrinolysis, maximum Japanese Association for Acute Medicine (JAAM) DIC score during the course of JSF, treatment and the prognosis were retrospectively reviewed.

Results   The median age of the patients (8 men, 12 women) was 68.3 years. There were significant differences in the alkaline phosphatase (ALP) and rothrombin time international normalized ratio (PT-INR) between the DIC and non-DIC groups using Mann-Whitney's U test. A multiple logistic regression analysis showed that the ALP and blood urea nitrogen (BUN) levels at the time of initial presentation were independent predictors of the occurrence of DIC.

Conclusion   We should pay special attention to JSF patients showing high levels of ALP at the initial presentation, since such patients may have a higher likelihood of developing DIC over the course of JSF and unfavorable outcomes than those with lower levels.

Key words: DIC, *Rickettsia japonica*, rickettsial infection, abnormal liver function tests

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Introduction

Japanese spotted fever (JSF) is a rickettsial infection occurring as the result of the percutaneous transmission of the *Rickettsia japonica* by a species of tick principally endemic to Japan. Typically, JSF occurs in coastal areas of southwestern and central Japan during the warm season from April to October (1). The three representative symptoms are a high fever, diffuse erythema and eschar at the point of the tick bite. The laboratory findings include elevated levels of C-reactive protein (CRP), abnormal liver function tests and a decreased platelet count (1). Several cases have been reported with disseminated intravascular coagulation (DIC) and abnormal liver function tests, resulting in unfavorable outcomes (2-6). However, few reports have investigated the relationship between the patients' clinical factors and the disease severity of JSF. Therefore, in the present study we retrospectively analyzed 20 cases of JSF focusing on the correlation between liver function test results and DIC

Table 1.   Clinical Characteristics of the Study Population at the Time of the Initial Presentation, Medications and the Prognosis.

	Total (n=20)	DIC (n=5)	non-DIC (n=15)	p value
Age (years)	68.3 (62.5-73.0)	68.8 (66.3-83.4)	68.0 (62.3-70.6)	0.45
Men (n, %)	8/20 (40%)	4/5 (80%)	4/15 (27%)	0.035
Disturbance of consciousness (n, %)	2/20 (10%)	2/5 (40%)	0/15 (0%)	0.010
Temperature (℃)	38.5 (37.0-39.5)	37.6 (36.1-39.3)	38.5 (37.5-39.5)	0.51
Pulse rate (bpm)	100 (96-105)	101 (92-106)	100 (96-105)	0.94
Diffuse erythema (n, %)	19/20 (95%)	4/5 (80%)	15/15 (100%)	0.076
Eschar (n, %)	17/20 (85%)	3/5 (60%)	14/15 (93%)	0.071
Swelling of lymph node (n, %)	3/20 (15%)	0/5 (0%)	3/15 (20%)	0.28
Admission (n, %)	16/20 (80%)	5/5 (100%)	11/15 (73%)	0.20
Medication (n, %)				0.001
M (n, %)	15/20 (75%)	0/5 (0%)	15/15 (100%)	
M, NQ (n, %)	1/20 (5%)	1/5 (20%)	0/15 (0%)	
M, NQ, RT (n, %)	1/20 (5%)	1/5 (20%)	0/15 (0%)	
M, NQ, AT III, GM (n, %)	1/20 (5%)	1/5 (20%)	0/15 (0%)	
M, NQ, mPSL, RT, SST (n, %)	1/20 (5%)	1/5 (20%)	0/15 (0%)	
M, mPSL (n, %)	1/20 (5%)	1/5 (20%)	0/15 (0%)	
Death (n, %)	2/20 (10%)	2/5 (40%)	0/15 (0%)	0.010

All continuous numeric variables are expressed as the median (interquartile range). The p values are the results of comparing the DIC and non-DIC groups by the χ^2 test or Mann-Whitney's U test.

M: minocycline, NQ: new quinolone, mPSL: methylprednisolone, RT: recombinant thrombomodulin, AT III: antithrombin III, GM: gabexate mesilate, SST: sivelestat sodium tetrahydrate

scores.

Materials and Methods

Twenty patients diagnosed with JSF between April 2010 and April 2014 at Onomichi Municipal Hospital were enrolled in this study. The diagnosis of JSF was based on an elevation in paired serum antibodies specific to *Rickettsia japonica* and/or detection of *Rickettsia japonica* DNA in the patients' blood or eschar by polymerase chain reaction. The Age, gender, disturbance of consciousness, body temperature, pulse rate, presence of diffuse erythema, eschar and swelling of lymph node, laboratory test results at the time of initial presentation, Japanese Association for Acute Medicine (JAAM) DIC score (7) during the course of JSF, medications and prognosis were retrospectively reviewed. The laboratory test results available at the time of initial presentation were the white blood cell count (WBC), platelet count (Plt), CRP, aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT), total bilirubin (T-bil), blood urea nitrogen (BUN), creatinine (Cre), prothrombin time international normalized ratio (PT-INR), fibrinogen degradation product (FDP) and fibrinogen levels. All clinical data were obtained from patients' medical records. The laboratory data and JAAM DIC score were evaluated every 1 to 3 days.

We divided the patients into DIC (DIC score ≥4) and non-DIC (DIC score ≤3) groups using maximum JAAM DIC score over the course of JSF and analyzed differences between the two groups using Mann-Whitney's U test. To identify the laboratory test parameters at the time of the initial presentation predictive of the occurrence of DIC, we conducted univariate and multivariate logistic regression analyses. Continuous numeric variables were expressed as the median and interquartile range (IQR).

The statistical analysis was performed using the SPSS software program, version 21.0 (IBM, Armonk, USA). A p value of <0.05 was considered to be statistically significant. The present study was approved by the Ethics Committee of Onomichi Municipal Hospital (Permission number: 14-10) and complied with the Declaration of Helsinki.

Results

The clinical characteristics of the patients in the present study are summarized in Table 1. The subjects were 8 men and 12 women, and the median age was 68.3 (IQR: 62.5-73.0) years. One case had no diffuse erythema, and three had no eschar. Sixteen cases were treated with minocycline alone. Regarding the antibiotics used to treat the five DIC cases, one and four cases were treated with minocycline alone and combination with minocycline and new quinolones, respectively. Two cases were treated with methylprednisolone. Regarding the DIC treatment of the DIC cases, one patient each was treated with recombinant thrombomodulin alone, combination with recombinant thrombomodulin and sivelestat sodium tetrahydrate, and combination with antithrombin III and gabexate mesilate. One fatal case was treated with minocycline and methylprednisolone, and the other fatal case was treated by combination therapy of minocycline and new quinolones. However, neither of the

Table 2. Differences in the Laboratory Test Results at the Time of the Initial Presentation.

	Total (n=20)	DIC (n=5)	non-DIC (n=15)	p value
WBC (×10³/μL)	5,500 (4,000-6,400)	6,100 (5,300-9,400)	6,800 (5,400-9,300)	0.87
Plt (×10⁴/μL)	11.2 (9.1-13.3)	10.7 (6.4-12.1)	12.5 (11.2-13.9)	0.23
CRP (mg/dL)	11.0 (8.6-19.6)	9.9 (8.1-20.9)	8.75 (5.32-11.95)	0.55
AST (IU/L)	69 (48-105)	101 (67-140)	67 (48-90)	0.45
ALT (IU/L)	55 (32-71)	70 (47-72)	54 (32-61)	0.55
LDH (IU/L)	340 (245-369)	354 (237-357)	332 (264-371)	0.67
ALP (IU/L)	301 (200-454)	530 (278-774)	262 (187-414)	0.042
GGT (IU/L)	91 (30-132)	119 (78-214)	37 (25-115)	0.27
T-bil (mg/dL)	0.90 (0.65-1.55)	1.00 (0.70-1.20)	0.70 (0.45-1.10)	0.27
BUN (mg/dL)	19.0 (16.2-33.2)	46.0 (31.2-69.2)	16.9 (14.4-18.8)	0.066
Cre (mg/dL)	0.90 (0.76-1.14)	1.22 (1.09-4.65)	0.87 (0.74-1.01)	0.066
PT-INR	1.17 (1.08-1.27)	1.30 (1.29-1.31)	1.10 (1.07-1.20)	0.002
FDP (μg/dL)	10.2 (5.3-17.0)	15.1 (7.6-22.5)	10.2 (5.8-15.1)	1.00
Fibrinogen (mg/dL)	397 (324-424)	328 (327-330)	405 (322-425)	0.58

All variables are expressed as the median (interquartile range). The p values are the results of comparing the DIC and non-DIC groups by Mann-Whitney's U test.

WBC: white blood cell count, Plt: Platelet count, CRP: C-reactive protein, AST: aspartate aminotransferase, ALT: alanine aminotransferase, LDH: lactate dehydrogenase, ALP: alkaline phosphatase, GGT: gamma-glutamyl transpeptidase, T-bil: total bilirubin, BUN: blood urea nitrogen, Cre: creatinine, PT-INR: prothrombin time international normalized ratio, FDP: fibrinogen degradation product

fatal cases was treated for DIC.

The laboratory test results at the time of initial presentation and the differences between the DIC and non-DIC groups are listed in Table 2. All cases showed elevated levels of AST, and 16 cases showed elevated levels of ALT. The maximum JAAM DIC score during the course of JSF was 2.0 (IQR: 0.8-3.0). While 18 surviving cases showed lower levels of ALP (≤500 IU/L) at the time of the initial presentation and a maximum DIC score ≤6 points, 1 fatal case showed high levels of ALP (1,016 IU/L) at the time of initial presentation and a maximum DIC score of 8 points. Significant differences between the DIC and non-DIC group were noted in ALP [530 (IQR: 278-774) vs. 262 (IQR: 187-415), p=0.042] and PT-INR [1.29 (IQR: 1.27-1.32) vs. 1.10 (IQR: 1.07-1.20), p=0.011]. The values on renal function tests, such as BUN and Cre, also tended to be higher in the DIC group than in the non-DIC group. A univariate regression analysis identified four significant variables at the time of initial presentation, including ALP and BUN (Table 3). A multivariate regression analysis found that only ALP and BUN remained statistically significant factors predicting the development of DIC over the course of JSF.

Discussion

In the present study, the value of ALP at the time of the initial presentation was significantly higher in the DIC group than in the non-DIC group and was a significant factor, along with BUN, predicting the development of DIC in a multivariate analysis. These results suggest that the ALP value might be a useful predictor of the clinical course in JSF patients.

It has been reported that temporary mild elevation of liver function tests in JSF patients is frequent and that liver function tests return to normal ranges within a few weeks (1). Furthermore, the disease severity and clinical characteristics of JSF patients have been investigated in several retrospective studies. One study defined severe JSF as respiratory failure, disturbance of consciousness and/or a DIC state and analyzed the aggravating factors in 28 JSF patients. Those authors reported that the initiation of adequate therapy was delayed for 6 or more days after onset in severe cases and that severe cases had significantly higher levels of WBC, FDP and CRP on admission and of soluble interleukin-2 receptor before the initiation of treatment (8). Nakamura defined severe JSF as in-hospital death and/or a DIC state and examined the correlation between disease severity and clinical parameters of 51 JSF patients. That study reported that AST, T-bil, LDH, CRP and Plt at the time of the initial presentation were significantly different between the DIC and non-DIC groups on a univariate analysis (9). These results suggest that inflammation and coagulopathy are representative parameters of the disease progression of JSF.

In the present study, the disease severity at the time of initial presentation did not appear to differ markedly between the DIC and non-DIC groups, as inflammation and coagulopathy at the time of initial presentation were not markedly different between the two groups. Nakamura also noted that a possible predictor of severity in JSF patients by a multivariate analysis was Cre ≥1.5 mg/dL at the time of the initial presentation (9). Similarly, our results showed that BUN at the time of the initial presentation was an independent predictor of the development of DIC. Another study compared the severity, serum cytokines and chemokines levels in 21 JSF patients and 37 tsutsugamushi disease patients. That study found that the clinical severity score (10) of JSF pa-

Table 3.   Logistic Regression Analyses of the Laboratory Test Results at the Time of the Initial Presentation Predicting the Development of DIC.

	Univariate analysis			Multivariate analysis		
	Odds ratio	95% confidence interval	p value	Odds ratio	95% confidence interval	p value
WBC (×10³/μL)	1.000	(1.000-1.000)	0.54			
Plt (×10⁴/μL)	0.949	(0.739-1.218)	0.68			
CRP (mg/dL)	1.067	(0.930-1.223)	0.35			
AST (IU/L)	1.015	(0.994-1.036)	0.18			
ALT (IU/L)	1.013	(0.975-1.053)	0.50			
LDH (IU/L)	0.997	(0.987-1.008)	0.63			
ALP (IU/L)	1.007	(1.000-1.015)	0.037	1.004	(1.000-1.012)	0.048
GGT (IU/L)	1.001	(0.994-1.008)	0.71			
T-bil (mg/dL)	2.495	(0.616-10.10)	0.20			
BUN (mg/dL)	1.098	(1.004-1.201)	0.021	1.078	(1.003-1.189)	0.036
Cre (mg/dL)	4.683	(0.241-90.94)	0.31			
PT-INR	132.3	(0.883-178.0)	0.17			
FDP (μg/dL)	1.055	(0.889-1.251)	0.54			
Fibrinogen (mg/dL)	0.993	(0.975-1.012)	0.46			

WBC: white blood cell count, Plt: Platelet count, CRP: C-reactive protein, AST: aspartate aminotransferase, ALT: alanine aminotransferase, LDH: lactate dehydrogenase, ALP: alkaline phosphatase, GGT: gamma-glutamyl transpeptidase, T-bil: total bilirubin, BUN: blood urea nitrogen, Cre: creatinine, PT-INR: prothrombin time international normalized ratio, FDP: fibrinogen degradation product

tients was higher than that of tsutsugamushi disease patients (11). It also noted that, in the acute phase, the levels of cytokines, such as tumor necrosis factor-α, interleukin-6 and interferon gamma, and chemokines, such as interleukin-8, interferon-inducible protein 10, monocyte chemoattractant protein 1, macrophage inflammatory protein 1α and macrophage inflammatory protein 1β, were significantly higher in JSF patients than in tsutsugamushi disease patients. However, in these previous studies, the correlation of DIC scores with the values of ALP and GGT were not examined. Therefore, to our knowledge, the present report is the first to suggest that the value of ALP at the time of the initial presentation may be a useful predictor of the occurrence of DIC over the course of JSF. In our study, one fatal case had an elevated ALP level of 1,016 IU/L at the initial presentation and a DIC score of 8 points, while the 18 surviving cases showed lower levels of ALP (≤500 IU/L) at the time of initial presentation and a DIC score ≤6 points. Another fatal JSF case with a high level of ALP (821 IU/L) was also reported elsewhere. A high ALP level may also be a useful predictor of mortality.

Rickettsiaea primarily infects the endothelium lining of vital organs, leading to tissue factor production that directly activates coagulation (12, 13). Recent studies have shown that JSF patients with hypercytokinemia develop cytokine storm or systemic inflammatory response syndrome, resulting in a severe status. Antibiotic treatments, including tetracycline and new quinolones, might modulate the cytokine production. Combination therapy of tetracycline and new quinolones is recommended in severe JSF patients (14, 15). Furthermore, the usefulness of the short-term administration of methylprednisolone, which may be able to modulate lym-

phocyte hyperactivation, has been noted in several reports of severe JSF patients (2, 3, 16). In our study, among the five DIC cases, one and four cases were treated with minocycline alone and combination with minocycline and new quinolones, respectively. Two cases were treated with methylprednisolone. One fatal case was treated with minocycline and methylprednisolone, and the other fatal case was treated by combination therapy of minocycline and new quinolones. While both fatal cases experienced severe septic shock and DIC at the initial presentation, they were not treated for DIC, and the outcomes were unfavorable.

Little is known about the mechanisms underlying hepatic injury in JSF patients. *Rickettsiae* may infect the liver sinusoidal endothelial cells but not hepatocytes, leading to mild focal hepatitis and periportal inflammation (17). In patients with "Rocky Mountain spotted fever" rickettsial infection, jaundice is a predictor of mortality and is likely the result of a combination of inflammatory bile ductular obstruction and hemolysis (18, 19). Little has also been reported on the relationship between the elevation of values on liver function tests and DIC in rickettsial infection, including JSF. It was reported that the values of ALP and GGT during early-stage sepsis-associated liver injury are useful predictors of a poor prognosis (20). Those results may indicate an association between cholestasis and the prognosis under these conditions. Liver sinusoidal hypercoagulability and microcirculatory disturbance are important mechanisms of sepsis-associated liver injury (21). Abnormal liver function test values, especially ALP values, have been suggested to reflect these pathogeneses in early-stage JSF patients. However, in our study, the value of GGT at the time of the initial presentation did not differ significantly between the DIC and non-

DIC groups, suggesting that another mechanism might be involved. Elevation of the GGT level is induced by not only cholestasis but also other causes, such as nonalcoholic fatty liver disease (NAFLD) (22), suggesting that elevation of ALP rather than GGT may reflect cholestasis in JSF patients. Further studies are needed to clarify the pathogenesis of the elevation of liver function test values and DIC in JSF patients.

It was also reported that hypercytokinemia, coagulation disorder and microcirculatory disturbance play roles in sepsis-associated acute kidney injury (23, 24). Kidney injury in JSF patients might also reflect these mechanisms, as the value of BUN as well as that of ALP at the time of the initial presentation were independent predictors of the occurrence of DIC, according to a multivariate analysis in our study.

Several limitations associated with the present study warrant mention. First, our sample size was relatively small. Second, we did not examine the serum levels of cytokines or chemokines. Although prospective studies at a single center are unlikely to be conducted due to the infrequency of the disease, we believe that future multi-center observational studies will reveal whether or not ALP levels or other biomarkers, including cytokines and chemokines, are useful for predicting DIC in JSF patients.

In conclusion, the elevation of liver function test values in JSF patients was significantly associated with a higher DIC score. In particular, the value of ALP at the time of the initial presentation was significantly higher in the DIC group than in the non-DIC group and was a significant factor, along with BUN, in predicting the development of DIC on a multivariate analysis. These results suggest that the value of ALP at the time of initial presentation might be a useful predictor of the clinical course in JSF patients. We should suspect JSF when we encounter a patient showing elevated liver function test values, a fever, and erythema. We should pay particular attention to JSF patients showing high levels of ALP at the time of initial presentation, since such patients may be more likely to develop DIC in the course of JSF than those with lower values.

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

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