Short Communication

Optimal Cutoff Value of Serum Adenosine Deaminase Activity for Diagnosing Acute Scrub Typhus

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SUMMARY: The objective of this study was to determine the optimal cutoff value of serum adenosine deaminase (ADA) activity for diagnosing scrub typhus in acute febrile patients, especially in the autumn. A total of 715 febrile patients were included; 286 of them were diagnosed with acute scrub typhus. The mean serum ADA activities in patients with and without scrub typhus were 42.8 ± 13.4 IU/L and 17.2 ± 11.5 IU/L, respectively. The optimal cutoff value for serum ADA activity was determined to be 25.0 IU/L with a sensitivity of 92.3%, specificity of 86.6%, and area under the curve of 89.4% using a receiver-operating characteristic curve analysis. The possibility of scrub typhus must be considered when acute febrile patients present with high ADA activity (>25 IU/L), especially in the autumn. Determining the optimal serum ADA activity cutoff value may help clinicians diagnose acute scrub typhus prior to serological confirmation.

Scrub typhus is mainly diagnosed by serological testing (1). However, since antibody titers increase several days after illness onset (2), diagnosing acute scrub typhus on the basis of serological testing is limited, because a definite diagnosis can only be made after a few days to a few weeks. Therefore, for patients in the acute phase of scrub typhus, the condition must be diagnosed prior to laboratory confirmation on the basis of clinical presentation and patient history. Most physicians can easily diagnose acute scrub typhus if a patient exhibits an eschar typical of scrub typhus. However, without the presence of an eschar, diagnosing scrub typhus and administering appropriate antibiotics may be delayed. Delayed administration of appropriate antibiotics is associated with an increased risk of complications (3). The evaluation of adenosine deaminase (ADA) activity in serum and other biologic fluids is useful for accurately diagnosing some pathological conditions (4). *Orientia tsutsugamushi*, an obligate intracellular bacterium, usually infects endothelial cells, macrophages, and polymorphonuclear leukocytes. *O. tsutsugamushi* can survive and replicate inside macrophages (5). ADA is released by macrophages upon stimulation by live microorganisms within them (6). Measuring ADA activity is an inexpensive, minimally invasive, quick, and readily accessible test method. Moreover, patients with scrub typhus have been reported to exhibit high ADA activity in serum (7,8).

In this study, we determined the optimal cutoff value of serum ADA activity for diagnosing scrub typhus in acute febrile patients. Scrub typhus was defined as an increased titer in the indirect immunofluorescence an-

tibody (IFA) test against *O. tsutsugamushi* ($\geq 1:256$) in a single serum sample or a ≥ 4 -fold increased titer in the follow-up (9). Patients were diagnosed with acute scrub typhus if they had a history of fever with either an eschar or a maculopapular skin eruption and >2 vague symptoms (e.g., headache, malaise, myalgia, coughing, nausea, and abdominal discomfort). Severe complications included the following conditions: pneumonia, renal failure with estimated creatinine clearance <50 using the Cockcroft-Gault formula, meningoencephalitis, shock (defined as systolic blood pressure <90 mmHg or systolic blood pressure reduced by >40 mmHg), myocarditis, gastrointestinal bleeding, and death (10). Serum ADA activity was measured by a fully automated analyzer (Modular Extension P800; Hitachi, High Technologies Cooperation, Tokyo, Japan) using a commercial kit (Diasyme Laboratories, San Diego, Calif., USA). This study was approved by the institutional review board of Wonkwang University Hospital.

This retrospective study was conducted at Wonkwang University Hospital, an 822-bed tertiary care hospital located in Iksan, Korea. Between September 2008 and December 2011, 1,469 adult febrile patients (≥ 18 years old) were serologically tested for scrub typhus. Among them, the 768 who were also tested for serum ADA activity were initially selected. We then excluded 53 patients who were clinically suspected of having scrub typhus without serological confirmation or were transferred to our hospital after having already been diagnosed with scrub typhus. Thus, a total of 715 patients were ultimately included in this study. During the study period, 293 patients were diagnosed with acute scrub typhus; 286 patients (97.6%) of them were included in the study. The mean ages of the patients with and without scrub typhus were 63.5 and 61.7 years, respectively. The mean serum ADA activities in patients with and without scrub typhus were 42.8 ± 13.4 IU/L and 17.2 ± 11.5 IU/L, respectively (Table 1). Receiver-operating characteristic (ROC) curve analysis deter-

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Table 1. Characteristics of the study population

	Scrub typhus (<i>n</i> = 286)	Non-scrub typhus (<i>n</i> = 429)	<i>P</i>
Age y, mean (SD)	61.7 ± 23.4	63.5 ± 14.5	0.269
Female (%)	59.1	47.3	0.002
Initial laboratory findings			
Leukocyte (/mm ³)	7,696 ± 3,555	10,575 ± 6,521	<0.001
C-reactive protein (mg/L)	94 ± 120.7	112.9 ± 462.7	0.503
Serum ADA activity (IU/L)	42.8 ± 13.4	17.2 ± 11.5	<0.001

ADA, adenosine deaminase.

Table 2. Cutoff values of serum ADA activity for the diagnosis of acute scrub typhus

Cutoff value of serum ADA activity (IU/L)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	AUC (%)
≥ 24	93.7	84.7	95.3	80.2	89.2
≥ 25	92.3	86.6	94.4	82	89.4
≥ 26	90.2	88.2	93.2	83.5	89.2

ADA, adenosine deaminase; PPV, positive predictive value; NPV, negative predictive value; AUC, area under curve.

mined the optimal cutoff value of serum ADA to be 25.0 IU/L with a sensitivity of 92.3% and a specificity of 86.6% (Table 2). The area under the ROC curve was 0.894 (95% confidence interval, 0.866–0.918). Among the 286 patients with acute scrub typhus, 219 (76.6%) presented with typical eschars. There was no significant difference in serum ADA levels between the patients with and without eschars (42.6 ± 13.1 IU/L versus 43.5 ± 14.5 IU/L; $P = 0.6$). Among the patients with acute scrub typhus, 31% (89/286) had severe scrub typhus-related complications. The serum ADA levels of the patients with severe complications were higher than those of the patients without severe complications (44.7 ± 13.1 IU/L versus 42.0 ± 13.5 IU/L), although the difference was not significant ($P < 0.113$). A total of 69.6% (199/286) of patients with acute scrub typhus had positive IFA test results on admission. Patients with positive initial IFA test results had higher serum ADA levels than those with negative initial IFA test results (44.7 ± 13.2 IU/L versus 38.6 ± 13.1 IU/L; $P < 0.001$).

In the present study, the optimal cutoff value of serum ADA for a scrub typhus diagnosis was 25 IU/L with a sensitivity of 92.3% and specificity of 86.6%. Patients with scrub typhus had significantly higher serum ADA activities than patients without scrub typhus. This finding indicates serum ADA activity is a useful parameter for differentiating between patients with and without acute scrub typhus. In particular, serum ADA activity is useful for the early diagnosis of scrub typhus in cases not presenting with eschar formation. In the present study, the IFA test (results were considered positive if the titer was $\geq 1:40$) was used to confirm the diagnosis; 30.4% of patients with scrub typhus had negative initial IFA test results. This finding suggests that physicians should perform follow-up IFA testing on acute febrile patients who initially have negative IFA results and a serum ADA level > 25 IU/L, especially during the endemic season. In Korea, scrub typhus shows that seasonal variation occurs in patients with fever, peaking

during October and November (11). Most of the patients (82.9%, 593/715) in the present study were enrolled between September and December. Various genotypes including the Boryong, Kato, Neimeng-65, Kawasaki, and Gilliam strains were present (12). The Boryong serotype was predominant among them and was distributed nationwide (12,13). As expected, patients with other acute febrile illnesses (13.3%, 57/429) such as pulmonary tuberculosis, acute viral hepatitis, malaria, hemophagocytic syndrome, and lymphoma also exhibited elevated serum ADA activity (> 25 IU/L). Thus, serum ADA levels should be considered in addition to clinical findings to establish a diagnosis of scrub typhus. The present study has some limitations. First, we could not theoretically exclude a history of scrub typhus when a patient was diagnosed with acute scrub typhus solely on the basis of a single increased IFA titer ($\geq 1:256$). Second, this study was performed in an endemic region of scrub typhus; therefore, most patients were enrolled during the endemic season of scrub typhus. Third, other acute febrile illnesses can also increase serum ADA activity. Therefore, the applicability of the present results to other regions may be limited, because other diseases that increase serum ADA levels are also prevalent. Accordingly, epidemiological information such as endemic seasons and other endemic diseases in this research site should be considered if serum ADA activity is evaluated to diagnose scrub typhus. However, scrub typhus must be considered a possible cause when acute febrile patients present with high ADA activity (> 25 IU/L), especially in the autumn. Furthermore, serum ADA activity should be considered a part of the clinical examination to establish a firm diagnosis of scrub typhus. The determination of serum ADA activity may help with the diagnosis of acute scrub typhus prior to laboratory confirmation during the endemic season.

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Conflict of interest None to declare.

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