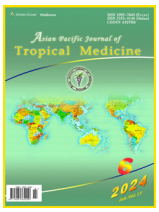

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Real-world utility of serological tests in patients with suspected scrub typhus in the Republic of Korea: A single-center, retrospective, observational study

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ABSTRACT

Objective: Serological tests are widely used for scrub typhus diagnosis; however, their limitations are evident. This study aims to assess their practical value in clinical settings.

Methods: We analyzed the data of adult patients with suspected scrub typhus who visited a tertiary care hospital in the Republic of Korea from September to December from 2019 to 2021. The included patients had an acute fever and at least one of the following ten secondary findings: myalgia, skin rash, eschar, headache, thrombocytopenia, increased liver enzyme levels, lymphadenopathy, hepatomegaly, splenomegaly, and pleural effusion. The diagnoses were grouped as scrub typhus or other diseases by two infectious disease physicians.

Results: Among 136 patients who met the eligibility criteria, 109 had scrub typhus and 27 had different diseases. Single and paired total antibodies using immunofluorescence assay (IFA), and total antibodies using immunochromatography-based rapid diagnostic testing (ICT) were measured in 98%, 22%, and 75% of all patients, respectively. Confirmation using paired samples for scrub typhus was established at a median of 11 [interquartile range (IQR) 10-16] days following the first visit. Among the 82 admitted patients, the median admission time was 9 (IQR 7-13) days. According to IFA, 58 (55%) patients with scrub typhus had total immunoglobulin titers ≥ 1:320, while 23 (85%) patients with other disease had titers < 1:320. Positive ICT results were observed in 64 (74%) patients with scrub typhus and 10 (67%) patients with other diseases showed negative ICT results.

Conclusions: Serological testing for scrub typhus is currently insufficient for decision-making in clinical practice.

KEYWORDS: Scrub typhus; Serological test; Immunofluorescence assay; Immunochromatography; Rapid detecting test

1. Introduction

Scrub typhus is caused by *Orientia (O.) tsutsugamushi*, a Gram-negative intracellular bacterium, and it is a serious public health problem in endemic areas known as the "Tsutsugamushi triangle", which extends from Southeast Asia to the Pacific Ocean[1]. It is transmitted by the larvae of trombiculid mites and typically presents as an acute febrile illness with rash, eschar at the bite site, headache,

Significance

For diagnosis of scrub typhus, serological tests are the most widely used in clinical practice; however, they have limitations. The findings of this study provide further insight into the real-world utility of serological tests in patients with suspected scrub typhus and encourage the development of more useful alternative microbiological testing methods.

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myalgia, generalized lymphadenopathy, and elevation of liver transaminases[2].

Diagnosis of scrub typhus is difficult because its clinical characteristics mimic other acute febrile diseases, such as dengue fever, malaria, chikungunya, typhoid fever, and leptospirosis[3,4]. It is one of the representative vector-borne diseases in the Republic of Korea, and their similar clinical presentations make distinguishing them challenging[5-7]. Therefore, a definitive diagnosis of scrub typhus relies on serological, molecular, and microbiological methods. Several methods have been developed for the diagnosis of scrub typhus, including immunofluorescence assays (IFA), enzyme-linked immunosorbent assays (ELISA), immunochromatography-based rapid diagnostic tests (ICT), and polymerase chain reaction (PCR)[8]. In most cases from the Republic of Korea, physicians request IFA from commercial or reference laboratories or conduct ICT at their hospitals to diagnose scrub[9-11]. IFA is considered the gold standard technique for diagnosing scrub typhus; however, it requires paired samples and sophisticated instrumentation. Additionally, there is no standard antibody titer cutoff value for the prompt diagnosis of scrub typhus[11,12]. ICT has recently been considered a point-of-care diagnostic system for detecting scrub typhus[13]; however, poor sensitivity and significant heterogeneity in diagnostic accuracy between ICT kits have been reported[14,15].

Several studies have evaluated the performance of various diagnostic tests using samples from patients with PCR-confirmed scrub typhus[16,17]. However, implementation of these diagnostic tests in clinical practice has been difficult because of issues regarding standardization, reproducibility, robustness, and cost. Therefore, in clinical practice, the diagnosis of scrub typhus depends on various factors, including the patient's medical history, physical examination, detailed account of the clinical features, and diagnostic test results. In addition, several studies have reported the limitations of serological tests that are widely used at present[8,11,12,18]. However, these limitations have not been properly evaluated in clinical situations; thus, the significance of these tests has been overlooked. This study aimed to investigate the role of these serological tests in the diagnosis of scrub typhus at a single medical center in the Republic of Korea.

2. Subjects and methods

2.1. Study design and data collection

All adult patients with suspected scrub typhus who visited the hospital during the autumn season (September–December) of 2019, 2020, and 2021 were screened retrospectively. This study was performed at a 1 300-bed, university-affiliated tertiary-care teaching hospital in Gyeongsangnam-do, Republic of Korea. Gyeongsangnam-do was recognized as an endemic area for scrub typhus, with an average of 37.45 cases/100 000 population/year in 2019–2021, and approximately 90% of patients were reported in the autumn season[19]. Information regarding demographics, pre-existing medical conditions, symptoms and signs at presentation, microbiological and imaging findings, type and duration of antimicrobial therapy, and patient outcomes was collected following a chart review of patient data.

Scrub typhus was defined by the presence of (1) acute onset fever; (2) at least one of these symptoms or signs, including skin rash, eschar, headache, thrombocytopenia, elevated liver enzymes, lymphadenopathy, hepatosplenomegaly, or pleural effusion; and (3) symptom improvement after appropriate antibiotics (*e.g.*, doxycycline, rifampin, azithromycin, or clarithromycin)[20]. We classified patients as having other diseases when alternative clinical diagnoses were obtained from the diagnostic criteria using clinical, laboratory, imaging, or histopathological findings. Additionally, patients without a definitive diagnosis who demonstrated no significant change in IFA antibody titers between the two samples obtained from the acute and convalescent phases were included in the other disease group. The clinical diagnoses were independently determined by two infectious disease physicians and classified into two groups: (1) scrub typhus and (2) other diseases. Patients who could not be followed up and classified into either group were excluded from the analysis.

2.2. Ethics

This study was approved by the Institutional Review Board of the Pusan National University Yangsan Hospital (05-2023-142). The need for informed consent was waived because of the retrospective nature of the study.

2.3. Serological testing

Diagnostic tests for scrub typhus were performed using commercially available IFA and ICT kits. Serum samples were sent to the Green Cross Reference Laboratory in the Republic of Korea for IFA. For IFA, two-fold serial dilutions from a 1:40 dilution of each patient's serum were reacted with mixed *O. tsutsugamushi* antigens to detect the total immunoglobulins (IgM/IgG/IgA). The IFA-positive cutoff value for scrub typhus was ≥1:40 for total immunoglobulins (Ig)[9,11,18]. ICT was performed in our hospital laboratory using a commercially available kit (BiolineTM Tsutsugamushi, Abbott Molecular Inc., Des Plaines, IL, USA) that measures the total antibody (IgM/IgG/IgA) in serum samples using *O. tsutsugamushi* antigens.

2.4. Statistical analysis

The clinical and laboratory test results were compared between patients with scrub typhus and other diseases. Continuous data were described as medians and interquartile ranges (IQR), and the Mann–Whitney *U* test was used to compare continuous variables. Fisher's exact test was used to compare categorical variables. All tests were two-tailed, and differences were considered statistically significant at *P* values of <0.05. Statistical analyses were performed using the SPSS software (version 23.0; SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Patient characteristics

In total, 172 patients with suspected scrub typhus were enrolled in this study (Figure 1). Thirty-two patients were lost to follow-up, and four with indeterminate diagnoses were excluded from the final analysis. Of the remaining 136 patients, 109 were included in the scrub typhus group and 27 in the other disease group. The other disease group exhibited bacterial infections (*n*=15, 55%), including urinary tract infection (*n*=7), primary bacteremia (*n*=2), cellulitis (*n*=1), endocarditis (*n*=1), enterocolitis (*n*=1), liver abscess (*n*=1), pneumonia (*n*=1), and vertebral osteomyelitis (*n*=1); malignancy (*n*=4, 15%); adverse drug reactions (*n*=3, 11%); dengue fever (*n*=1, 4%); nontuberculous mycobacteria lung infection (*n*=1, 4%); and no definitive diagnosis warranted by serological test results (*n*=3, 11%). The clinical characteristics of the 109 patients with scrub typhus and the 27 with other diseases are shown in Table 1. Patients in the scrub typhus group engaged in significantly more outdoor activities within 3 weeks of symptom onset than those in the other disease group did (69% *vs.* 44%, *P*=0.03) and had a significantly higher incidence of skin rash (56% *vs.* 19%, *P*<0.01) and liver enzyme elevation (66% *vs.* 37%, *P*<0.01). None of the patients in the other disease group had an eschar. All patients with scrub typhus received appropriate antimicrobial treatment for *O. tsutsugamushi*. Most patients received doxycycline (97%), and some patients who discontinued doxycycline owing to adverse effects received clarithromycin (2%) or azithromycin (1%). More than half (*n*=16; 59%) of the patients in the other disease group received effective antimicrobial agents against *O. tsutsugamushi*. Additionally, 72 (66%) patients with scrub typhus received other agents without antimicrobial effects against *O. tsutsugamushi*.

The median time from illness onset to hospital visit was 3 (IQR, 2–6) days, with no significant difference between the scrub typhus and other disease groups [4 (IQR 2–6) days *vs.* 3 (IQR 0–7) days, *P*=0.17]. Exactly 54 (40%) patients received oral antimicrobials

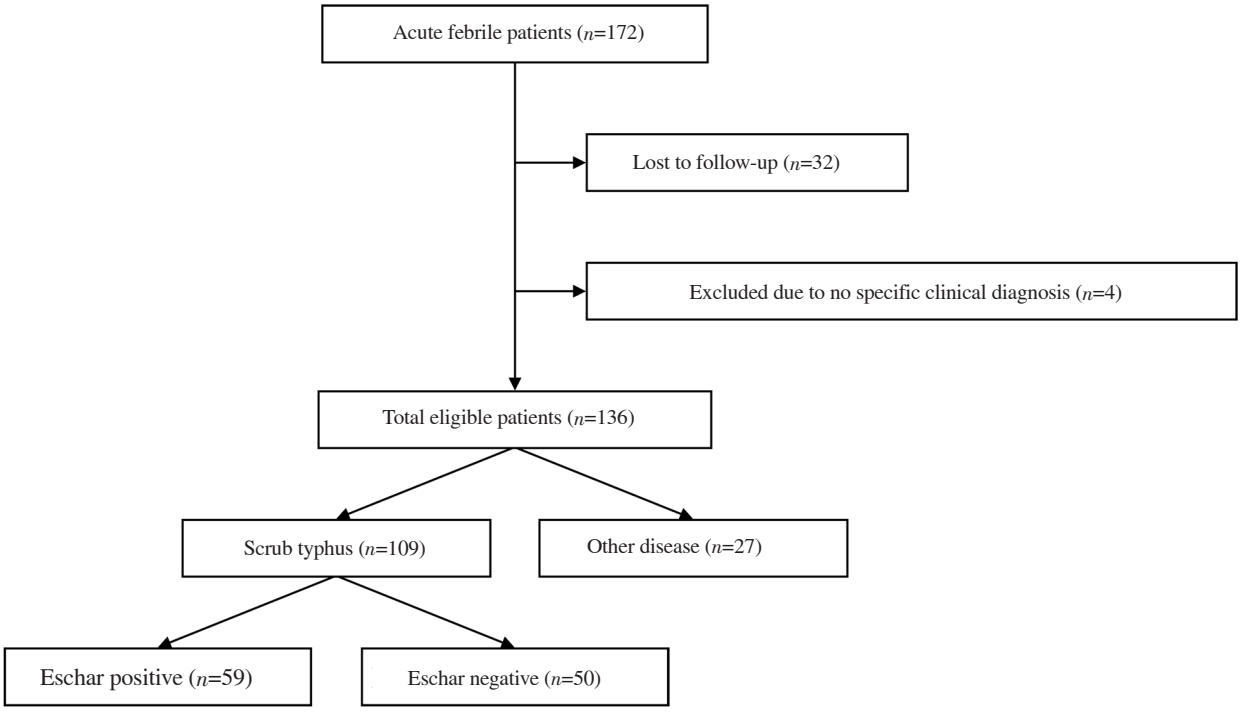

Figure 1. Flow chart of the patient selection process.

Table 1. Clinical characteristics of 136 patients with suspected scrub typhus.

Characteristics	Total (*n*=136)	Scrub typhus (*n*=109)	Other diseases (*n*=27)	*P* value*
Age, median (IQR), year	66 (60–76)	66 (60–74)	69 (65–74)	0.27
Male sex	64 (47)	50 (46)	14 (52)	0.67
Exposure to field	87 (64)	75 (69)	12 (44)	0.03
Underlying disease†	37 (27)	28 (26)	9 (33)	0.47
Clinical symptoms				
Eschar	59 (43)	59 (54)	0 (0)	<0.01
Myalgia	42 (31)	35 (32)	7 (26)	0.65
Skin rash	66 (49)	61 (56)	5 (19)	<0.01
Headache	17 (13)	16 (15)	1 (4)	0.19
Lymphadenopathy	20 (15)	16 (15)	4 (15)	0.99
Hepatomegaly	2 (2)	2 (2)	0 (0)	0.99
Splenomegaly	15 (11)	12 (11)	3 (11)	0.99
Pleural effusion	48 (35)	38 (35)	10 (37)	0.99
Laboratory findings‡				
Thrombocytopenia	74 (54)	62 (57)	12 (44)	0.28
Increased liver enzyme	82 (60)	72 (66)	10 (37)	<0.01
Ward admission	62 (46)	42 (39)	20 (74)	<0.01
ICU admission	20 (15)	16 (15)	4 (15)	0.99
Mortality	5 (4)	3 (3)	2 (7)	0.26

Abbreviations: ICU: intensive care unit; IQR: interquartile range. Data are expressed as the number (%) of patients unless otherwise indicated. †Underlying diseases included diabetes mellitus, chronic respiratory disease, chronic renal failure, congestive heart failure, liver cirrhosis, malignancy, and immunosuppressive treatment. *Mann-Whitney *U* test or Fisher's exact test; *P*<0.05 were considered statistically significant. ‡Thrombocytopenia was defined as a platelet count of < 140 000/mm^3. Increased liver enzyme levels were defined as serum AST or ALT levels that were >1.5 fold the upper limit of normal.

and underwent outpatient follow-up visits; 62 (46%) required admission to the general ward, and 20 (15%) required admission to the intensive care unit (ICU). The proportion of outpatient follow-up visits significantly differed between the scrub typhus and other disease groups (47% *vs.* 11%, *P*<0.01, respectively). Among the 82 patients admitted to the general ward or ICU, the median admission time was 9 (IQR 7-13) days. The median follow-up time for all patients was 13 (IQR 8-19) days. There was no significant difference in mortality between the two groups (3% *vs.* 7%, *P*=0.26).

Of the 109 patients with scrub typhus, 59 (54%) had eschar, and the remaining 50 (46%) did not. The clinical characteristics of patients with and without eschar are shown in Supplementary Table 1. On comparing patients with and without eschar, it was found that the number of outpatient follow-up visits was significantly higher in patients who had eschar (58% *vs.* 34%, *P*=0.02), and the proportion of ICU admission was significantly lower in patients who had eschar (7% *vs.* 24%, *P*=0.02). Furthermore, the use of other agents without antimicrobial effects against *O. tsutsugamushi* tended to be less frequent in patients with eschar than in those without eschar (59% *vs.* 74%, *P*=0.16). Among patients who received other agents without antimicrobial effects against *O. tsutsugamushi*, the median duration of antimicrobial administration was 7 (IQR 7-12) days. Other clinical characteristics were not significantly different between the two groups.

3.2. IFA and ICT results in patients with suspected scrub typhus

Single and paired total Ig using IFA and total antibody using ICT were measured in 98%, 22%, and 75% of all the patients, respectively (Table 2). In the scrub typhus group, the total Ig titer measured by IFA increased from 0-7 days after symptom onset, peaked at 14-20 days, and subsequently declined (Figure 2). Seroconversion, or more than a four-fold rise in the antibody titer, was observed in 20 patients. In the other disease group, paired sampling of 10 patients was performed, and no significant serological changes were observed (Table 2). The median interval between the first and second sampling was 7 (IQR 6-8) days. The final laboratory confirmation using paired samples for scrub typhus was established at a median of 11 (IQR 10-16) days following the first visit. There were 81 (76%) patients with total Ig titers ≥1:40 in the scrub typhus group and 4 (15%) with total Ig titers <1:40 in the other disease group. When the cutoff value for total Ig titer was changed to 1:320, 58 (55%) patients had titers ≥1:320 in the scrub typhus group, while 23 (85%) had titers <1:320 in the other disease group. Sixty-four (74%) patients showed positive ICT results in the scrub typhus group while 10 (67%) showed negative ICT results in the other disease group.

Table 2. Results of commercially available serological tests for scrub typhus in 136 patients with suspected scrub typhus.

Variables	Scrub typhus		Other diseases	
	No. of samples	No. of patients	No. of samples	No. of patients
IFA single test results	126	106*	37	27
Single positive results, titer ≥1:40	93	81 (76%)	31	23 (85%)
Single negative results, titer <1:40	33	25 (24%)	6	4 (15%)
Single positive results, titer ≥1:320	61	58 (55%)	5	4 (15%)
Single negative results, titer <1:320	65	48 (45%)	32	23 (85%)
Paired test results	20	20	10	10
Seroconversion or 4-fold rise	20	20 (100%)	0	0 (100%)
No rising antibody titer	0	0 (0%)	10	10 (0%)
ICT test results	87	87†	15	15†
Positive	64	64 (74%)	5	5 (33%)
Negative	23	23 (26%)	10	10 (67%)

Abbreviations: ICT, immunochromatography-based rapid diagnostic tests; IFA, immunofluorescence assay; *The 3 patients who did not undergo IFA test were excluded; Of 136 patients, 133 patients (106 patients with scrub typhus and 27 patients with other diseases) underwent IFA testing at least once. Of 106 patients with scrub typhus, 86 had one (first) IFA result per patient, and 20 had two (first & second) IFA results per patient. Of 27 patients with other diseases, 17 had one (first) IFA result per patient, and 10 had two (first & second) IFA results per patient. Therefore, the number of samples and the number of patients are not the same; †Of the 136 patients, 102 (87 patients with scrub typhus and 15 patients with other diseases) recived the ICT test.

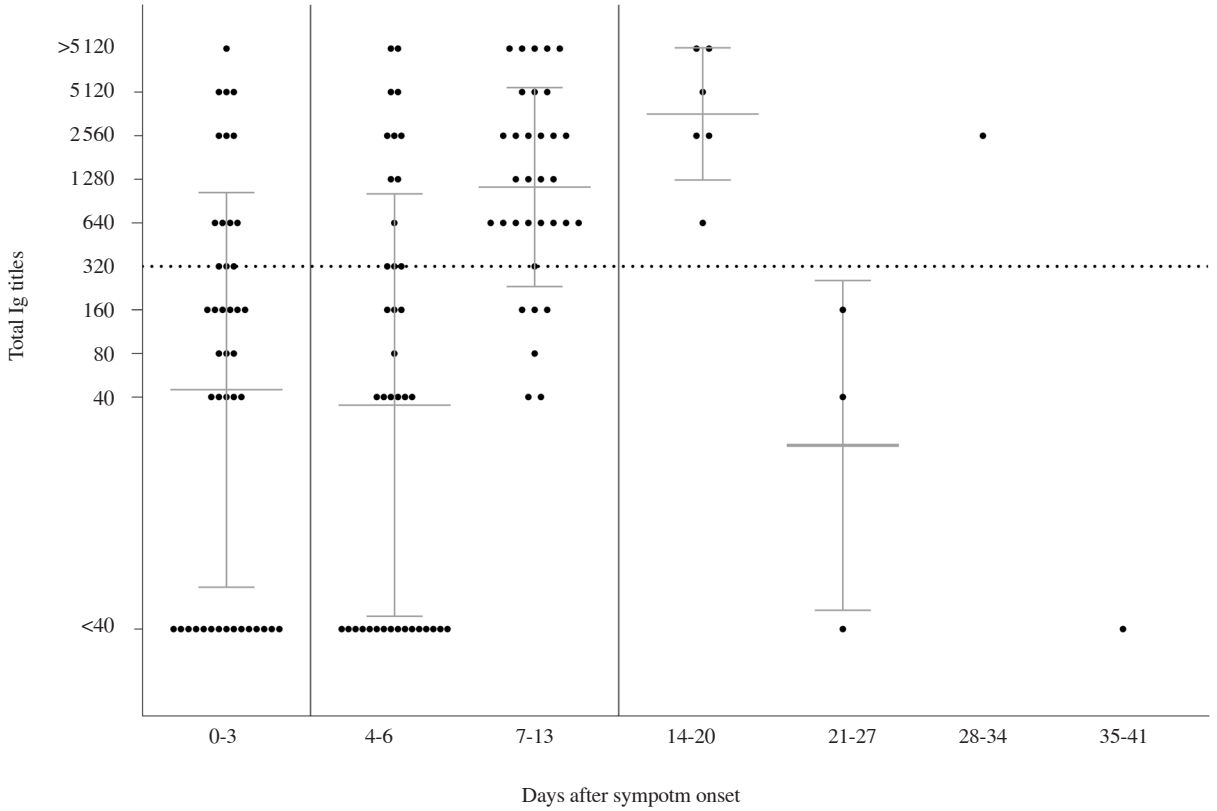

Figure 2. Total Ig titer against *Orientia tsutsugamushi* in blood samples throughout the disease course. Blood samples were obtained from patients with scrub typhus between 0-39 days after symptom onset. Medians and quartiles are represented by the gray lines.

4. Discussion

In this study, we investigated the usefulness of serological tests in the diagnosis of scrub typhus. Of the 136 patients with suspected scrub typhus, we diagnosed 109 with scrub typhus and 27 with other diseases. In clinical practice, single and paired total Ig using IFA and total antibody using ICT were measured in 98%, 22%, and 75% of the patients, respectively. Neither serological test was useful for diagnosing scrub typhus because of its long turnaround time or low accuracy.

Several previous studies have developed clinical prediction rules to improve diagnosis, as laboratory testing is required for a definitive diagnosis of scrub typhus[21,22]. Both prediction models include eschar as a parameter, and the presence of eschar significantly contributes to the accuracy of diagnosis[4]. In this study, none of the patients in the other disease group had eschar, and the use of unnecessary antimicrobial agents in patients without eschar in this group was significantly higher than in patients with eschar in the scrub typhus group (38% *vs.* 17%, P=0.02). These findings suggest that the presence of eschar could serve as a highly definitive diagnostic indicator in patients with suspected scrub typhus and affect the treatment in clinical practice. However, only half of the patients in the scrub typhus group had eschar, similar to the findings of previous reports[21,23,24].

IFA is the most commonly used diagnostic method for scrub typhus, and our study also showed that 98% of all the included patients suspected of having scrub typhus underwent IFA. In a previous study evaluating the diagnostic accuracy of antibody assays for scrub typhus in the Republic of Korea, the sensitivity and specificity of the commercial IFA for total Ig were 70.10% (95% *CI* 60.05%-79.0%) and 74.59% (95% *CI* 67.7%-80.7%), respectively, with a cutoff value of ≥1:40[11]. When the cutoff value was ≥1:320 in the same study, the sensitivity decreased to 55.67% (95% *CI* 45.2%-65.8%), while the specificity increased to 95.14% (95% *CI* 91.0%-97.8%). Similarly, this study showed 81 (76%) patients had total Ig titers ≥1:40 and 58 (55%) had total Ig titers ≥1:320 in the scrub typhus group. On the other hand, 4 (15%) and 23 (85%) patients had total Ig titers <1:40 and <1:320, respectively, in the other disease group. Although there were differences in values between the two studies, they were similar in that the sensitivity and specificity values considerably depended on the cutoff value. In summary, IFA has several limitations when single acute-phase serum samples are collected: 1) wide variation in cutoff titers of scrub typhus seropositivity across different countries, 2) lag time between infection and antibody formation, 3) inter-individual variation in antibody response to scrub typhus, and 4) indistinguishability between current and past infections, especially in endemic areas[11,12,18]. To overcome these limitations, it is essential to collect two sequential samples to determine antibody response dynamics. An least a four-fold increase in antibodies in paired samples could definitively diagnose acute scrub typhus infection, but this is difficult in clinical practice. In our study, paired samples for IFA were collected from only 22% of all the included patients, and the median duration from the first visit to final laboratory confirmation was 11 (IQR 10-16) days. The median admission time was 9 (IQR 7-13) days, and the median follow-up time for all patients was 13 (IQR 8-19) days. In other words, the final laboratory confirmation was reported to physicians when patients had already been discharged and did not affect clinical decision-making.

ICT is a simple and rapid serological test that does not require sophisticated instrumentation; thus, it is considered a point-of-care diagnostic system[8]. Of all the enrolled patients, 102 (75%) received ICT for scrub typhus, 64 (74%) had positive results for ICT in the scrub typhus group, and 10 (67%) had negative results for ICT in the other disease groups. A recent meta-analysis revealed that the sensitivity and specificity of commercially available ICTs were 66.0% (95% *CI* 0.37-0.86) and 92.0% (95% *CI* 0.83-0.97), respectively. However, they also found a significantly high degree of heterogeneity among the studies[14]. Even within the same study population, there were differences in sensitivity and specificity among commercial ICTs[11].

In the Republic of Korea, physicians often empirically prescribe doxycycline to patients with suspected scrub typhus based on their clinical experience because of the low clinical applicability of commercial diagnostic methods. Empirical therapy has reduced the complications associated with scrub typhus, corresponding to a decrease in morbidity and mortality over several decades[25]. However, newly emerging diseases such as severe fever with thrombocytopenia syndrome, anaplasmosis, and Q fever have become major public health concerns in the Republic of Korea. The incidence of other vector-borne infections is also continuously increasing due to global warming and increased outdoor activities[5,7]. Moreover, although the incidence of murine typhus seemed to be on the decline, it has been consistently reported every year[19], additionally, reports of spotted fever group rickettsiosis cases have recently increased[26-29]. Therefore, rapid and accurate differentiation between these diseases is critical because a strategic treatment approach is required for each infectious disease to improve clinical outcomes. PCR detection of specific DNA for *O. tsutsugamushi* has been increasingly used to diagnose scrub typhus with high specificity and the advantage of early diagnosis. PCR sensitivity for scrub typhus varies widely, ranging from 20.6% to 91.9%, depending on the detection method and study design[17,30-32]. Currently, only specialized laboratories perform PCR using in-house protocols, and there is a lack of reliable and commercially available diagnostic test kits[11,33]. Nevertheless, PCR will become the standard test for diagnosing scrub typhus, ultimately replacing

serological tests[8].

This study had a few limitations. First, we could not enroll a sufficient number of patients with scrub typhus to describe the real-world clinical settings. Second, because we enrolled patients who visited a tertiary-care hospital, patients with scrub typhus who presented with mild disease may not have been included. Third, we initially enrolled 172 patients with suspected scrub typhus; however, 36 (21%) patients were excluded from the study due to loss-to-follow-up or intermediate diagnoses. This may have introduced selection bias that affected our study outcomes. Fourth, we used the IFA test which measured total Ig instead of measuring IgM and IgG separately. Because of the consistency and relatively short turnaround time of the test, we selected the IFA test; however, there was no significant difference in diagnostic accuracy between IgM-IFA, IgG-IFA, and total Ig-IFA tests according to previous reports[11]. The area under the curve values for the sensitivity and specificity of IgM-IFA, IgG-IFA, and total Ig-IFA were 0.763 (95% *CI* 0.709-0.813), 0.714 (95% *CI* 0.657-0.767), and 0.788 (95% *CI* 0.735-0.835), respectively[11]. We considered that the measurement of total Ig did not significantly impact the results; nonetheless, further evaluation is needed.

In summary, we analyzed 136 patients with suspected scrub typhus and investigated the diagnostic process for scrub typhus in an endemic area during an epidemic in the Republic of Korea. Neither IFA nor ICT was sufficient to diagnose scrub typhus. Further, they had low clinical usefulness and did not contribute to clinical decision-making. There have been several reports that serological tests show limitations in the diagnosis of scrub typhus; nonetheless, serological methods are still used in clinical practice and the need for the introduction of a new standard test for scrub typhus has been neglected. Therefore, this study could be helpful as it re-recognized the limitations of serological testing and to promote the development of an alternative microbiological testing method for rapid and accurate diagnosis of scrub typhus.

Conflict of interest statement

The authors declare no potential conflicts of interest.

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Authors' contributions

Both S.K. and A.R.K. authors contributed to the data curation. M.B. performed the analytic calculations. Both S.K. and M.B. authors contributed to the final version of the manuscript. Both S.L. and S.J.L. supervised the project and reviewed the manuscript.

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