EVALUATION OF A NEWLY DEVELOPED DIPSTICK TEST FOR THE RAPID DIAGNOSIS OF SCRUB TYPHUS IN FEBRILE PATIENTS

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Abstract. Scrub typhus is a potentially fatal, febrile disease prevalent in rural Asia. The etiological agent, *Orientia tsutsugamushi*, is transmitted to humans by the bite of a larval trombiculid mite. No current diagnostic test is sufficiently practical for use by physicians working in rural areas. A new dipstick test using a dot blot immunoassay format has been developed for the serodiagnosis of scrub typhus. We evaluated this test on 83 patients presenting with acute fever of unknown origin at Maharaj Hospital, a tertiary care medical center in Nakhon Ratchasima, Northeast Thailand. The diagnosis of scrub typhus was confirmed in 30 of these patients (36%) by the indirect immunoperoxidase test. The sensitivity of the test was 87% and its specificity was 94%. The dot blot immunoassay dipstick is accurate, rapid, easy to use, and relatively inexpensive. It appears to be the best currently available test for diagnosing scrub typhus in rural areas where this disease predominates.

INTRODUCTION

Scrub typhus is an acute febrile disease caused by *Orientia tsutsugamushi*. The organism is transmitted to humans by the bite of a larval trombiculid mite (chigger). It is a common cause of febrile illness in rural areas of Asia in both indigenous inhabitants and visitors who have contact with rice fields, scrub or secondary growth forest (Grown *et al*, 1976; Olson and Bourgeois, 1977). The number of reported cases of scrub typhus increased in Thailand between 1984 and 1995, with annual rats ranging from 1,000 to 1,500 cases. Fifty-one cases occurring during the rainy season (Ministry of Public Health, 1993).

A typical eschar is pathognomonic for scrub typhus but is present in a minority of patients. In the absence of an eschar, the clinical picture of scrub typhus is nonspecific and the diagnosis is often based on a therapeutic trial with doxycycline or chloramphenicol. The diagnosis is generally confirmed serologically by either an immunofluorescent assay (IFA) or an indirect immunoperoxidase (IIP) test. However, these techniques require a specialized laboratory and their use is generally limited to reference centers (Kelly *et al*, 1988; Brown *et al*, 1983; Sugita *et al*, 1992). The Weil-Felix test is commercially available, simple to perform and widely used but lacks both sensitivity and specificity (Brown *et al*, 1983; Chouriyagune *et al*, 1992). The absence of a practical confirmatory test for scrub typhus in rural areas, coupled with its wide range of clinical manifestations (Olson and Bourgeois, 1977) has led to underdiagnosis and a large discrepancy between the reported and the true incidence of scrub typhus in Thailand (Takada *et al*, 1984; Strickman *et al*, 1994; Johnson *et al*, 1982).

A practical commercially available test would both improve patient care and provide more accurate data on the true incidence of scrub typhus. A dipstick test, using a dot blot immunoassay format, has recently been developed for the rapid diagnosis of *O. tsutsugamushi* infection. The dipstick was 90%

sensitive and 83% specific compared to IFA in diagnosing scrub typhus on pre-collected Malaysian sera (Weddle *et al*, 1995). However, there are few data on the usefulness of this test in the clinical setting. We therefore performed a prospective investigation of the dipstick test in patients presenting to hospital with acute febrile diseases.

MATERIALS AND METHODS

Dipstick test for scrub typhus

The dipstick dot blot immunoassay (Dip-S-ticks^{R}, Integrated Diagnostics, Baltimore, MD, USA) was performed as described by Weddle *et al* (1995) with antigen bound to a nitrocellulose membrane. Each dipstick incorporated 4 antigen dots diluted to be approximately equivalent in reactivity to reciprocal anti-Karp antibody titers measured by the indirect immunoperoxidase test (IIP) of 400, 1,600, 6,400; and titers to a 1:1 mixture of Kato and Gilliam strains. The dipstick procedure detects total antibody (IgG and IgM). A positive and negative control dot are incorparated onto every stick. Positive dots were purple with a distinct border within the window of the mask. Negative dots were usually completely white, but were sometimes indistinct or lacked a distinct border. The number of positive dots was counted for each dipstick such that a positive test could have between 1 and 4 positive dots.

Patients

Three physicians (FC, KA, HL) without previous laboratory experience evaluated the dipstick test on patients presenting to Maharaj Nakhon Ratchasima Hospital in Northeast Thailand with acute FUO. The study began in mid-November of 1995 and was completed in early January 1996. Inpatients and outpatients 14 years or older with an oral temperature of at least 37.8ºC were included in the study. Patients were allocated into 3 groups. Group A comprised patients with a positive dipstick test result. Group B included patients admitted to the medical ward with a negative dipstick result. Group C as made up of patients who tested negative by dipstick were not admitted to hospital. The decision to admit patients with a negative dipstick result was made by a physician not involved in the study. All patients with a positive dipstick were admitted. Serotesting was also performed on healthy farmers in a village 60 km from Nakhon Ratchasima to assess baseline anti-*O. tsutsugamushi* antibody titers.

Laboratory Investigations

CBC, routine biochemistry, blood cultures, thick and thin film for malaria, HIV serology, electrocardiogram and chest radiography were performed on the first day of admission on hospitalized patients. The Widal test and the Weil-Felix test were performed on day 0 and at follow-up.

Indirect immunoperoxidase test

The indirect immunoperoxidase (IIP) test was performed on day 0 and at follow-up. Antibodies to *O. tsutsugamushi* were measured using a pool of Karp, Kato and Gilliam antigen (Kelly *et al*, 1988). Briefly, diluted serum was incubated on spots of antigen at 37ºC for 45 minutes. The slide was then rinsed and incubated with goat affinity purified peroxidase-conjugated anti-human IgG or IgM (Kirkegaard and Perry, Gaithersburg, MD, USA). After another rinse, the color reaction was developed with diaminobenzidine tetrahydrochloride (DAB) substrate and contrast green counterstaining (Kirkegaard and Perry, Gaithersburg, MD, USA). A positive reaction results in brown staining of rickettsial particles. The diagnosis of scrub typhus was made if there was either a four-fold or greater rise in IIP titer to at least 1:200, or if a single IIP test detected IgM antibody titers of $\geq$ 1:400 and/or IgG antibody titers of $\geq$ 1:1,600.

RESULTS

The dipstick test result was positive on admission in 29 patients and negative in 54

patients. The diagnosis of scrub typhus was confirmed in 30 individuals whose admission characteristics are listed in Table 1.

Four patients (13.3%) had evidence of complicated scrub typhus. Two individuals developed mild respiratory failure. One patient had electrocardiographic signs consistent with myocarditis and one patient had an aseptic meningitis-like illness proven by lumbar puncture. An additional 3 patients had neck stiffness but no lumbar puncture was performed.

Patients were treated with either oral doxycycline 21 cases, oral chloramphenicol 1 case, intravenous chloramphenicol plus oral doxycycline 3 cases, tetracycline included in an antimalarial regimen 2 cases or clarithromycin in one pregnant patient. One patient from group A with concomitant falciparum malaria and did not receive anti-rickettsial therapy. Sufficient follow-up was obtained in 20 patients, all of whom recovered. After exclusion of 3 patients with concomitant malaria, the mean fever clearance time was 2.6+/-1.1 days.

Scrub typhus was more prevalent in farmers than in non-farmers (48% *vs* 22%; $p < 0.01$). A specific cause of fever was determined in 23 of the 53 patients (43%) without scrub typhus (Table 2).

The dipstick had a sensitivity of 86.7% and a specificity of 94.3%. The prevalence of scrub typhus in both Thailand and Malaysia has been measured at 20% (Grown *et al*,1976; Takada *et al*, 1984). The positive predictive value of the dipstick test assuming a 20% prevalence rate would be 79.2% while the negative predictive value would be 79.2%. For the prevalence of 36.1% measured during the present study, positive and negative predictive values would be 89.6% and 92.6% respectively.

Three patients had positive dipstick results that were not confirmed by IIP. An alternative diagnosis was found in two of the three patients: acute pyelonephritis due to *E. coli* and tuberculous meningitis. No specific etiology of fever could be determined in the third case. Four patients with a negative dipstick return were diagnosed by IIP as having scrub typhus. Two of these patients had very high acute IIP IgM titers (>1:3,200). The other 2 patients had concomitant falciparum malaria and were given the diagnosis of scrub typhus based on a four fold increase of IgM titers between acute and convaledcent sera.

Table 1
Baseline characteristics of 30 scrub typhus patients.

Characteristic	Number of patients
Male/Female	20/10
Age (years)	42 (14-69)
Farmer (%)	22 (73.3)
Non-farmer (%)	8 (26.7)
Exposure to rice fields (%)	23 (76.7)
- forest (%)	12 (40)
- none (%)	1 (3.3)
Chigger bite (%)	5 (16.7)
Anti-rickettsial drug prior to admission	
- yes (%)	7 (23.3)
- no (%)	14 (46.7)
- unknown (%)	9 (30)

Table 2
Diagnosis in 22 patients without scrub typhus.

Diagnosis	Number of cases
Bronchopneumonia	4
Amebic liver abcess	1
Acute pyelonephritis	2
Angiostronglyloidiasis	1
AIDS+tuberculosis	1
Acute bronchitis+hemoptysis	1
Tuberculous pericarditis	2
Pulmonary tumor	1
Tuberculous meningitis	2
Brain abcess	1
AIDS+cryptococcal meningitis	1
Stevens-Johnson syndrome	1
AIDS+tuberculous meningitis	1
Polymyalgia rheumatica	1
Tuberculous spondilodiscitis	1
Subacute cholangitis	1

Village serosurvey

Sera from 24 healthy farmers, 17 women and 7 men, were examined by dipstick and IIP. One of the 24 (4.2%), a 50 year asymptomatic woman, had a positive dipstick and IIP serology (IgG 1:1,600 and IgM < 1:50). The 23 other farmers had a negative dipstick and either no detectable or low levels of anti-*Orientia tsutsugamushi* antibody by IIP.

DISCUSSION

We found from a prospective investigation conducted in a scrub typhus endemic area that the dipstick test was an accurate, practical tool for diagnosing *O. tsutsugamushi* infection. The sensitivity of the dipstick 87%, its specificity was 94.3%, and positive and negative predictive values were satisfactory. The test was easy to perform, even in the hands of investigators without previous laboratory experience. Results were generally known within an hour, making it suitable for an outpatient or community hospital setting.

There is no single "gold standard" for the diagnosis of scrub typhus, so we therefore used a panel of criteria to confirm the diagnosis in the 83 patients investigated. We relied heavily on the results of the IIP test in the presence of typical clinical findings. The presence of a typical eschar is virtually pathognomonic for scrub typhus in Thailand. Spotted fever group rickettsia is rare and would not give a positive IIP test result. All 13 patients with a typical eschar had a positive dipstick result and positive IIP serology. High titers of antibodies measured by IIP have been shown to be > 95% specific (Kelly *et al*, 1988) but such titers can occur rarely in healthy individuals from endemic areas (Strickman *et al*, 1994) and were present in 1 of the 24 (4.2%) healthy farmers in our village survey.

IIP serotesting was not performed on convalescent sera in 15 patients. Some cases of scrub typhus might have been missed but the number would have been low. The diagnosis of *O. tsutsugamushi* infection was made by seroconversion in only 5 of the 30 cases of scrub typhus. Dipstick test results were interpreted on site by the investigators in a non-blinded way. This potential bias did not appear to have much influence on our results since they were identical to those reported by a blinded expert in all but one case. This one incorrectly read dipstick was initially considered to be negative in a patient later confirmed to have scrub typhus. The vast majority of the dipstick results were clear cut. Three weakly reactive dipstick required a discussion in order to reach a consensus. Thus, the subjectivity of the interpretation of results is a definite minor limitation of the dipstick. Interpretation of IFA and IIP tests appear even more subjective and difficult, even for skilled technicians.

The dipstick assay requires a refrigerator to store the reagents, distilled water and a waterbath to keep the temperature at 50ºC during the procedure. The cost effectiveness of these items would depend on the prevalence of scrub typhus in the area. According to the manufacturer, the kit comprising the dipstick and the reagents can be kept for 2 years if stored in proper conditions. No data about a potential loss of sensitivity of the dipstick with time of storage have been published.

The diagnosis of scrub typhus could not be confirmed in 3 patients with a positive dipstick. All had IgM or IgG titers on IIP above 1:50 but below the cut-off diagnostic titers. This shows that the dipstick sometimes detects low antibody titers. However, no patients in whom IIP was negative (IgG and IgM <1:50) tested positive by dipstick. The prevalence of anti-*O. tsutsugamushi* antibodies in rural Thailand reaches 77% (Johnson *et al*, 1982). This is another limitation of the dipstick test; higher specificity might be expected if the dipstick test were applied to non-indigenous visitors to an endemic area such as soldiers or travellers.

CONCLUSION

The newly developed dot blot immunosassay for scrub typhus is rapid, reliable, and easy

to perform. It is the most suitable test to date for rural community hospitals in areas of Asia where *O. tsutsugamushi* infection is most prevalent. The sensitivity and specificity of the dipstick were 86.7% and 94.3% respectively in 83 patients with acute FUO presenting to Maharaj Nakhon Ratchasima Hospital in Northeast Thailand. However, as with any serodiagnostic test, physicians should interpret the results of this dipstick assay in the context of the clinical and epidemiologic picture. Routine screening for malaria should not be overlooked in endemic areas regardless of the results of other seroassays. Assays such as the dipstick test for scrub typhus which are well adapted for use in rural areas should help improve the standard of care outside large urban centers in the tropics.

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