

Effects of Antibiotic Treatment on the Results of Nested PCRs for Scrub Typhus[∇]

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In order to determine the effects of antibiotic treatment on the results of nested PCRs for scrub typhus, we investigated the frequency of positive outcomes at various times after antibiotic administration in patients with scrub typhus. The rate of detection by nested PCR with blood buffy coat before antibiotic administration was 90.5%. However, this decreased to 60.5% within 3 days after administration of antibiotics such as doxycycline and rifampin and to 10% by the fourth day following antibiotic administration. Since the sensitivity of nested PCR can be affected by antibiotic administration, clinicians should perform this PCR before antibiotic administration and at the latest within 3 days after antibiotic administration.

Scrub typhus is transmitted to humans by chiggers infected with *Orientia tsutsugamushi* that attach to the skin and suck tissue fluid, and it is characterized by eschar, fever, headache, and skin rash (1, 3). It can be diagnosed by isolating *O. tsutsugamushi* from patients' blood during the febrile stage or by measuring antibody titers against *O. tsutsugamushi* during the acute and convalescent stages (5). However, it takes several weeks to isolate *O. tsutsugamushi* from cultured cells or infected rats (7, 9), and the sensitivity of isolation is reported to be only 46.7% (6). Scrub typhus can be diagnosed on the basis of a fourfold or greater increase in antibody titer using the passive hemagglutination assay, indirect immunofluorescent-antibody assay (IFA), indirect immunoperoxidase assay, or enzyme-linked immunosorbent assay (2, 5). However, it is difficult to make an early diagnosis using these assays for patients who visit a clinic, because antibodies may not have appeared and a follow-up visit may be needed.

Scrub typhus has also been diagnosed by PCR for *O. tsutsugamushi*-specific genes, and this has been shown to be of considerable use in the early diagnosis of scrub typhus (4, 5, 7). However, its potential disadvantages include false positivity due to DNA contamination and a decline in sensitivity following antibiotic administration.

This study was designed to determine the effects of antibiotic administration on the results of nested PCR in patients with scrub typhus; to this end, we measured the positivity rate of the nested PCR at various times after antibiotic administration.

MATERIALS AND METHODS

Patients with acute febrile diseases who visited Chosun University Hospital between September 2006 and December 2007 were included in the study. The patients were ≥ 18 years old and had a history of fever together with eschar or a maculopapular skin rash, as well as more than two of the following symptoms: headache, general weakness, myalgia, cough, nausea, and abdominal discomfort. Informed consent was obtained from the patients or their parents after they were

given a complete description of the study protocol. The definitive diagnosis of scrub typhus was defined as an immunoglobulin M (IgM) antibody titer of $\geq 1:160$ or a fourfold or greater increase in the IgM or IgG titer measured by IFA. Patients who were diagnosed with diseases with clinical features similar to those of scrub typhus, such as murine typhus, leptospirosis, epidemic hemorrhagic fever, and systemic lupus erythematosus, were excluded from the study, which was approved by the Institutional Review Board of our hospital. Nested PCR was performed with blood buffy coat, and IFA was conducted with serum by a method described previously (5).

RESULTS

Of 141 patients with confirmed scrub typhus between September 2006 and December 2007, 129 were investigated and 12 were excluded because they had received an antibiotic(s) with antimicrobial effects against *O. tsutsugamushi*, such as tetracyclines, macrolides, quinolones, and rifamycins, before admission. We had blood samples prior to antibiotic administration from 116 of the 129 patients, and 105 of these (90.5%) proved positive in nested PCR for scrub typhus (Table 1). The positivity rate had declined to 60.5% in blood drawn within 3 days after administration of antibiotics such as doxycycline and rifampin and was only 10% at approximately 4 to 9 days after antibiotic administration. *O. tsutsugamushi* DNA was detected by nested PCR for a maximum of 13 days after antibiotic administration (Table 1).

DISCUSSION

Scrub typhus may fail to be diagnosed on the basis of serum antibody titers during the acute and convalescent stages because antibody formation and a fourfold or greater increase in specific antibody titer require several weeks to occur. In contrast, PCR can detect *O. tsutsugamushi* DNA in blood from the onset of symptoms, thus allowing physicians to make an early diagnosis (5). Furthermore, since life-threatening complications or death can occur as a result of delays in antibiotic therapy, PCR could be very useful for early diagnosis of scrub typhus in the clinical setting (5).

It has been reported that the sensitivity and specificity of conventional PCR targeting the 16S rRNA gene of *O. tsutsugamushi* were 44.8% and 99.7%, respectively, in Thai patients

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TABLE 1. Positivity rates for nested PCR at various times after antibiotic treatment in 129 patients with confirmed scrub typhus

Days following antibiotic administration	No. with nested PCR result:		Positivity rate, % (SE)
	Positive	Negative	
0	105	11	90.5 (2.7)
1-3	23	15	60.5 (7.9)
4-6	7	63	10 (3.6)
7-9	2	17	10.5 (7.0)
10-12	1	17	5.6 (5.4)
13-15	1	24	4 (3.9)
16-30	0	21	0 (0)

(8). We previously reported that the sensitivity and specificity of nested PCR targeting the gene that encodes the 56-kDa major outer membrane protein were 82.2% and 100%, respectively. In our study, we used the buffy coat for PCR assay since *O. tsutsugamushi* is an intracellular organism, and we used nested PCR to enhance the sensitivity. This nested PCR method is known to be 100 times more sensitive than use of a single PCR for detecting *Orientia* DNA (7).

The sensitivity of PCR for the diagnosis of scrub typhus, unlike that of antibody detection methods, can be affected by antibiotic therapy. In this study, when patients who received antibiotic therapy prior to presentation were excluded, nested PCR showed a positivity rate of 90.5% at diagnosis. However, the positivity rate decreased to 10% by the fourth day following antibiotic administration in the patients who received antibiotic therapy.

In scrub typhus patients, eschars develop at the mite biting sites, where *O. tsutsugamushi* multiplies and spreads throughout the whole body. Since such eschars contain a large number of microorganisms, PCR performed using eschar specimens can detect *Orientia* DNA even 1 to 2 weeks after antibiotic administration. Hence, PCR using eschars is less affected by antibiotic therapy. In our previous study (4), we reported that PCR using eschars was positive in 92% (46/52) of patients who had been treated with doxycycline for 7 days. In contrast, the present data indicate that nested PCR with blood buffy coat should be conducted within at most 3 days after the onset of

antibiotic administration, because *Orientia* DNA is more easily eradicated by antibiotics in blood than in eschars.

In conclusion, the sensitivity of PCR with blood buffy coat can be affected by antibiotic treatment. The rate of detection by nested PCR with blood buffy coat before antibiotic administration was 90.5%. It had fallen to 60.5% within 3 days of antibiotic administration and was only 10% by the fourth day of antibiotic administration. Therefore, clinicians should perform PCR with blood drawn before antibiotic administration or at the latest 3 days after antibiotic administration.

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