2007년 8월
박사 학위 논문

쯔쯔가무시증 환자의 피부조직에서 면역조직화학 염색법의 임상적인 유용성

조선대학교 대학원

의 학 과

이 승 현

쯔쯔가무시증 환자의

피부조직에서 면역조직화학

염색법의 임상적인 유용성

Diagnosis of Scrub Typhus by Immunohistochemical Staining of *Orientia tsutsugamushi* in Cutaneous Lesions

2007년 8월 24일

조선대학교 대학원

의 학 과

이 승 현

쯔쯔가무시증 환자의

피부조직에서 면역조직화학

염색법의 임상적인 유용성

지도교수 박 상 기

이 논문을 의학박사 학위신청 논문으로 제출함.

2007년 4월

조선대학교 대학원

의 학 과

이 승 현

이승현의 박사학위 논문을 인준함

위원장	조선대학교 교수	인
위원	조선대학교 교수	인
위원	조선대학교 교수	인
위원	서남대학교 교수	인
위원	조선대학교 교수	인

2007년 4월

조선대학교 대학원

목차

Page

표 목차

그림 목차

ABSTRACT

쯔쯔가무시증 환자의 피부조직에서 면역조직화학 염색법의 임상적인 유용성

Lee Seung-hyun

Advisor : Prof. Park Sang-kee Ph.D.

Department of medicine,

Graduate School of Chosun University

배 경: 현재까지 쯔쯔가무시증의 진단에 있어서 파라핀고정 조직에서 *O. tsutsugamushi* 의 확인을 위한 면역조직화학염색법(IHC) 의 유용성과 확진법인 면역형광 항체 검사(IFA) 와의 비교연구에 대한 보고는 아직까지 없는 실정이다. 저자는 진단적 방법으로써의 IHC 의 임상적 유용성을 확인하기 위해 쯔쯔가무시증 의심 환자들을 대상으로, IFA 와 IHC 의 비교 연구를 수행하였다.

방 법: 2005년 9월 1일부터 2006년 8월 31일 사이의 조사 기간 동안 조선 대학교 병원에 한달 이내에 시작된 급성 발열성 질환으로 방문한 환자들을 대상으로, 피부 발진 및 가피 존재 유무를 철저히 조사하였고, 피부 발진과 가피가 확인된 경우 환자의 허락하에 피부조직검사를 시행하여 면역조직화학염색법의 임상적 유용성을 확인하였다

결 과: 항생제 투여 전에 피부조직검사를 시행 받은 환자는 총 46 명이었고 이들을 확진 검사법인 IFA 와 비교해 보았을 때, 민감도가 64.7%, 특이도가 100% 이고, 가피를 이용한 경우 민감도 및 특이도가 모두 100% 를 나타냈다; IFA 양성인 환자 30 례 중 피부발진을 이용한 면역조직화학검사를 시행한 18 례 중 11 례에서 양성, 가피를 이용한 면역조직화학검사를 시행한 27 례 중 부적절한 표본 5 례를 제외한 22 례 모두 양성을 보였으며, IFA 음성인 환자 13 례 중 피부발진을 이용한 면역조직화학검사를 시행한 8 례에서 모두 음성이 나왔고, 가피를 이용한 4 례의 경우 모두 음성이었다. 연구에 참여한 63 명중 항생제 투여 여부에 상관 없이 IFA 검사상 쯔쯔가무시증으로 확진된 44 명중 한 환자를 제외한 43 명의 환자에서 가피가 확인되었으며, 40 명의 환자에서 가피 조직검사를 시행하였고, 7 검체는 부적절하였다. 33 명의 가피 조직 IHC 검사상 32 명의 환자는 항생제 투여 4 일 이내에 조직검사를 시행하였고 모든 환자에서 가피 IHC 양성을 확인 할수 있었으나, 한 환자에서 항생제 투여후 13 일째 시행한 IHC 검사상 음성을 보였다.

결론: 쯔쯔가무시증의 급성기 동안 가피를 이용한 IHC 가 임상 진단을 확진 하는 검사법으로 유용하게 사용될 수 있다.. 특히 가피를 이용한 IHC 는 단 한번의 방문으로 수일 내에 진단할 수 있으며. 단기간 항생제 투여 후 에도 이러한 민감도와 특이도는 영향을 받지 않아 매우 민감하고 특이적인 확진 진단법으로 이용될 수 있다.

Key words: Immunohistochemical staining, Scrub typhus, *Orientia tsutsugamushi*

I. INTRODUCTION

Scrub typhus is an acute febrile disease that is characterized by high fever, headache and rash; these symptoms are caused by the intracellular gram negative bacteria, *Orientia tsutsugamushi*. It is a major febrile disease in Korea, Japan, China, Thailand etc. The reservoir of *O. tsutsugamushi* is wild rats, and chiggers are the vector. Bacterial infection occurs when chiggers bearing *O. tsutsugamushi* bite human beings and aspirate human tissue fluid. Eschars are formed on the sites bitten by the chiggers; fever, maculopapular rash, muscle ache, headache, anorexia, lymph node enlargement appear at the time of the formation of eschar. The presence of eschar has been reported to be an important finding for the diagnosis of rickettsial pox or scrub typhus [1].

Scrub typhus can be diagnosed by isolating *O. tsutsugamushi* from the blood of patients during the febrile period or by the confirmation of an elevated antibody titer against *O. tsutsugamushi* during the acute phase or the recovery period [2]. However, to isolate *O. tsutsugamushi* from cultured cells or infected rats is feasible only in those institutions that are equipped for Biosafety level 3, and it takes a minimum of several weeks to culture *O. tsutsugamushi* [3, 4]. For serologic testing, it may take from several days to several weeks for the antibody titer to become elevated after the onset of symptoms for scrub typhus. Hence, in many cases, the diagnosis made by indirect immunofluorescence assay requires follow-up tests for achieving an accurate definite diagnosis. However, convalescent-phase serum samples are not available in many cases, thus making an accurate diagnosis can be difficult.

Rickettsial pox has been diagnosed by using paraffin-embedded skin biopsy specimens, and the usefulness of immunohistichemical staining using an anti-*R. rickettsii* antibody has been reported [5, 6]. For suspected Rocky Mountain spotted fever patients, immunohistichemical

staining of formaldehyde-fixed tissues has been reported to be useful as a sensitive definite diagnosis method [7]. Nervertheless, the usefulness of immunohistochemical staining paraffin-embedded tissue for the diagnosis of scrub typhus has not yet been reported. To assess the clinical usefulness of immunohistochemical staining as a diagnostic method, author conducted a comparative study of IFA and immunohistochemical staining on possible scrub typhus patients.

II. MATERIALS AND METHODS

Selection of the patient group

During the study period from September 1, 2005 to August 31, 2006, among the patients who visited Chosun University Hospital located in southwest Korea for acute febrile disease that developed within one month prior to their visit, author selected the adult patients over 18 years old who were suspected to have scrub typhus based on eschars or maculopapular skin rash, or the diagnosis was based on the determination of the clinicians. Author obtained a written consent from all patients or their guardians. For the patients participating in the study, the presence or absence of maculopapular skin rash as well as eschars was examined; in the cases that skin rash and eschars were detected, if the patient allowed, skin biopsy specimens from the eschars or maculopapular lesions were obtained using a 3 mm punch. The definitive diagnosis of scrub typhus was that the IgM titer to *O. tsutsugamushi* was increased to over 1:80 by indirect immunofluorescence or that case's IFA titer was increased more than 4 times [8]. Tests for other diseases similar to scrub typhus such as murine typhus, leptospirosis, hemorrhagic fever with renal syndrome and systemic lupus erythematosus were performed on the patients participating in the study. This study was approved by the Ethics in Human Research Committee of Chosun University Hospital.

Immunohistochemical staining (IHC)

All the cases investigated in the study were tested with ICR mouse polyclonal anti-*O. tsutsugamushi* antibody (dilution 1:200) against the *O. tsutsugamushi* Boryong strain. Immunolocalization was performed using a streptavidin-biotin immunoperoxidase method, according to the supplier's protocol (LSAB kit, DAKO, Carpinteria, California, USA). Briefly,

the 4 ㎛ thick sections that were obtained after formalin fixation and paraffin embedding were deparaffinized in xylene and then they were rehydrated with distilled water through graded concentrations of ethanol. The sections were then placed in a glass jar with 10 mM citrate buffer (pH 6.0), irradiated in a microwave oven for 15 minutes and allowed to cool down in the jar at room temperature for 20 minutes. The slides were next rinsed with Tris buffered saline (TBS). Blocking reagent was added for 10 minutes after quenching the endogenous peroxidase activity in 0.3% hydrogen peroxide for 10 minutes. The slides were then washed as before and they were subsequently subjected to the primary antibody reaction. Primary antibody was applied in a moist chamber overnight at 4 ℃. After washing with TBS, a biotinylated link antibody was applied to the slides for 10 minutes; this was followed by applying streptavidin peroxidase for an additional 10 minutes. After washing out the excess complex, the localization of antibody was visualized by incubating the sections for 15 minutes with diaminobenzidine (DAB, Santa Cruz Biotechnology, Santa Cruz, CA, USA), and counterstaining was done with Mayer's hematoxylin. An isotype matched control antibody was also used. The positive control for *O. tsutsugamushi* was a serologically proven case of skin tissue. Instead of the primary antibody, TBS was used for the negative control. Proven cases of other inflammatory skin lesions such as erythema nodosum and varicella zoster were also stained.

Analysis and interpretation of staining

Staining for *O. tsutsugamushi* was determined to be positive when intracytoplasmic or extracellular discrete coccobacilli and aggregates of fragmented, granular or coalesced rickettsial antigen were stained brown under an optical microscope (BX 50, Olympus, Japan).

Immunofluorescent antibody assay (IFA)

IgM and IgG antibody to the standard *O. tsutsugamushi* antigen in the patients' sera were determined by the method described by Robinson et al. with some modification (Gilliam, Karp, Kato, and Boryong) [2, 8, 9]. Briefly, the serum was diluted with phosphate buffered saline to 1:32, and a two-fold serial dilution solution was applied onto slides on whch Orientia antigen was seeded. Sera that were already known to be positive or negative were used as the internal quality controls. Experiment slides were incubated in a humidified chamber at 37 degrees for 30 minutes, and they were subsequently washed with PBS and distilled water. Fluorescent-conjugated goat anti-human IgM and IgG diluted with PBS to 1:300 were applied to each slide; after incubation, they were washed as described above. The slides were air dried with FA mounting solution (Bacto, USA), and examined under an immunofluorescent microscope (Axioskop 2; Carl Zeiss, Germany) at 400X magnification. The highest titer among the titers against the Gilliam, Karp, Kato and Boryong species obtained by IFA test was used.

III. RESULTS

1. Characteristic of the subjects

Among the patients who visited the hospital for acute febrile diseases that developed within one month prior to their visits, 125 patients were suspected to have scrub typhus based on the determination of clinicians, or the lesions suspected to be eschars or maculopapular skin rash. Among those patients, 63 patients gave me permission to perform skin biopsy tests. Of the 63 patients who underwent skin biopsy test, 25 patients (39.7%) were males and 38 patients (60.3%) were female. Their mean age was 59 years (range: 22-89 years). For 44 of these patients, the titer of *O. tsutsugamushi*-specific IgM was higher than 1:80, or the antibody value during the acute phase and the recovery phase was elevated more than 4 times, and so they were definitely diagnosed as having scrub typhus. For 14 patients, the antibody value during the acute and recovery phases was confirmed to be negative, and they were definitely diagnosed as having other diseases. Five patients were undetermined because there were not obtained patients' sera during the recovery phase due to follow-up loss. The demographic data of the patients was shown in table 1.

2. Immunohistochemistry as a diagnostic method for scrub typhus

Among 63 patients who underwent skin biopsy, 4 patients were treated with antibiotics that were active against *O. tsutsugamushi* prior to their visit to our hospital, and 59 patients were treated with antibiotics after their visit to our hospital: 46 patients underwent skin biopsy prior to the administration of antibiotics and 13 patients underwent skin biopsy after the administration of antibiotics (Figure 1). Considering the effect of antibiotics on IHC, the results of only 46 patients who underwent histological testing prior to the administration of antibiotics

were compared with their IFA results. Thirteen patients were diagnosed with diseases other than scrub typhus by IFA testing and by the judgement of clinician. In 30 out of 33 suspected scrub typhus patients, they were confirmed as suffering from scrub typhus because the titer of *O. tsutsugamushi*-specific IgM was higher than 1:80 or the antibody titer during the recovery phase was elevated more than 4 times. Three patients could not be definitely diagnosed as scrub typhus due to follow-up loss.

Among the 30 definitely diagnosed patients, 18 patients underwent skin biopsy on their maculopapular lesions: one sample was an inadequate. Among the appropriate 17 samples, 11 samples (64.7 %) presented with positive findings on IHC. On the other hand, among the 13 patients definitely diagnosed as having a disease other than scrub typhus, 8 patients were underwent skin biopsy on their maculopapular lesions. IHC was performed on the maculopapular skin lesions of 8 patients, and a negative result was obtained for all the samples. For 2 of 3 patients who could not be definitely diagnosed due to the follow-up loss, skin biopsy was performed on a maculopapular lesion; positive IHC findings were confirmed in both cases.

Regarding the results of skin biopsy of eschars, eschar skin biopsy was performed on 27 of the 30 definitely diagnosed cases; among them, 5 samples were found to be inadequate samples that contained only necrotic debri. On the IHC performed on 22 patients, positive findings were detected for all 22 patients (100%). Among the 13 patients diagnosed as having a disease other than scrub typhus, eschar-like lesions were detected on 5 patients (2 ecthyma gangrenosum, chickenpox, metastatic *S. aureus* infection and fluid injection sites of meningitis patients); IHC was performed using the eschar-like lesions obtained from these 5 patients. The sample from one patient was inadequate, and a negative result was obtained for the remaining 4 patients. Skin biopsy on the eschars was performed for 3 patients who could not be definitely diagnosed due to follow-up loss: Positive IHC findings of the eschars were detected for all 3 patients.

Compared with the IFA, gold standard for the definite diagnosis of scrub typhus, in regard to the sensitivity and specificity of IHC on biopsy specimens performed prior to the administration of antibiotics, in the case of maculopapular lesions, the sensitivity was 64.7% and specificity was 100%, and the sensitivity and speficity of IHC testing on eschars were shown to be 100% (Table 2).

For the four patients who had already been treated with antibiotics prior to visiting our hospital, they were admitted our hospital after treatment with doxycycline for 2 to 5 days. Skin biopsy was performed on both the eschar and papules of one patient, and biopsy was performed on only eschar in 3 cases. Positive IHC findings were detected for the eschars and papules of these 4 patients. Among the 13 patients who underwent skin biopsy after treatment with antibiotics at our hospital, one patient was confirmed to have erythema nodosum. The test results of the remaining 12 scrub typhus patients are summarized in Table 3.

Among the 63 enrolled patients, irrespective of antibiotics administration, 44 patients were confirmed to be cases of scrub typhus by IFA ; among them, eschars were detected in 43 patients excluding one patient. Histological tests on eschar was performed for 40 patients, and 7 samples were deemed inadequate. Concerning the IHC performed on eschar for 33 patients, histological testing was done on 32 patients within 4 days after the administration of antibiotics, and the positive IHC finding of eschar could be confirmed. Nevertheless, in one patient, Oriential Ag could not be detected by the IHC performed at 13 days after the administration of antibiotics.

3. Histological findings

On hematoxylin and eosin staining, the typical eschar showed confluent epidermal necrosis with dermal vascular dilatation and perivascular inflammation. The ulcerated lesions

showed heavy infiltration of neutrophils in and around the small blood vessels and there were frequent thrombosed vessels in the ulcer base. The common pattern of inflammation showed a superficial and deep perivascular mononuclear cell infiltrate. Lobular or septal panniculitis that consisted mainly of mononuclear cell infiltration was frequently identified (Figure 2). Most of the uppermost part of the eschar lesions consisted of non-viable acellular denatured components. The papular lesions showed a very wide range of inflammatory changes that consisted of various degrees of papillary dermal edema and a sparse or heavy infiltration of perivascular mononuclear cells in the superficial and mid-dermis (Figure 3). None of the lesions showed epidermal necrosis or leukocytoclastic vasculitis.

According to the IHC findings, positive staining for Oriential Ag was most apparent within and associated with the vascular endothelium of capillaries, arterioles, venules and veins. We observed intracytoplasmic positive staining of the lining epithelia of the sweat ducts and glands in the mid- and deep-dermis. Oriential Ag and discrete coccobacilli were primarily located within the cytoplasm of the infected endothelial cells, and these were also identified within the macrophages around the blood vessels (Figure 2, Figure 3). Aggregates of fragmented, granular or coalesced Oriential Ag were also demonstrated in some interstitial areas. Orientia were identified more frequently in the areas of the more inflamed lesion, and eschar that consisted of non-viable tissue usually failed to demonstrate positive stainability. Generally, immunohistochemical positive staining was much more apparent for the eschar lesions than for the maculopapular lesions.

IV. DISCUSSION

For scrub typhus patients, an eschar approximately 5-20 mm in diameter is formed at the site bitten by thrombiculid mites, and this may be considered to be the most important clinical finding for the diagnosis of scrub typhus. The site bitten by chiggers is initially a papule followed by a blistered ulcer and then this is covered with black colored crust; the border of the eschar is surrounded by reddish erythema. Such a typical eschar is formed at the time when symptoms are manifested [10].

In the past, the clinical diagnosis of scrub typhus was dependent on detecting eschar and rash, and the past history of the outdoor activity [10-12]. Nevertheless, under actual clinical conditions, only eschar without rash may be seen in some cases. As was shown in previous case report, for febrile patients showing a lesion similar to eschar, distinguishing whether such an eschar-like lesion is actually a simple crust or eschar is required [13]. Furthermore, eschars are also detected for rickettsialpox or cutaneous antrax. Travel and other population migrations are currently often occurring. Scrub typhus has been reported in western countries [14, 15], and it is difficult to definitely diagnose scrub typhus only by the assessment of eschar-like lesions in many cases, so more accurate diagnostic evaluation may be required.

Regarding the IHC method with using skin tissues, the advantages are that it requires the patient to visit the hospital only once, and the result can be obtained within a few days. For other rickettiosis such as Rickettsialpox, Rocky Mountain spotted fever, the diagnostic usefulness of IHC has already been reported and IHC can be applied as the definite diagnostic method [16]. Nevertheless, the clinical usefulness of IHC for scrub typhus patients has not yet been reported, so author conducted this study on the usefulness of IHC. The responsiveness of scrub typhus to antibiotics is relatively good, and it has been reported that within 48 hours after

the administration of antibiotics that are effective on rickettia such as doxycycline, azithromycin, etc., fever was controlled in most patients [17]. Therefore, to minimize the effect of antibiotics on the IHC results, we excluded 4 patients who were treated with antibiotics prior to hospitalization and 13 patients who underwent skin biopsy after treatment of antibiotics in our hospital, and the IHC results were compared with the IFA results. The result showed that the sensitivity and specificity of IHC performed on maculopapular lesions were 64.7% and 100%, respectively, and the sensitivity and specificity were 100% for IHC performed on eschar.

Among the 63 enrolled patients irrespective of the administration of antibiotics, 44 patients were confirmed to have scrub typhus via the IFA ; for 25 patients among them, IHC was performed with using papules. One patient's sample among these 25 patients was inadequate. Among the remaining 24 patients, 16 patients (66.6%) were positive IHC finding and 8 patients (33.3%) were negative IHC finding. In other words, 33.3% showed as false negative. Nevertheless, the impact of prior administration of antibiotics or the period from the onset of the symptom to the time of performing skin biopsy on IHC positivity didn't result in any statistically significant difference ($p > .05$).

Among the 63 patients on whom we performed histological testing, 14 patients were confirmed to have a disease other than scrub typhus. 5 patients could not be definitely diagnosed as scrub typhus by IFA test due to follow-up loss (IgM lower than 1:80 or the increase of the IgG titer of more than 4 times could not be proven), and eschars were detected on all 5 patients. For 3 patients among these 5 patients, positive IHC findings on both the papules and eschars were confirmed, and a positive finding on eschars was confirmed for the other two patients. 49 patients were confirmed to have scrub typhus by IHC performed on both the eschar and papule tissues; among these patients, 21 patients had an IgM titer lower than 1:80 at the time of visit. 42.9% were patients who required follow up observations for the

definite diagnosis; the Ig G was also lower than 1:128 for 10 patients among them. However, for the patients who underwent IHC, it was confirmed that an accurate diagnosis could be made by a single test performed at the time of the first hospital visit.

Both papule and eschar can be used as diagnostic samples. The eschar formed on the site of the mite bite is the site where *O. tsutsugamushi* is proliferating; hence, the eschar is theoretically the site where the inoculum size is quite large. The sensitivity of IHC on eschar was confirmed to be 100%, so it was confirmed that eschar could be applied as a more sensitive diagnostic sample for the IHC than the maculopapular lesions.

For the IHC performed on eschar or maculopapular lesion after 3-4 days administration of antibiotics that are effective for rickettia, the antibiotics didn't greatly influence the sensitivity of diagnosis. Yet for one patient, Oriential Ag could not be detected on IHC testing performed on the 13th day after the administration of antibiotics. The number of patients on whom we performed IHC after the administration of antibiotics was small; hence, additional studies are required concerning the usefulness of IHC performed after the administration of antibiotics.

The hematoxylin and eosin histological finding of scrub typhus is vasculitis. Dilation of the capillary blood vessels and infiltration of monocytes to the vicinity of capillary blood vessels can be detected. The histologic findings of eschar is the infiltration of monocytes to the vicinity of blood vessels together with coaulation necrosis, and such findings are known to be the characteristic finding of scrub typhus. Yet such findings can also be detected in other eschar-like lesions (Figure 2), and so such findings can not be diagnostic for scrub typhus. We found that eschar is not always a pathognomic finding of scrub typhus. Scrub typhus should be definitely diagnosed by assessing *Orientia* coccobacilli with IHC. For IHC, more abundant Orientia coccobacilli were generally detected in the eschars than in the skin maculopapular lesions, and Oriential Ag was assessed primarily in the perivascular inflammatory infiltration in

the deep and superficial dermis. Therefore, IHC on a skin biopsy with using eschar during the acute phase of scrub typhus could be used as the best test method for confirming the clinical diagnosis. This test method, and particularly IHC using eschar, could diagnose scrub typhus within few days after a single visit, and this could be applied as a very sensitive and specific definite diagnosis method, and the sensitivity and specificity are not influenced even after the short term administration of antibiotics.

In conclusion, it was suggested that for possible scrub typhus patients, the IHC with using skin tissues, and especially eschar, could be applied as a useful, sensitive and specific diagnostic method for making the early diagnosis.

V. SUMMARY

Backgroud: The aim of this study is to assess the clinical usefulness of performing immunohistochemical staining (IHC) on paraffin-embedded skin biopsy specimens for the diagnosis of scrub typhus in comparison to indirect immunofluorescent antibody (IFA) assay.

Methods: We conducted a prospective study of patients with possible scrub typhus from September, 2005 to August, 2006 to assess the clinical usefulness of IHC.

Results: 125 potential scrub typhus patients were prospectively studied. Skin biopsy specimens were obtained from 63 patients. To minimize the effects caused by antibiotics on the IHC, 46 patients were assessed prior to the administration of antibiotics (except for the 4 patients who had received prior antibiotic therapy before admission and the 13 patients who underwent skin biopsy after the administration of antibiotics at our hospital). Compared with the results of IFA, which is the definite diagnostic method for scrub typhus, the results of IHC on the maculopapular skin lesions demonstrated a sensitivity of 0.65 and a specificity of 1. The results of IHC on the eschars demonstrated a sensitivity of 1 and a specificity of 1. For the IHC test performed on eschar or maculopapular lesion after 3-4 days administration of antibiotics that are effective for rickettia, the antibiotics didn't greatly influence the sensitivity of diagnosis.

Conclusions: The IHC of skin biopsy specimens, and particularly that of eschars, is both sensitive and specific, and this technique can be a reliable test for confirming the diagnosis of scrub typhus.

Key words: Immunohistochemical staining, Scrub typhus, *Orientia tsutsugamushi*

REFERNCE

1. Saini R, Pui JC, Burgin S. Rickettsialpox: report of three cases and a review. J Am Acad Dermatol **2004**;51(5 Suppl):S137-42.
2. Bozeman FM, Elisberg BL. Serological diagnosis of scrub typhus by indirect immunofluorescence. Proc. Soc. Exp. Biol. Med **1963**;112:568-73.
3. Murai K, Tachibana N, Okayama A, Shishime E, Tsuda K, Oshikawa T. Sensitivity of polymerase chain reaction assay for Rickettsia tsutsugamushi in patients' blood samples. Microbiol Immunol **1992**;36:1145-53.
4. Kim DM, Kim HL, Park CY, Yang TY, Lee JH, Yang JT, Shim SK, Lee SH. Clinical usefulness of eschar polymerase chain reaction for the diagnosis of scrub typhus: a prospective study. Clin Infect Dis **2006** ;43:1296-300.
5. Koss T, Carter EL, Grossman ME, Silvers DN, Rabinowitz AD, Singleton J Jr, Zaki SR, Paddock CD. Increased detection of rickettsialpox in a New York City hospital following the anthrax outbreak of 2001: use of immunohistochemistry for the rapid confirmation of cases in an era of bioterrorism. Arch Dermatol **2003**;139:1545-52.
6. Kannangara S, DeSimone JA, Pomerantz RJ. Rickettsialpox in a patient with HIV infection. J Am Acad Dermatol **2003**;48:286-9.
7. Paddock CD, Greer PW, Ferebee TL, Singleton J Jr, McKechnie DB, Treadwell TA, Krebs JW, Clarke MJ, Holman RC, Olson JG, Childs JE, Zaki SR Hidden mortality attributable to Rocky Mountain spotted fever: immunohistochemical detection of fatal, serologically unconfirmed disease. J Infect Dis **1999**;179:1469-76.
8. Kim DM, Yun NR, Yang TY, Lee JH, Yang JT, Shim SK, Park MY. Usefulness of

Nested PCR for the Diagnosis of Scrub typhus in Clinical Practice: A Prospective study. Am J Trop Med Hyg **2006**;75:542-45.

9. Robinson DM, Brown G.,Gan E, Huxsoll DL. Adaptation of a microimmunofluorescence test to the study of human *Rickettsia tsutsugamushi* antibody. Am J Trop Med Hyg **1976**:25, 900-5.
10. Berman SJ, Kundin WD. Scrub typhus in South Vietnam. A study of 87 cases. Ann Intern Med **1973**;79:26-30.
11. Sayen JJ, Pond HS, Forrester JS, et al: Scrub typhus in Assam and Burma. Clinical study of 616 cases. Medicine(Baltimore) **1946**;25:155-214.
12. Blake FG, Maxcy KF, Sadusk JF Jr, et al: Studies on tsutsugamushi disease(scrub typhus, mite-borne typhus) in New Guinea and adjacent islands. Epidemiology, clinical observations, and etiology in the Dobadura. Am J Hyg **1945**; 41:243-373.
13. SH Lee, DM Kim, Y Cho, S Yoon, and S Shim. The usefulness of eschar PCR for the diagnosis of scrub typhus. J Clin Microbiol **2006** ;44:1169-71.
14. Jensenius M, Fournier PE, Raoult D. Rickettsioses and the international traveler. Clin Infect Dis **2004**;39:1493-9.
15. Jensenius M, Montelius R, Berild D, Vene S. Scrub typhus imported to Scandinavia. Scand J Infect Dis. **2006**;38:200-2.
16. Kass EM, Szaniawski WK, Levy H, Leach J, Srinivasan K, Rives C. Rickettsialpox in a New York City hospital, 1980 to 1989. N Engl J Med **1994**;331:1612-7.
17. TW Sheehy, D. Hazlett R E Turk Scrub typhus. A comparison of chloramphenicol and tetracycline in its treatment. Archives of Internal Medicine **1973**;132:77 - 80.

Table 1. Demographic and clinical data for the enrolled patients

Characteristics	Value
Age, median years (range)	59 (22 – 89)
Gender	
Male	25 (39.7%)
Female	38 (60.3%)
Diagnosis	
Scrub typhus (confirmed case)	44
Scrub typhus (undetermined case)	5
Drug reaction	3
Ecthyma gangrenosum	2
Typhoid fever	1
Paratyphoid fever	1
Varicella Zoster	1
Pemphigus	1
Erythema nodosum	1
Adult onset Still's disease	1
Meningitis	1
Erysipelas	1
Metastatic *S. aureus* infection	1

NOTE. Values are reported as no. (%) of patients, unless otherwise indicated.

Table 2. Results of IHC in comparison with the IFA for patients who underwent skin biopsy prior to the administration of antibiotics

Test	Screening tests			
	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
Papule IHC	0.65 (0.38-0.85)	1 (0.60-1)	1 (0.68-1)	0.57 (0.29-0.81)
Eschar IHC	1 (0.82-1)	1 (0.40-1)	1 (0.82-1)	1 (0.40-1)

* IFA indicates indirect immunofluorescent; IHC, immunohistochemical staining; CI confidence interval; PPV, positive predictive value; NPV, negative predictive value

Table 3. Results of IHC in comparison with the IFA for patients who underwent skin biopsy after the administration of antibiotics in our hospital

Pati-ent No.	Age /sex	Admission time after Onset of symptoms	Serum IFA titer Acute (Ig M/IgG)	Covalescent (Ig M/IgG)	Biopsy days after antibiotics administration	IHC Maculo-papule	Eschar
1	73/M	10 days	80/1,024	160/16,384	1 days	Not done	Inadequate
2	60/F	4 days	80/32	80/2,048	1 days	-	+
3	89/F	5 days	<10/ <32		1 days	Not done	+
4	54/F	5 days	<10/ <32		1 days	Not done	Inadequate
5	37/M	15 days	80/ 512	40/16,384	1 days	+	+
6	54/F	7 days	80/32	40/1,024	2 days	+	+
7	73/M	5 days	160/256	160/2,048	3 days	+	+
8	71/M	9 days	160/512		3 days	+	+
9	81/F	7 days	<10/ <32		3 days	+	Inadequate
10	85/F	6 days	80/128		3 days	Not done	+
11	44/M	11 days	80/512		4 days	-	+
12	79/F	7 days	80/1,024		13 days	Not done	-

* IFA indicates indirect immunofluorescent; IHC, immunohistochemical staining

Figure 1. Patients flow in scrub typhus patients underwent skin biopsy

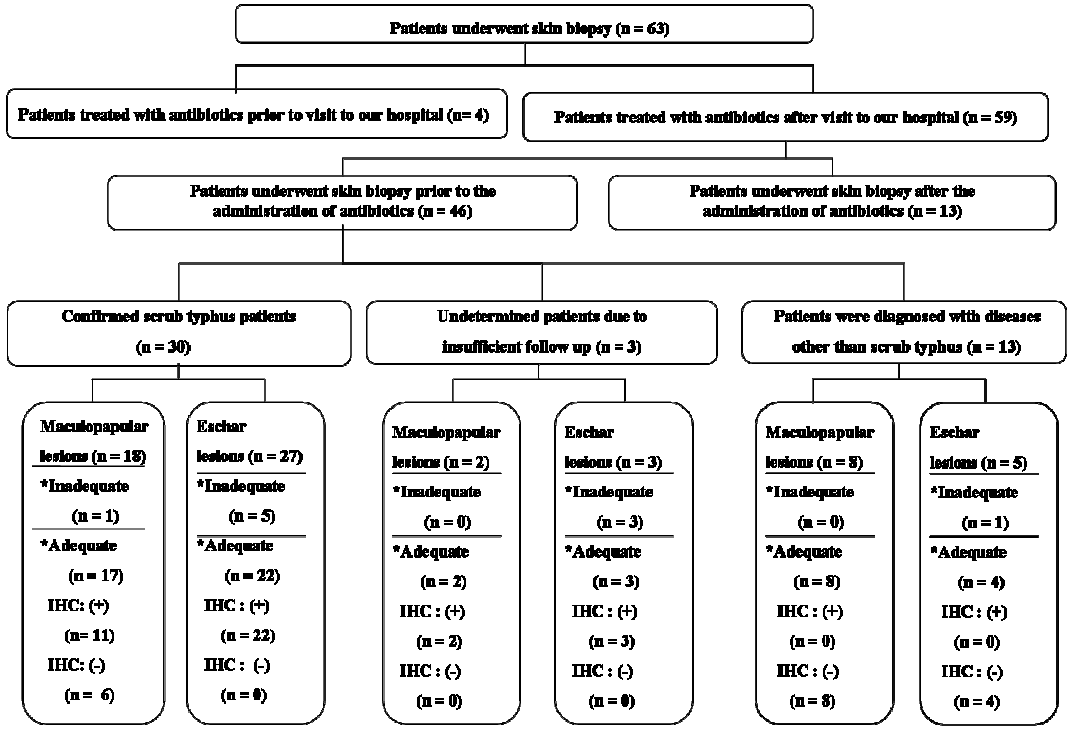


Figure 2. **Histopathologic findings and immunohistochemical staining of the eschar of a scrub typhus patient (a~c) and for the eschar-like lesions on a patient with chickenpox (d~f).** Eschar from the right shoulder of the patient with scrub typhus (a). Confluent epidermal necrosis with dermal vascular dilatation and perivascular inflammation was identified. Infiltration of mononuclear cells such as lymphocyes and macrophage was observed. H&E, x 40 (b). Positive immunohistochemical staining of the vascular endothelial cells, sweat glands and a few scattered mononuclear cells around the small blood vessels and sweat glands was demonstrated. The LSAB method and counterstaining with Mayer's hematoxylin, x 400 (c). Eschar-like lesion from the submental area of the patient with chickenpox (d). Confluent epidermal necrosis with dermal vascular dilatation and perivascular inflammation was identified. Mononuclear cells infiltration such as lymphocyes and macrophage were observed. H&E, x 40 (e). Negative immunohistochemical staining of the vascular endothelial cells, sweat glands and a few scattered mononuclear cells around the small blood vessels and sweat glands was demonstrated. The LSAB method and counterstaining with Mayer's hematoxylin, x 400 (f).

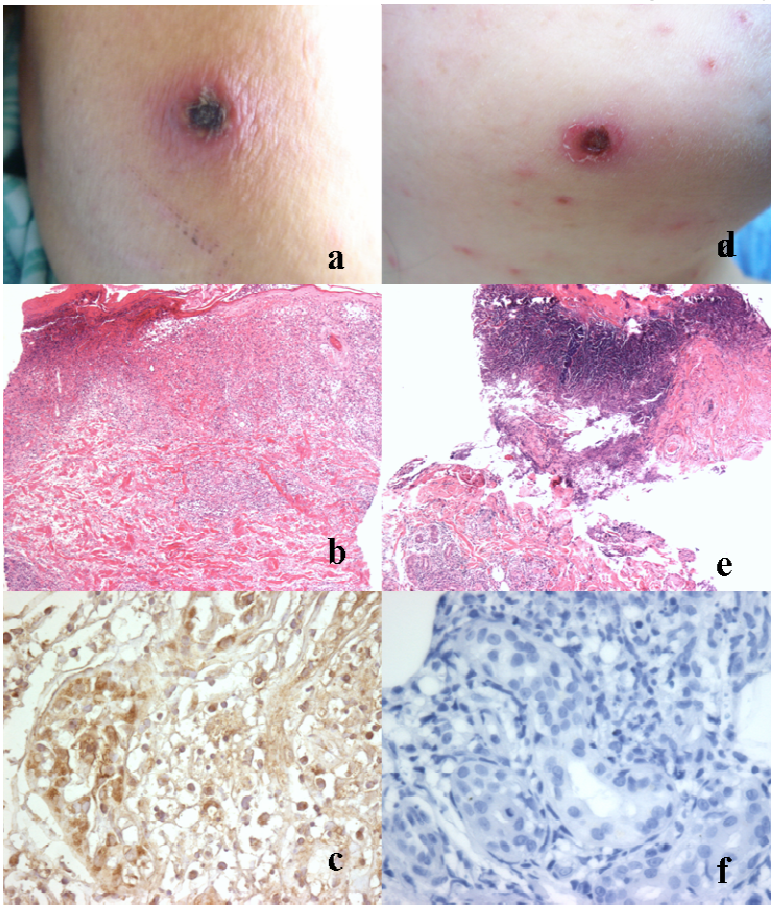
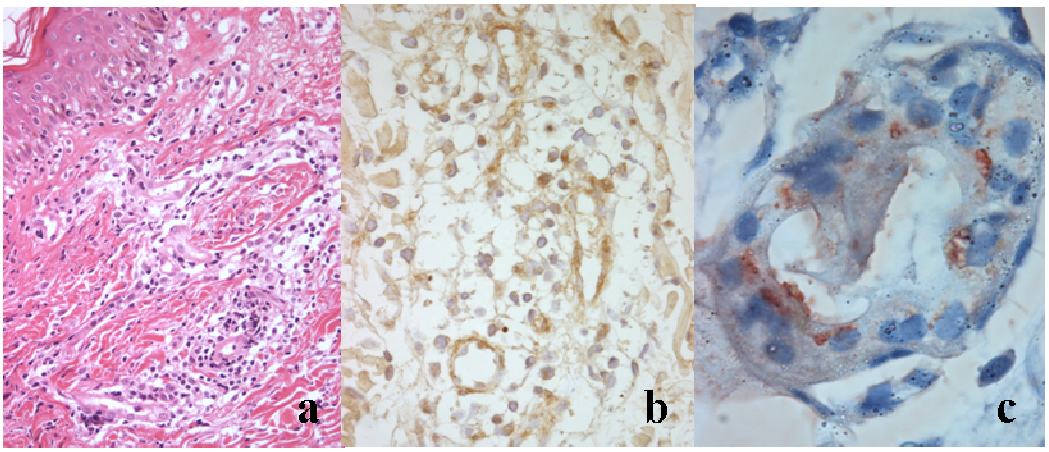


Figure 3. **Histopathologic findings and immunohistochemical staining of the maculopapular lesions of a patient with scrub typhus.** Heavy infiltration of perivascular mononuclear cells in the mid- and deep-dermis was demonstrated. H&E, x 200 (a). Positive immunohistochemical staining of the vascular endothelial cells and a few scattered mononuclear cells around the small blood vessels was demonstrated. The LSAB method and counterstaining with Mayer's hematoxylin, x 1,000 (b). Strong intracytoplasmic immunohistochemical staining of the epithelia lining the sweat ducts was found. LSAB method and counterstaining with Mayer's hematoxylin, x 400 (c).



저작물 이용 허락서

학 과	의학과	학 번	20057456	과 정	박사
성 명	한글: 이 승 현 한문 : 李 勝 賢 영문 : Seung-Hyun Lee				
주 소	광주시 남구 봉선동 포스코 아파트 102-102				
연락처	E-MAIL : 31gmlakd@hanmail.net				
논문제목	한글 : 피부조직에서 면역조직화학 염색법을 이용한 쯔쯔가무시증 진단의 유용성 영문 : Diagnosis of Scrub Typhus by Immunohistochemical staining of *Orientia tsutsugamushi* in Cutaneous Lesions				

본인이 저작한 위의 저작물에 대하여 다음과 같은 조건아래 -조선대학교가 저작물을 이용할 수 있도록 허락하고 동의합니다.

- 다 음 -

1. 저작물의 DB 구축 및 인터넷을 포함한 정보통신망에의 공개를 위한 저작물의 복제, 기억장치에의 저장, 전송 등을 허락함
2. 위의 목적을 위하여 필요한 범위 내에서의 편집·형식상의 변경을 허락함. 다만, 저작물의 내용변경은 금지함.
3. 배포·전송된 저작물의 영리적 목적을 위한 복제, 저장, 전송 등은 금지함.
4. 저작물에 대한 이용기간은 5년으로 하고, 기간종료 3개월 이내에 별도의 의사 표시가 없을 경우에는 저작물의 이용기간을 계속 연장함.
5. 해당 저작물의 저작권을 타인에게 양도하거나 또는 출판을 허락을 하였을 경우에는 1개월 이내에 대학에 이를 통보함.
6. 조선대학교는 저작물의 이용허락 이후 해당 저작물로 인하여 발생하는 타인에 의한 권리 침해에 대하여 일체의 법적 책임을 지지 않음
7. 소속대학의 협정기관에 저작물의 제공 및 인터넷 등 정보통신망을 이용한 저작물의 전송·출력을 허락함.

동의여부 : 동의(O) 조건부 동의( ) 반대( )

2007 년 4 월 18 일

저작자: 이 승 현 (서명 또는 인)

조선대학교 총장 귀하