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CASE REPORT

Undifferentiated tropical fever: a case series of three Rickettsial infections

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ABSTRACT

We report three cases of rickettsial infections diagnosed by performing a multiplex molecular syndromic panel, in patients who presented with undifferentiated tropical fever with non-specific clinical signs and symptoms. All three patients were from regions endemic to agents of tropical fever, such as dengue fever, leptospirosis, and typhoid fever, which were considered as differential diagnoses in the initial investigative workup. These cases highlight the need for a rapid syndromic diagnostic approach for tropical fever to enable timely diagnosis and institution of appropriate antibiotic therapy, as the typical empirical antibiotics for undifferentiated febrile illness, targeting the bacterial cell wall are ineffective in treating rickettsial infections.

Keywords: rickettsia; fever; tropical; Malaysia.

INTRODUCTION

Tropical fevers are prevalent in the tropical and subtropical areas, and commonly present as undifferentiated febrile illness. Etiologies of tropical fever include diseases like dengue, leptospirosis, malaria, typhoid fever, and less commonly, rickettsial infections. Rickettsial infections are zoonotic infection caused by intracellular bacterial agents, namely from the genus *Rickettsia* and *Orientia.* Diagnosis of rickettsial infections is frequently neglected if not delayed due to reduced awareness among clinicians as well as the limitations of serological diagnosis. Laboratory diagnosis using a syndromic molecular approach is essential for the timely administration of targeted antimicrobial therapy.

CASE REPORT

Case 1

A 48-year-old lady with no known medical history, was admitted to general medical ward for fever and lethargy for 6 days. Her symptoms were associated with nonproductive cough, diarrhea and loss of appetite for 3 days. She denied joint pain, petechial rash, dyspnea, vomiting and abdominal pain. Physical examination was unremarkable, and she had no hypotensive episode or tachycardia at presentation. Laboratory investigation revealed leukocytosis (WBC 19.4 x 10^9/L), thrombocytopenia (platelet count of 26 x 10^9/L) and hemoconcentration (hematocrit 44%). She also had a deranged liver function test, with an AST of 152 U/L and ALT of 107 U/L.

Since she stays in a neighborhood with recent reported dengue cases, dengue fever was suspected. Bacterial infection, including possible co-infection with leptospirosis was also considered. She was started on maintenance IV fluids and empiric IV ceftriaxone 2g once daily. Dengue serology showed positive IgG, negative IgM and a negative NS-1 antigen, suggesting past or secondary dengue infection. Leptospira serology was negative, and two sets of blood cultures showed no growth. A multiplex real-time PCR for the detection of bacterial febrile illness was performed on the day of admission from a blood sample using the GenoAmp Real-Time PCR Tropical Fever II kit (MEDIVEN, Malaysia). This assay was used to detect *Borrelia* spp., *Rickettsia/Orientia* spp., *Salmonella* Typhi and *Salmonella* Paratyphi using whole blood sample. The PCR process is carried out using 8 µL of extracted DNA in a total reaction volume of 20 µL, with an annealing temperature set at 61.3°C. The assay is optimized for 50 amplification cycles.

Rickettsia/Orientia DNA was detected, while *S.* Typhi, *S.* Paratyphi and *Borrelia* spp. DNA were not detected. She was treated for rickettsial fever and doxycycline was initiated on day 3 of admission for the treatment of rickettsial infection. Her condition improved with resolution of her abnormal liver function,

thrombocytopenia, leukocytosis resolved after 3 days, and she was discharged well. The antibiotic therapy was continued for a total of 2 weeks duration.

Case 2

The second case was a 26-year-old man with no known medical illness. He was a farmer living in the rural area with proximity to forest area. He presented with fever associated with chills, rigors, and intermittent frontal headache for 1 week duration. He denied bleeding tendencies, rashes, joint pain, or any respiratory and gastrointestinal symptoms. On examination, he was tachycardic and had body temperature of 39°C with multiple enlarged anterior cervical lymph nodes and eschar over the right lower abdomen. Liver and spleen were not enlarged. Laboratory investigation showed thrombocytopenia (platelet 121 x 10^9/L), elevated creatinine kinase (360 IU/L) and elevated liver transaminases (AST 154 U/L, ALT 204 U/L). WBC was normal.

He was treated empirically for leptospirosis with IV ceftriaxone 2g OD, with the differential diagnoses of scrub typhus. Oral doxycycline was also given empirically for the possibility of scrub typhus. Leptospira serology, dengue IgM antibody and NS1 antigen were negative. However, dengue IgG antibody was positive indicating past dengue infection. A molecular panel for acute febrile illness was done on day 3 of admission, and *Rickettsia/Orientia* DNA was detected. The nucleic acid for other pathogens, namely *S. Typhi*, *S. Paratyphi* and *Borrelia* spp. were not detected. Two sets of blood culture sent had no growth. The patient was then diagnosed with rickettsial fever and IV ceftriaxone was stopped after 5 days of admission, and oral doxycycline was continued for a 2-week duration for the treatment of scrub typhus. He became afebrile after 3 days of oral doxycycline, and was discharged after 6 days of hospitalization, with the plan to complete oral doxycycline for 14 days.

Case 3

A 23-year-old man with bronchial asthma presented to the emergency department for fever of 2 weeks duration. The fever was intermittent and was associated with lethargy and anorexia. He also had 3 episodes of vomiting and abdominal discomfort and loose stools for 2 days. He had been treated with oral ciprofloxacin by a general practitioner a week earlier but his fever persisted. He denied bleeding tendencies, joint pain, rashes or any respiratory symptoms. He also denied any water activity to suggest exposure to leptospirosis. On examination, he was having temperature of 38°C and tachycardic with pulse rate of 110 beats per minutes. Vital signs were normal. Other physical examinations were unremarkable. Laboratory investigation showed thrombocytopenia (PLT, 145 x 10^9/L), leukocytosis (TWBC, 11 x 10^9/L) and hemoconcentration (HCT, 48%), and deranged liver enzymes with the AST of 143 U/L and ALT of 220 U/L. During admission, he developed hypotension associated with fever and chills, which resolved promptly with intravenous fluid resuscitation. He was empirically treated with IV ceftriaxone 3g OD with the differential diagnoses of leptospirosis and typhoid fever. Leptospira IgM serology, dengue NS1 antigen and IgM antibody, and blood film for malaria were negative and two sets of blood culture showed no growth. A multiplex molecular panel for tropical fever was done (GenoAmp Real-Time PCR Tropical Fever II kit (MEDIVEN, Malaysia) on day 4 of admission. Rickettsia/*Orientia* DNA was detected. Further testing at the national reference laboratory revealed that the genetic material for the causative agent of scrub typhus, *Orientia tsutsugamushi,* was present. The nucleic acid for other pathogens, namely *S.* Typhi, *S.* Paratyphi and *Borrelia* spp. were not detected. He was treated for scrub typhus and started on doxycycline at day 5 of admission for 2 weeks duration. The patient became afebrile, and the symptoms improved with the antibiotic therapy and was discharged after 7 days of admission.

Table 1. Summary of the main clinical manifestation of the rickettsia patients

Case		Clinical presentations	Provisional/ differential diagnosis at presentation	Relevant laboratory findings		Treatment
1	48 years old female	Fever and lethargy for 6 days, nonproductive cough, diarrhea and anorexia	Dengue fever	Leukocytosis (WBC count, 19.4 x 10^9/L) Thrombocytopenia (PLT, 26 x 10^9/L) Hemoconcentration (HCT, 44%) Deranged liver function test (AST, 152 U/L; ALT, 107 U/L)	Dengue IgG positive and IgM negative, Dengue NS1 antigen positive *Rickettsia/ Orientia* spp. DNA detected	IV ceftriaxone (Empirical) IV fluid therapy
2	26 years old male	Fever, chills, rigors, on and off frontal headache for 1 week Cervical lymphadenopathy and eschar over the right lower abdomen	Leptospirosis Scrub typhus	Thrombocytopenia (PLT, 121 x 10^9/L) Creatinine kinase (360 IU/L) Transaminases (AST, 154 U/L; ALT 204 U/L)	Negative Leptospira serology, dengue IgM and NS1 antigen. Negative blood culture Positive for dengue IgG antibody *Rickettsia/ Orientia* spp. DNA detected	IV ceftriaxone (Empirical) Oral doxycycline IV fluid therapy
3	23 years old male	Fever for 2 weeks, lethargy, anorexia, abdominal discomfort, vomiting & diarrhea for 2 days	Typhoid fever, Leptospirosis	Leukocytosis (TWBC count, 11 x 10^9/L) Thrombocytopenia (PLT, 145 x 10^9/L) Hemoconcentration (HCT, 48%) Deranged liver function test (AST, 143 U/L; ALT, 220 U/L)	Negative Leptospira IgM serology, dengue NS1 antigen and IgM antibody, and blood film for malaria Negative blood culture *Rickettsia/ Orientia* spp. DNA detected	IV ceftriaxone (Empirical) Oral doxycycline IV fluid therapy

DISCUSSION

Tropical fevers are community-acquired infections unique to the tropical regions. The clinical manifestations of tropical fever are often non-specific, leading to a wide array of differential diagnoses, such as dengue fever, leptospirosis, malaria, scrub typhus and typhoid fever (Singhi *et al*., 2014). History of contact, travels and other epidemiological clues are necessary for initial evaluation of patient, and timely laboratory diagnoses are crucial in the timely management of these infections, as the specific administration of an antimicrobial is often required for the successful treatment of the infections.

Dengue fever and leptospirosis are common causes of undifferentiated fever in Malaysia while zoonotic malaria cases are also seen in the rural areas. As highlighted in the reported cases, the non-specific nature of the clinical presentations may lead to diagnostic dilemma in the treatment of these cases. Often, broad spectrum antibiotics such as third-generation cephalosporins are empirically administered. These antibiotics are usually β-lactam antibiotics that have poor activity against rickettsial disease caused by an intracellular bacterium.

Scrub typhus is a zoonotic disease endemic in Southeast Asia, with the estimated seroprevalence of 9.3-27.9%, contributes significantly to the burden of tropical fevers, with the mortality rate of up to 24% (Peter *et al*., 2015). However, available literature and diagnostic modalities for scrub typhus remain limited, leading to missed clinical and laboratory diagnoses, and inability to administer effective antibiotic for the treatment.

The *Rickettsiaceae* family consists of 2 genera, *Rickettsia* and *Orientia.* The genus Rickettsia has numerous designated species, each causes a different febrile illness, has distinct geographical distribution, vector, natural cycle, and mode of transmission. The genus can be broadly divided into Typhus group (*R. prowazekii*, *R. typhi*), Spotted fever group (*R. rickettsii, R. conorii, R africae, R. parkeri, R. japonica, R. sibirica, R. honei, R. heilongjiangensis, R. slovaca*) and Transitional group (*R. akari, R. australis*, *R. felis*) (Weinert *et al*., 2009). The causative agent of scrub typhus, *Orientia tsutsugamushi* diverges from Rickettsia by approximately 10% in the 16S rRNA and has a distinct cell wall structure (thicker outer membrane, lack lipopolysaccharide, and different cell wall proteins) (Vitorino *et al*., 2007).

Serological diagnosis (such as IFA or EIA) was previously considered the gold standard for the diagnosis of scrub typhus, requiring acute- and convalescent-phase sera. However, serological diagnosis has several drawbacks, including delayed seroconversion leading to missed acute infection, subjective interpretation of endpoint titers, need for paired specimens, and consideration of cut-off based on regional seroprevalence (Paris & Dumler, 2016).

Nucleic acid amplification tests (NAAT) have been increasingly used for the diagnosis of rickettsial infections. It has the advantage of detecting the rickettsial DNA in the acute phase of febrile illness, enabling the determination of etiological agent and successful administration of effective antibiotic therapy (Prakash *et al*., 2009). However, the limited quantities of circulating rickettsial DNA may result in reduced sensitivity when peripheral blood specimens are used (Yun *et al*., 2021). Skin biopsy specimen, and swabs or crust from eschars provide a better sensitivity for the detection of rickettsial DNA (Kim *et al*., 2006). Real-time PCR has added advantage of high sample throughput, rapid turnaround time (within same day of sample collection), ability for quantification, reduced risk of contamination, and multiplexing capability, and is considered to be the preferred NAAT method for diagnosis of scrub typhus (Prakash *et al*., 2009).

The GenoAmp RT-PCR Tropical Fever assay has a good analytical sensitivity of 95%, with a limit of detection of less than 10 copies/µL. Analytical specificity is also good with no cross reactivity with other pathogens tested. The assay is not able to differentiate between *Rickettsial* and *Orientia* spp., as both genera are closely related and belong to the family of *Rickettsiaceae*. *Orientia* diverges from *Rickettsia* by approximately 10% in the 16S rRNA gene. Therefore, in suspected cases of typhus fever group, the diagnosis may need to be confirmed by molecular test to differentiate the various typhus fever diseases, namely endemic typhus, murine typhus and scrub typhus. However, a *Rickettsia*/*Orientia* genus NAAT detection is usually sufficient for clinical and treatment purpose, as effective differentiation of the causes of tropical fever can be achieved by employing a multiplex syndromic panel. Administration of antibiotics with activity against intracellular bacteria such as doxycycline and azithromycin will lead to the successful treatment.

CONCLUSION

The availability of multiplex panel for molecular detection of the agents of tropical fever enables the early diagnosis and treatment of rickettsial infections, such as scrub typhus.

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Authorship Contribution Statement
Wei Chuan Chua: Data curation, Writing - original draft, Writing - review & editing. **Ahmad Adebayo Irekeola:** Data curation, Investigation, Writing - original draft, Writing - review & editing. **Muhammad Izzat Abdul Hadi:** Investigation, Writing - review & editing. **Wan Syahmi Wan Mohamad:** Investigation, Writing - review & editing. **Nor Irma Mohd Nasir:** Investigation, Writing - review & editing. **Norsarwany Mohamad:** Methodology, Supervision, Writing - review & editing. **Mohd Hashairi Fauzi:** Methodology, Supervision, Writing - review & editing. **Alwi Muhd Besari @ Hashim:** Methodology, Supervision, Writing - review & editing. **Chan Yean Yean:** Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Writing - review & editing.

Competing Interests
None declared. The funder of this study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Ethical approval
Ethical approval was received from the Human Research Ethics Committee of Universiti Sains Malaysia (USM/JEPeM/KK/23010113). The authors certify that they have obtained all appropriate patient consent forms.

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