Successful diagnosis and treatment of scrub typhus associated with haemophagocytic lymphohistiocytosis and multiple organ dysfunction syndrome: A case report and literature review

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\textbf{ABSTRACT}

Scrub typhus is a natural foci disease caused by the bacteria \textit{Orientia tsutsugamushi}. Symptoms of the disease range from fever to severe multiple organ dysfunction. The diagnosis is based on clinical signs and antibody serological tests, which has poor sensitivity and specificity. Scrub typhus is rarely associated with multiple organ dysfunction syndrome (MODS) and haemophagocytic lymphohistiocytosis (HLH). In this paper, we report a 17-year-old Asian male who was characterized with a persistent fever without eschar. He was diagnosed with scrub typhus using metagenomic next-generation sequencing (mNGS) of the blood after negative of routine examinations. The patient was progressed to HLH and MODS but had a good recovery following anti-rickettsial therapy, dexamethasone, and advanced life support. Besides, we present a brief overview of the literature about scrub typhus and associated complications.

1. Introduction

Scrub typhus is a natural foci disease induced by the bacteria \textit{Orientia tsutsugamushi}. Symptoms of the disease range from mild fever to multiple system dysfunction. An eschar is the most pathognomonic sign \cite{1, 2}. Clinical signs and serological tests (eg. the Weil–Felix test) are used to diagnose scrub typhus, despite the poor sensitivity and specificity. Serum immunoglobulinM (IgM) antibody, immunoglobulinG (IgG) antibody, enzyme-linked immunosorbent assay (ELISA), and polymerase chain reaction (PCR) are used as alternative tests. Metagenomic next-generation sequencing (mNGS) is a novel technology to identify the special infectious pathogens. To date, few cases of scrub typhus have been confirmed by mNGS.

There have been an increasing number of scrub typhus cases reported in the past years. However, few studies have reported scrub typhus-associated haemophagocytic lymphohistiocytosis (HLH) and multiple organ dysfunction syndrome (MODS). HLH is a rare and deadly hyperinflammatory syndrome, characterised by the over-activation of T lymphocytes and macrophages, along with the weakening of natural killer (NK) cells and cytotoxic T lymphocytes. This results in uncontrolled histiocytic phagocytosis of blood cells and cytokine-mediated multiple organ dysfunction \cite{3, 4}. Primary HLH is caused by genetic mutations. Secondary HLH is mainly initiated by immunodeficiency, autoimmune diseases, infections, and malignancies \cite{1, 5, 6, 7, 8}. Here, we report a case of scrub typhus with no eschar diagnosed by mNGS of blood. The patient was progressed to HLH and MODS but had a good recovery following anti-rickettsial therapy, dexamethasone, and advanced life support. Besides, we present a brief overview of the literature about scrub typhus and associated complications.

2. Case presentation

The patient with scrub typhus-associated HLH and MODS was admitted to the intensive care unit of West China Hospital, Sichuan University. The present study was confirmed by the Committee on Medical Ethics of West China Hospital, Sichuan University. The study was accomplished in conformity to the Declaration of Helsinki and CARE guidelines. The guardians of the patient signed the written informed consent.

The patient was a 17-year-old Asian male who was first admitted to another hospital with persistent fever. He had a history of mountain
cycling, but with no eschar or rash. After empirical anti-infection (tazobactam-piperacillin and follow-up meropenem) and anti-inflammatory (injectable dexamethasone) treatment, the patient developed dyspnoea and multiple organ dysfunction. The patient was then transferred to the intensive care unit (ICU) of our hospital for further treatment. The vital signs at admission were as follows: temperature, 39.2 °C; blood pressure, 119/75 mmHg (continuous intravenous pumping of norepinephrine 0.389 μg/kg/min); respiratory rate, 40 bpm; heart rate, 165 bpm; and saturation of haemoglobin with oxygen (SpO2), 90 % (fraction of inspired oxygen, FiO2, 61%). Physical examination revealed chemosis, wet voice, wheezing, hepatosplenomegaly, lymphadenopathy, and no bite or eschar. Information on the vital signs and arterial blood gas analysis at admission are summarized in Table 1.

Laboratory examination showed anemia (haemoglobin, 72 g/L), thrombocytopenia (platelets, 12,000 cells/mm³), raised serum ferritin (5249 U/mL). The laboratory examination results at admission are summarized in Table 1. mNGS revealed a total of 738 sequences of O. tsutsugamushi in the blood sample with a total coverage of 2.70 % (Figure 1).

Bone marrow examination revealed phagosome-containing platelets and in the blood sample with a total coverage of 2.70 % (Figure 1). Bone marrow examination revealed phagosome-containing platelets and erythrocytes (Figure 2). FCM of the bone marrow revealed negative results. The mutation genes of the primary HLH were negative. The Weil–Felix agglutination test was not available in our hospital. Tests for human immunodeficiency virus (HIV), hepatitis B and C, Epstein–Barr virus (EBV), cytomegalovirus (CMV), mycoplasma pneumonia, and TORCH toxoplasma were negative. Bacterial and fungal cultures of blood, sputum, and bone marrow were negative. Ultrasonography revealed lymphadenopathy. CT showed bilateral diffuse effusion shadows and consolidations in the lungs and splenomegaly (Figure 3A, 3B and 3C). Bronchoscopy revealed normal tracheal and bronchial lumens.

Intravenous azithromycin (0.5 g intravenous per day) was commenced on the 5th day of his disease for 12 consecutive days. Doxycycline (4 mg/kg daily) was added following azithromycin withdrawal. The patient received intravenous dexamethasone (10 mg/day) for 12 days and then tapered to 5 mg/day for 15 days. Respiratory failure, septic shock, blood coagulation dysfunction, upper gastrointestinal bleeding, elevated transaminase levels, low albumin levels, and cardiac insufficiency were observed during the course of the disease. The patient received invasive mechanical ventilation, vascular active pharmaceutical, blood transfusion, procoagulation inhibitors, somatostatin, and treatment of the liver and heart. During the course of the therapy, a culture of bronchoalveolar lavage fluid (BALF) revealed Aspergillus and Acinetobacter baumannii. Meropenem and amoxicillin of amphetamine B and injection of caspofungin were commenced at the time of positive BALF culture. The patient recovered completely and was discharged after 25 days of treatment. Information on the vital signs and laboratory examination results at discharge are summarized in Tables 1 and 2. Clinical course of the patient is showed in Figure 4.

3. Discussion

This study describes a 17-year-old Asian male who was characterized by persistent fever but with no eschar. The patient was diagnosed with scrub typhus by mNGS. The patient was progressed to HLH and MODS. He had a good recovery following anti-rickettsial, immunomodulatory, and supportive treatments.

Scrub typhus is an acute febrile disease induced by chiggers in endemic areas. An eschar is a special sign. The diagnosis of scrub typhus was based on clinical signs and serological tests [9, 10, 11, 12, 13, 14]. Diagnosis of scrub typhus is summarized in Table 3. Serological tests of scrub typhus are usually possible 5–10 days after disease onset. The Weil–Felix test is the most commonly used serological test, but it has poor specificity and sensitivity [15]. One study reported that the sensitivity and specificity of the Weil–Felix test and IgM enzyme-linked immunoabsorbent assay (ELISA) were 47 %, 96 %, 91 %, and 100 %, respectively [16]. Studies have reported that IgM ELISA and IgG capture ELISA techniques [17], and PCR-based tests [18] have better predictive value in the diagnosis of scrub typhus. mNGS, also known as high-throughput sequencing, is a newer method for evaluating infections. mNGS sequences millions of DNA fragments simultaneously by a massively

Table 1. Diagnosis and treatments of scrub typhus and HLH.

| Scrub typhus # | HLH + |
|----------------|-------|
| **Diagnosis**  |       |
| Clinical Characteristics: |       |
| (1) history of outdoor activities; |       |
| (2) persistent fever, with or without eschar; |       |
| (3) rash, splenomegaly, and lymph node enlargement; |       |
| **Serological tests:** |       |
| (1) Weil–Felix test: OXK titre ≥1:160 or an increase by 4-fold; |       |
| (2) more than 4-fold rise in serum IgG antibody titre; |       |
| (3) positive PCR of O. tsutsugamushi in clinical samples; |       |
| (4) isolation of O. tsutsugamushi from clinical samples. |       |
| **A)** family history or gene mutations of PRF, SAP, or MUNC1; |       |
| **B)** presence of five of eight diagnostic criteria: |       |
| (1) persistent fever |       |
| (2) splenomegaly |       |
| (3) unexplained cytopenia, at least two cell lines (haemoglobin < 90 g/L, neutrophils < 100,000 cells/mm³, platelets < 1000 cells/mm³); |       |
| (4) hypofibrinogenemia (≤150 mg/dL) and/or hypertriglyceridaemia (≥265 mg/dL); |       |
| (5) hyperferritinaemia (≥500 mg/mL) |       |
| (6) haemophagocytosis (in bone marrow, liver, spleen, or lymph nodes without evidence of malignancy) |       |
| (7) low activity of NK cells |       |
| (8) high concentration of soluble CD25 (interleukin-2Rα chain ≥ 2,400 U/mL) |       |

| **Treatments** |       |
| Doxycycline | Dexamethasone |
| Azithromycin | Esposide |
| Chloramphenicol | IT Therapy |
| Clarithromycin | HSCT |

* The diagnosis of scrub typhus was made once three of the criteria were positive;
* The diagnosis of HLH can be established if one of the either A or B criteria below is fulfilled; HLH, haemophagocytic lymphohistiocytosis; IT. Therapy, intrathecal methotrexate and corticosteroids; HSCT, hematopoietic stem cell transplantation; NK, natural killer cell.

Table 2. Findings from vital signs and arterial blood gas analysis at admission and discharge.

| Findings                  | Parameter | At admission | At discharge |
|---------------------------|-----------|--------------|--------------|
| Vital signs               | Temperature (°C) | 39.5 | 37.1          |
|                           | heart rate (times/min) | 135 | 105          |
|                           | respiratory rate (times/min) | 35 | 18          |
|                           | blood pressure (mmHg) | 76/40 | 120/65          |
| Arterial blood gas analysis | PH (mmHg) | 7.400 | 7.431          |
|                           | PO2 (mmHg) | 76 | 104.8          |
|                           | PCO2 (mmHg) | 15.9 | 34.9          |
|                           | PO2/FiO2 | 76 | 316          |
|                           | Lac (mmol/L) | 5.4 | 2.1          |

PH, Pondus Hydrogeni; PO2, partial pressure of oxygen; PCO2, partial pressure of carbon dioxide; PO2/FiO2, partial pressure of oxygen/fraction of inspire oxygen; Lac, lactic acid.
parallel sequencing platform for a few hours [19, 20, 21]. With this technology, DNA fragments can be sequenced from a clinical specimen. However, to date, there is few cases of scrub typhus have been confirmed using mNGS [22, 23, 24, 25, 26, 27]. Li et al. [23] reported a 51-year-old patient who had unexplained fever and with no eschar was diagnosed by mNGS of blood and sputum samples. The patient received minocycline and was discharged from the hospital without any complications. Liu et al. [24] applied mNGS, Weil–Felix reaction, indirect immunofluorescence test (IIFT), respiratory tract profile IgM and culture for routine bacteria to identify scrub typhus. Nine of the 10 patients were finally diagnosed as scrub typhus. All patients were positive of mNGS. For other methods, only Weil–Felix reaction of one patient detected the pathogen. From these results, we considered that mNGS as a better diagnostic method than conventional clinical methods in early diagnose of scrub typhus.

HLH is a syndrome of immune system activation which leads to phagocytosis of blood cells and proliferation of histiocytes in solid organs. Scrub typhus invades endothelial cells and macrophages, activating various chemokine genes [1, 2, 28, 29, 30, 31, 32, 33]. These inflammatory cytokines are responsible for HLH and MODS in patients with severe infections [1, 2, 29, 30, 34, 35]. The HLH-2004 criteria are used to identify HLH, which are summarized in Table 3 [36]. The prevalence of HLH associated with scrub typhus has been previously reported. About 3% of associated HLH cases were reported in one report of scrub typhus in children [16]. But sporadic cases of associated HLH have been reported in another study of infants [37]. However, HLH and MODS associated

Figure 1. mNGS of the patient. The total base number of the species of Orientia tsutsugamushi is 1932116 bps, and the measured sequences covering the total length are 52234 bps. The coverage of Orientia tsutsugamushi is 2.70 %, and the average depth is 1.061 X in blood; mNGS, metagenomic next-generation sequencing.

Figure 2. The bone marrow smear test of the patient. Smear image of the bone marrow shows phagosome containing platelets and erythrocytes hemophagocytic.
Figure 3. CT scan of lung and spleen. (A) pulmonary window of CT scan shows consolidation and atelectasis of both the under lung; (B) mediastinal window of CT scan shows consolidation and atelectasis of both the under lung; (C) CT scan shows lymph stasis of liver and splenomegaly.

Figure 4. Clinical course of the patient. ARDS: acute respiratory distress syndrome; HLH: haemophagocytic lymphohistiocytosis; mNGS: metagenomic next-generation sequencing; MODS: multiple organ dysfunction syndrome; BALF: bronchoalveolar lavage fluid.
with scrub typhus in adult is rarely. Basheer et al. [38] reported that 5 patients had scrub typhus-associated HLH and MODS. All patients were adults, among them an 81-year-old female patient who died after supportive and antibiotics therapy. Three patients combined with sepsis and one patent with DIC. No patient had eschar or rash. Besides, no one received immunosuppressive and/or immunomodulatory therapy. During infection, Orientia tsutsugamushi targets the vascular endothelial cells of the small to medium-sized blood vessels and invades smooth muscle cells, perivascular macrophages, and monocytes [1, 2]. In patients with severe infections, widespread vasculitis/perivasculitis is the characteristic pathophysiologic mechanism in MODS. For a few reports, we considered old age, without eschar or rash, delayed diagnosis and more complications may be risk factors to progress HLH and MODS in the patients diagnosed with Scrub Typhus. Immunosuppressive and immunomodulatory therapy should be rigorously assessed in these severe patients.

The treatment of scrub typhus and HLH are summarized in Table 3. The treatment response rate of doxycycline, azithromycin, and clari-thromycin in scrub typhus is ranging from 64% to 100% [39]. In endemic areas, doxycycline has been suggested as an empirical therapy for patients with a central nervous system (CNS) infection [16]. Chloramphenicol has limited use in bone marrow suppression. Intravenous azithromycin was the preferred treatment in all the cases. As mentioned, profound hypercytokinaemia plays an important effect in the pathophysiology of HLH. Managing hypercytokinaemia is the first and most important step in the therapy of HLH [40, 41]. Etoposide, cyclosporin A (CSA), and dexamethasone are recommended as initial treatments. Supportive care and appropriate broad-spectrum antibiotics are recommended. Continuation therapy and/or haematopoietic stem cell transplantation (HSCT) are recommended if the disease is active after the initial therapy. The management of primary HLH has been well proved in the HLH protocol, but the treatment strategy of secondary HLH has been controversial [4]. Kleynberg and Schiller [42] emphasised the importance of etoposide in the treatment of HLH associated with EBV infection. In our study, the patient made a complete recovery after being treated with anti-rickettsial antibiotics, immunomodulatory therapy (dexamethasone), and supportive treatment but without chemotherapy (etoposide, or CSA). Therefore, chemotherapy in the management of scrub typhus-associated HLH may not be necessary. Patients with secondary HLH may require additional immunomodulatory therapies. For the cytoketic effect of lymphocytes and inhibition of the expression of cytokines, corticosteroids and intravenous Ig are suitable for the treatment of hyperinflammation [43, 44, 45]. These studies showed that anti-rickettsial treatment and immunomodulatory therapy may be effective in patients with scrub typhus-associated HLH. It may be hard to utilize cytoketic agents in patients with severe diseases (such as sepsis or multiple organ dysfunction). For anti-inflammatory effects, immunosuppression remains as the basis of early management.

Previous studies suggest that infection-associated HLH may have a poor clinical prognosis [46, 47]. Additionally, patients with a delay in anti-rickettsial therapy had a higher risk of complications [47]. Early diagnosis and therapy are important for disease management. The mortality rate of scrub typhus varies widely. In one study, the mortality was up to 10% in patients with significant risk factors, including shock, renal failure, thrombocytopenia, MODS, and CNS involvement [48]. In another study, up to 30% mortality was reported in patients with severe complications without proper treatment [15]. A recent study from Japan showed that old age is an independent risk factor for mortality in scrub typhus cases.

The patient in our study was unusual for diagnosing by mNGS and recovering under treatment of anti-rickettsial, immunoregulatory and supportive therapy, without chemotherapy and HSCT. From our case report, we may highlight the following: (1) mNGS may be a good choice when routine serological tests cannot be obtained; (2) eschar or bites are important for diagnosis, but patients with negative results cannot be ruled out from being diagnosed with scrub typhus; (3) anti-rickettsial therapy, anti-inflammatory therapy, and advanced life support are key therapies for scrub typhus and associated HLH and MODS; and (4) chemotherapy and HSCT may be not necessary in the management of secondary HLH.

4. Conclusions

This study suggests that mNGS is a novel method to identify scrub typhus in patients without eschar or convention methods are negative or inaccessible. Anti-rickettsial therapy, anti-inflammatory therapy, and advanced life support are key therapies for scrub typhus and associated HLH and MODS. Chemotherapy and HSCT may be not necessary in the management of secondary HLH.

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Data availability statement

No data was used for the research described in the article.

Declaration of interest’s statement

The authors declare no conflict of interest.

Table 3. Findings from laboratory examinations at admission and discharge.

| Findings | Parameter | At admission | At discharge |
|----------|-----------|--------------|--------------|
| General laboratory tests | WBC (cells/mm³) | 16,980 | 12,410 |
| | PLT (cells/mm³) | 12,000 | 463,000 |
| | N % | 95 | 49.6 |
| | HGB (g/L) | 72 | 87 |
| | PCT (ng/mL) | 18.4 | 0.05 |
| | CRP (mg/L) | 94 | 1.37 |
| | Albumin (g/L) | 15.3 | 41.7 |
| | AST (U/L) | 573 | 50 |
| | ALT (U/L) | 100 | 35 |
| | BUN (mmol/L) | 15.3 | 4.4 |
| | CR (umol/L) | 110 | 45 |
| | eGFR (ml/min/1.73m²) | 84.58 | 158.15 |
| | BNP (ng/L) | 6279 | 13 |
| | PT (s) | 17.7 | 10.8 |
| | APTT (s) | 87.4 | 26.5 |
| | INR | 1.61 | 0.98 |
| | FIB (g/L) | 0.59 | 1.84 |
| | TG (mmol/L) | 4.53 | 1.5 |
| | Ferritin (mg/dL) >2000 | 104 |
| | sCD25 (U/mL) | 5249 | 85.3 |
| | NK % | 3.6 | 7.2 |

WBC: white blood cells; N %, percentage of neutrophil; PLT: platelet; HGB: hemoglobin; PCT, procalcitonin; CRP: C-reactive protein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; BUN, blood urea nitrogen; CR, creatinine; eGFR, estimated glomerular filtration rate; PT, prothrombin time; APTT, activated partial thromboplastin time; INR, International Normalized Ratio; FIB, fibrinogen; TG, triglyceride; NK, natural killer cell; sCD25, soluble interleukin-2 receptor.
Additional information

No additional information is available for this paper.

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