Rickettsia japonica infections in Huanggang, China, in 2021

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ABSTRACT

Two patients from Huanggang, China, were diagnosed with spotted fever group (SFG) rickettsiosis—caused by spotted fever group rickettsiae (SFGR)—in 2021. This study aimed to investigate the clinical symptoms, laboratory examinations, epidemiological factors, and therapeutic responses in patients with SFG rickettsiosis—an emerging disease in this region. The patients showed a variety of clinical signs and symptoms, such as acute febrile illness with severe headache, myalgia, asthenia, anorexia, eschar, lymphadenopathy, and rash on the trunk and extremities. They exhibited increased neutrophil ratio, mild thrombocytopenia, liver dysfunction, and increased C-reactive protein and procalcitonin levels. Following treatment with doxycycline, the patients recovered completely.

This is the first report of Rickettsia japonica infection in Huanggang City, Hubei Province, China. SFGR infection is a tick-borne disease, which can be effectively treated with doxycycline; however, it has a mortality rate of approximately 10% with delays in treatment. The Huanggang area is also a high-risk area for tick-borne severe fever with thrombocytopenia syndrome (SFTS). Therefore, SFTS and SFG rickettsiosis should be carefully diagnosed in this area and clinicians should be alert with respect to the possibility of infections with both SFTS and SFG rickettsiosis.

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Introduction

Spotted fever group rickettsiae (SFGR) contains more than 20 species of rickettsiae that are pathogenic to humans and distributed worldwide. In recent years, cases of spotted fever group (SFG) rickettsiosis have been reported in Jiangsu, Jiangxi, Henan, Anhui, and Shandong provinces and the Inner Mongolia Autonomous Region in China [1–5]. Some cases were diagnosed by routine monitoring of wild mammals and ticks in China found a variety of SFGR that can cause human diseases, indicating that several regions in China could be natural foci [4,6]. However, relatively few clinical cases of SFG rickettsiosis have been reported in hospitals in these areas. Rickettsiae are gram-negative intracellular bacteria and may not be detected using nucleic acid tests due to false negative results, especially when rickettsial antibodies appear late. There are some similarities and differences among the clinical features of the disease found in different regions. These factors may lead to misdiagnosis and delays in initiation of treatment. SFG rickettsiosis has not been previously reported in Hubei Province, China where Haemaphysalis longicornis is widely distributed. The onset of illness for the two patients were highly consistent in time and space, indicating that the pathogen may have spread to the local population. The purpose of this study was to investigate the epidemiological characteristics, clinical manifestations, laboratory results, and treatment responses of patients with SFG rickettsiosis in this region. The findings of the study could be used as a basis to formulate prevention and treatment plans for the disease.

Case description

Two patients from Fuzihe Town, Macheng City, Huanggang City, Hubei Province, which is a hill area, presented to the Department of Infectious Diseases of Union Hospital Affiliated to Tongji Medical College of Huazhong University of Science and Technology in August 2021 with fever, fatigue, severe headache, and rash. One is a 62 year old male and the other is a 59 year old female. They all lived in hill region.
Laboratory tests

Peripheral blood samples were obtained from each patient according to standard procedures during admission, and procalcitonin (PCT) levels, C-reactive protein (CRP) levels, D-dimer, urinary sediment microscopy and differential blood cell counts were examined at the Clinical Diagnosis Laboratory of the Union Hospital. Plasma levels of creatinine, alanine aminotransferase, aspartate aminotransferase, and lactate dehydrogenase were determined at the Biochemistry Laboratory of the Union Hospital. Furthermore, the activated partial thromboplastin time and thrombin time of fresh plasma samples were measured at the Clinical Diagnosis Laboratory of the Union Hospital.

The patients had failed to respond to antiviral and antibacterial therapy in the primary hospital during the initial stage of illness. After admission to our hospital, we performed quantitative metagenomics next-generation sequencing (mNGS) tests. For mNGS tests, blood samples were transported to the molecular lab of Hangzhou Matridx Biotechnology Co., Ltd., within 24 h.

Library preparation and sequencing

Whole blood was centrifuged at 1600 g for 10 min and the supernatant was further centrifuged at 16,000 g for 10 min to separate the plasma. For cerebrospinal fluid, DNA or RNA sequencing was performed. DNA or RNA sequencing libraries were prepared by reverse transcription (for RNA), enzymatic fragmentation (except for plasma), end repair, terminal addition, and adapter ligation (NGS master™ library preparation, Matridx, Cat# MAR002) [7]. The concentration of libraries was quantified by real-time PCR (KAPA) and pooled. Shotgun sequencing was carried out on the Illumina NextSeq platform. Approximately 20 million 75 bp single-end reads were generated for each library. For each run, one negative control (artificial plasma mixed with fragmented human genomic DNA) and one positive control (a mixture of inactivated bacteria, fungi and pseudoviral particles containing synthesized DNA or RNA fragments of adenovirus and influenza A virus, respectively) were included for quality control.

Bioinformatics pipeline

Raw sequencing data were analyzed using a bioinformatics pipeline, which included the following steps: (1) Unnecessary adapter sequences and low-quality bases (Q-score cut-off of 20) were trimmed. (2) Human host sequences were eliminated by mapping to the human reference genome (GRCh38.p13) using BWA (Burrows-Wheeler alignment, http://bio-bwa.sourceforge.net). (3) After removal of low-complexity reads, the remaining sequencing data were simultaneously aligned by BWA to the reference databases (NCBI nt database and GenBank) to identify microbial species.

Clinical findings and laboratory examination

Both patients reported history of fieldwork, and one had a known tick bite. Both patients had fever, asthenia, severe headache, and anorexia and displayed lymphadenopathy and characteristic rash manifested as dark reddish spots (Fig. 1a,b). The characteristic rash of patient 1 transformed into ecchymosis (Fig. 1c) in convalescence stage. (Fig. 1). Patient 2 had eschars (Fig. 2), but neither of them had any severe complications (i.e. respiratory failure; or hemorrhagic or neurological signs or symptoms) (Table 1). None of their household contacts exhibited similar symptoms.

Peripheral blood examination in both patients suggested lymphopenia, eosinopenia, thrombocytopenia, and normal WBC levels, but the ratio of neutrophils was increased. The inflammatory indicators, including D-dimer, PCT, and CRP, were significantly increased. Biochemical examination showed that the levels of liver transaminase and lactate dehydrogenase increased upon admission (Table 2).

After 3–5 days of doxycycline treatment, the clinical symptoms disappeared, and laboratory test results returned to normal.

Diagnosis of patients with SFG Rickettsiosis

The pathogens detected using mNGS are listed in Table 3. In addition to common human symbiotic microorganisms, spotted fever

Fig. 1. A dark reddish, non-blanching, maculopapular rash was visible on the trunk and extremities of the patients (a, b). The characteristic rash of patient 1 transformed into ecchymosis (c) in convalescence stage.

Fig. 2. Eschar visible on the skin of the lateral surface of the right thigh of patient 2.
rickettsiae and herpes virus were detected. A small amount of Epstein-Barr virus was detected in one patient, and a small amount of human herpes virus types 1 and 5 was detected in the other patient. Six 50 bp Rickettsia fragments from the two patients were aligned with the reference database, and the results showed that all six fragments were consistent with the gene library of Rickettsia japonica (Fig. 3a, b). Due to the small number of fragments detected, the whole gene sequence analysis could not be carried out.

Therapeutic responses

After treatment with doxycycline, the patient’s conditions rapidly alleviated without sequelae.

Table 3
Pathogenic microorganisms detected by mNGS in blood (sorted by species).

| Patient no. | Name          | Genus name   | Genus reads accum |
|-------------|---------------|--------------|-------------------|
| 1           | Staphylococcus| Staphylococcus| 4                 |
|             | Staphylococcus aureus | Staphylococcus | 4                 |
|             | Staphylococcus capitis | Staphylococcus | 4                 |
|             | Rickettsia    | Rickettsia   | 2                 |
|             | Spotted fever group | Rickettsia | 2                 |
|             | Cutibacterium | Cutibacterium | 1                 |
|             | Cutibacterium acnes | Cutibacterium | 1                 |
|             | Herpesviridae | Herpesviridae | 14                |
|             | Gammaherpesvirinae | Gammaherpesvirinae | 14                |
|             | Lymphocryptovirus | Lymphocryptovirus | 14                |
| 2           | Moraxella     | Moraxella    | 18                |
|             | Moraxella osloensis | Moraxella | 18                |
|             | Cutibacterium | Cutibacterium | 10                |
|             | Cutibacterium acnes | Cutibacterium | 10                |
|             | Rickettsia    | Rickettsia   | 4                 |
|             | Spotted fever group | Rickettsia | 4                 |
|             | Staphylococcus | Staphylococcus | 2                 |
|             | Staphylococcus aureus | Staphylococcus | 2                 |
|             | Staphylococcus capitis | Staphylococcus | 2                 |
|             | Malassezia    | Malassezia   | 1                 |
|             | Malassezia restricta | Malassezia | 1                 |
|             | Herpesviridae | Herpesviridae | 2                 |
|             | Alphaherpesvirinae | Alphaherpesvirinae | 1                 |
|             | Simplexvirus  | Simplexvirus | 1                 |
|             | Human         | Simplexvirus | 1                 |
|             | alphaherpesvirinae 1 | alphaherpesvirinae 1 | 1                 |
|             | Betaherpesvirinae | Betaherpesvirinae | 1                 |
|             | Cytomegalovirus | Cytomegalovirus | 1                 |
|             | Human betaherpesvirina 5 (cytomegalovirus) | Cytomegalovirus | 1                 |

Table 2
Laboratory test results of the two patients on admission.

| Result                          | Normal range | Patient no. | 1 | 2 |
|--------------------------------|--------------|-------------|---|---|
| WBC count (×10⁹/l)             | 3.5–9.5      | 7.53        | 6.78 |   |
| %N                             | 40–75        | 74.6        | 86.5 |   |
| %E                             | 0.4–8.0      | 0           | 0   |   |
| %L                             | 20–50        | 17.6        | 12.2 |   |
| Thrombocytopenia (×10⁹/l)      | 125–350      | 89          | 113 |   |
| Proteinuria                    | negative     | positive    |     |   |
| Hematuria                      | negative     | positive    |     |   |
| Total Bilirubin (µmol/l)       | 5.1–19.0     | 7.7         | 9.6 |   |
| APPT (s)                       | 28.0–41.5    | 26.5        | 32.9 |   |
| ALT (U/l)                      | 5–40         | 95          | 41  |   |
| AST (U/l)                      | 8–40         | 99          | 29  |   |
| LDH (U/l)                      | 109–245      | 498         | 319 |   |
| →-dimer (mg/l)                 | <0.5         | 4.51        | 2.32 |   |
| PCT (µg/l)                     | <0.5         | 12.2        | 0.68 |   |
| CRP (mg/l)                     | <8.00        | 105.0       | 83.1 |   |
| CR (µmol/l)                    | 44.0–133.0   | 49.8        | 86.2 |   |

WBC count, white blood cell count; %N, neutrophil ratio; %E, Eosinophil ratio; %L, Lymphocyte ratio; APPT, activated partial thromboplastin time; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; PCT, procalcitonin; CRP, C-reactive protein; CR, creatinine.

Discussion

This is the first report of an SFG rickettsial infection in Huanggang City, Hubei Province. We used the second-generation sequencing method to detect the gene fragment of the SFG rickettsiae subgenus in the blood of two febrile patients from the same township in the Huanggang area of Hubei Province and found that the infections were caused by Rickettsia japonica by gene comparison. The patients presented with marked fever, rash, headache, and one patient also reported myalgia. They exhibited normal WBC levels, increased PCT and CRP, and slightly elevated transaminase and lactate dehydrogenase levels. The absence of severe complications in these two patients may be due to their low rickettsial load.

A small number of gene fragments from a variety of herpes viruses were detected in the blood of these two patients. These herpes viruses cause common latent infections in humans. As a natural pathogen, Rickettsia japonica can overactivate macrophages and T lymphocytes [8]. This leads to an immune imbalance and a subsequent loss of immune control over latent herpes viruses in cells resulting in a small amount of nucleic acid being detectable in the blood. However, the primary symptoms in both patients were not consistent with herpes virus infection, and herpes virus infection is not responsive to doxycycline.

In 2013, Rickettsia japonica bacteria were detected in a patient and Haemaphysalis longicornis tick, and the subsequent epidemiological investigation and serological tests revealed that 54.8% of 902 healthy people living in rural areas of Anhui Province tested positive for Rickettsia japonica specific antibodies [3]. The two patients we reported to reside in the same township. However, this is the first
cities in the Huaiyangshan mountain region of Hubei and Henan. Notably, the Huanggang area is also an epidemic area of research institutions. These factors have caused great difficulties in illness when most patients look for medical help. The isolation of seven to 15 days after the occurrence of the disease [12], therefore, the detection of nucleic acid of transmission to the local area and neighboring cities and bocytopenia syndrome virus the SFTS. Testing of skin rash and eschar tissue has not yet been a routine test different from SFTS in early stage, physicians often consider this to be due to secondary bacterial and fungal infections with significantly reduced granulocytes by SFTS-virus. Hence, physicians prescribe antibacterial and antifungal drugs therapeutically or prophylactically at that time, and they are less likely to choose doxycycline or clari-thromycin.

We recommend the use of mNGS for diagnosing patients with fever of unknown origin in natural foci where Haemaphysalis longicornis is present, to allow timely detection and treatment of SFG rickettsiosis. Patients with both SFTS and SFG rickettsiosis have increased PCT and CRP levels, which is very different from SFTS early stage, physicians often consider this to be due to secondary bacterial and fungal infections with significantly reduced granulocytes by SFTS-virus. Hence, physicians prescribe antibacterial and antifungal drugs therapeutically or prophylactically at that time, and they are less likely to choose doxycycline or clari-thromycin.

We recommend the use of mNGS for diagnosing patients with fever of unknown origin in natural foci where Haemaphysalis longicornis is present, to allow timely detection and treatment of SFG rickettsiosis, especially in the early epidemic stage in this region. To reduce medical costs when the number of affected patients increases, clinicians should summarize the clinical characteristics of SFG rickettsiosis in this area, and provide reference data for early diagnosis and treatment of local patients with SFG rickettsiosis. Although the laboratory diagnosis of SFG rickettsiosis is difficult, in the areas where Haemaphysalis longicornis is present, physicians in primary hospitals need to improve their awareness of SFG rickettsiosis, take the disease into account when treating patients with
headache, fever, mottled rash, and eschar, and use effective antimicrobial drugs to greatly reduce the risk of SFGR infections. They must be alert to signs of rash and eschar, and levels of PCT and CRP when treating patients with SFTS. Doxycycline should also be used empirically in patients with SFTS with no significant relief of symptoms or with severe headache.

Ethical approval

Written informed consent was obtained from patients. In our setting, ethical approval for case reports is not required if informed consent is given.

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Conflict of interest

No conflict of interest to declare.

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