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Letter to the Editor

Next-generation sequencing helped diagnosis and successful treatment of an atypical severe scrub typhus patient

Scrub typhus is an acute infectious disease caused by *Orientia tsutsugamushi*, a mite-borne obligate intracellular bacteria belonging to the genus *Orientia*, family Rickettsiaceae. In recent years, it is becoming a major public health threat due to its wide distribution, especially in Asia. Scrub typhus mainly manifests undifferentiated fever, eschar, headache, myalgia, and cough [1]. In severe cases, systematic complications such as acute respiratory distress syndrome, acute renal failure, encephalitis, meningoencephalitis, septic shock, pneumonia, and multiple organ failure were also observed [1]. The fatality rate of untreated scrub typhus patients varies from 6.3% to as high as 38.9%, especially in severe cases with multiple organ dysfunction syndrome [2]. Timely diagnosis and correct antimicrobial therapy are important for preventing complications and reducing mortality. However, clinical manifestations of scrub typhus are often nonspecific or misleading. Although pathognomonic eschar is the characteristic manifestation of scrub typhus, the low incidence of eschar in many cases may increase the difficulties of diagnosis. In this study, a severe patient without eschar was quickly diagnosed with scrub typhus by next-generation sequencing (NGS). The result contributed to the effective treatment and resulted in a favorable outcome.

On August 1, 2021, a 66-year-old woman in Yuxi City, an entrance guard without a history of fieldwork, complained of abdominal distension, asthenia, poor appetite, vomiting, and abnormal color of urine. On 5th August (day 0), she was admitted to the Tonghai County Hospital with a fever of 39.6 °C and then transferred to the Emergency Department of The People's Hospital of Yuxi City on the same day. On admission, laboratory test results showed thrombocytopenia (platelet count: 63×10^9/L), liver dysfunction (glutamic pyruvic transaminase [ALT]: 105 U/L, glutamic oxaloacetic transaminase [AST]: 130 U/L), and kidney injury (UREA: 9.7 mmol/L, UA: 394 μmol/L, K: 4.1 mmol/L, Na: 125 mmol/L, Ca: 2 mmol/L) (Table 1). Mild pneumonia was observed in the lower lobes of both lungs (Fig. S1). She has a history of type II diabetes and was negative for SARS-VoV-2, Hepatitis A, B, and C viruses. No eschars were observed on the body. From the 6th to the 9th of August (day 1 to day 4), Levofloxacin was tentatively used for anti-infection treatment, but the patient still had fever fluctuations between 38.5°C and 39.4 °C, and no significant improvement was observed in clinical indicators.

On 9th August, the patient was transferred to Intensive Care Unit (ICU) because of multiple organ dysfunction syndrome. TORCH test (targeting Rubella Virus, Cytomegalo Virus, herpesviruses, etc), Widal test (targeting typhoid and paratyphoid bacillus), and Weil-Felix test (targeting rickettsiae) were all negative. Cefoperazone sodium and sulbactam sodium were used for anti-infection. On 10th August (day 5), laboratory tests further suggested respiratory failure (PO2: 56 mmHg, SO2: 89.4%) and cardiac dysfunction (NT-proBNP: 754.3 ng/L). The platelet count (30×10^9/L) and inflammatory indicators (PCT: 2.62 μg/L, IL-6: 89.25 ng/L, CRP: 264.38 mg/L) also deteriorated (Table 1). The non-invasive ventilation was applied and doxycycline hydrochloride was additionally used. On the same day, blood samples were collected and subjected to next-generation sequencing (NGS) to make a precise diagnosis.

On 11th August (day 6), NGS results showed that *O. tsutsugamushi* was the causative agent and 601 sequence reads were identified, with a relative abundance of 96.78%. Doxycycline hydrochloride was used as the sole antibiotic based on the NGS results and invasive ventilation was applied. The temperature of the patient returned to normal on 12th August (day 7) and invasive ventilation was weaned on 14th August (day 9). Chest radiograph on 18th August showed that pneumonia in both lungs was alleviated compared to the result on 9th August (Fig. S2). On 19th August (day 14), most indicators of blood coagulation, respiratory function, cardiac function, and inflammation were apparently improved, and then the patient was transferred to the general ward. She was discharged on 28th August (day 23), without any complications.

Orientia tsutsugamushi, the etiologic agent of scrub typhus, is not susceptible to many commonly used antibiotics such as Penicillin and Cephalosporin [3]. Therefore, early diagnosis is crucial for the selection of antibiotics and correct treatment of scrub typhus patients. To date, epidemiology factors and clinical features are often used for reference in the diagnosis of scrub typhus. However, the current patient has no history of outdoor activities, no eschars or rashes, and the clinical characteristics are undifferentiated, which increased the difficulties and resulted in delayed diagnosis. In the laboratory, serological tests are often used for the diagnosis of scrub typhus. Of those, the Weil-Felix test is a commonly used serological test. However, its specificity and sensitivity are often unsatisfying [4]. In the current study, the Weil-Felix test still showed a negative result on the 9th of August, 8 days after the disease onset. Compared to serological tests, PCR test provides a fast, cheap, and precise method. However, laboratories in many county-level hospitals have not routinely applied this diagnostic test. Furthermore, when there are no typical manifestations, it is difficult to suspect a diagnosis of scrub typhus and perform a PCR test specific for *O. tsutsugamushi*. Compared to the tests above, NGS provides a fast (in 48 hours) and unbiased method which simultaneously detects a wide range of pathogens. In recent years, it has been reported that NGS be successfully applied to the diagnosis of scrub typhus [4,5]. In this study, an atypical patient who has no history of fieldwork, no eschars, and was negative for the Weil-Felix test, was

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Table 1
Results of laboratory tests of the patient on different days.

	Reference Value	Day 0 (5th Aug)	Day 5 (10th Aug)	Day 6 (11th Aug)	Day 7 (12th Aug)	Day 8 (13th Aug)	Day 10 (15th Aug)	Day 11 (16th Aug)	Day 12 (17th Aug)	Day 13 (18th Aug)	Day 14 (19th Aug)
Blood test											
WBC (10^9/L)	3.5–9.5	6.06	9.69	12.15	8.37	5.62	4.73	4.25	5.06	3.87	3.6
PLT (10^9/L	100–300	63	30	22	20	54	51	60	76	81	96
NEUT	50–70	93.2	84.6	69.9	55.6	53.5	59.7	72.9	62.9	53.3	56.9
GLU (mmol/L)	3.9–6.1		10.9	9.1	9.2	23.4	9.6	13.1	9.7	10.5	
Liver function											
ALT (IU/L)	5–40	105	79		45	37	35			50	
AST (IU/L)	0–40	130	144		63	33	38			56	
Kidney function											
UREA (mmol/L)	2.0–7.1	9.7		15	15.2		10.5	6.9			
UA (μmol/L)	100–300	394		509	406		291	151		97	
K (mmol/L)	3.5–5.5	4.1	3.1	3.1	3.9	5.2	3.3	3.8	3.8	3.7	3.9
Na (mmol/L)	135–145	125	135	139	141	141	147	144	142	139	136
CL (mmol/L)	96–106	96	106	110	113	113	111	109	110	108	108
Ca (mmol/L)	2.25–2.75	2	1.93	2.07	2.03	2.05	2.15	2.23	2.29	2.23	
Respiratory function											
PH	7.35–7.45		7.41	7.41	7.4	7.38	7.51	7.54	7.53	7.5	7.51
PO2 (mmHg)	80–100		56	60	76	95	86	75	80	79	79
PCO2 (mmHg)	35–45		31	29	26	35	39	33	31	34	31
SO2 (%)	95–99		89.4	92.4	97.9	99.3	98.2	97.3	97.6	98	98.2
CHCO3 (mmol/L)	22–27		19.6	18.4	16.1	20.7	31.1	28.2	25.9	26.5	24.7
LAC (mmol/L)	0.5–1.7		3	2.4	1	1.5	1.4	1.2	1.1	0.9	0.9
Cardiac function											
NT-proBNP (ng/L)	<300		754.3	1413	801.8		1274	1055	642.8	293.9	
Inflammatory indicators											
PCT (μg/L)	0–0.5	1.68	2.62	1.99	0.972	0.188	0.086	0.058	0.092	0.095	
IL-6 (ng/L)	<7	26.89	89.25	29.83	22.68	<1.50	10.39	4.35	<1.50		
CRP (mg/L)	<10	170.46	264.38	181.71	127.24	80.59	24.85	68.21	46.65		
Coagulation index											
AT III (%)	80–120	73		35	40		67	62	66		
D-Di (μg/mL)	0–0.5	1.99		3.4	3.35		2.9	2.94	2.91		2.28
FDP (μg/mL)	<5	7.14		11.29	8.37			6.12	5.89		5.55

diagnosed by NGS. Although her manifestations were severe with multiple organ dysfunction, the syndromes were rapidly alleviated once the agent was identified and the correct antibiotic was used. This result confirmed that NGS can be used for the diagnosis of atypical scrub typhus patients. There are still some shortcomings of NGS. For example, the price of NGS (approximately 500$ for mNGS DNA or mNGS RNA, and 800$ for both) is still much higher than routine tests such as PCR. Besides, NGS is still not available in many counties in China. With the development of NGS technology and the decrease in prices, we believe NGS would become a preferable choice in clinical diagnosis.

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Ethical approval

Since the data were anonymous and no human samples were collected specific for scientific research, the need for ethics approval was waived by the Ethics Committee of The People's Hospital of Yuxi City.

Declaration of competing interest

The authors have no competing interests to declare.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nmni.2023.101144.

References

[1] Varghese GM, Trowbridge P, Janardhanan J, Thomas K, Peter JV, Mathews P, et al. Clinical profile and improving mortality trend of scrub typhus in South India. Int J Infect Dis 2014;23:39–43. https://doi.org/10.1016/j.ijid.2014.02.009.
[2] Devasagayam E, Dayanand D, Kundu D, Kamath MS, Kirubakaran R, Varghese GM. The burden of scrub typhus in India: a systematic review. PLoS Neglected Trop Dis 2021;15(7):e0009619. https://doi.org/10.1371/journal.pntd.0009619.
[3] Reller ME, Dumler JS. Optimization and evaluation of a multiplex quantitative PCR assay for detection of nucleic acids in human blood samples from patients with spotted fever rickettsiosis, typhus rickettsiosis, scrub typhus, monocytic ehrlichiosis,

and granulocytic anaplasmosis. J Clin Microbiol 2020;58(9):e01802–19. https://doi.org/10.1128/JCM.01802-19.
[4] Wu H, Xiong X, Zhu M, Zhuo K, Deng Y, Cheng D. Successful diagnosis and treatment of scrub typhus associated with haemophagocytic lymphohistiocytosis and multiple organ dysfunction syndrome: a case report and literature review. Heliyon 2022; 8(11):e11356. https://doi.org/10.1016/j.heliyon.2022.e11356.
[5] Zhan Z, Li CF, Liu J, Cao CS, Huang L. Metagenomic next-generation sequencing to diagnose atypical severe scrub typhus. J Microbiol Immunol Infect 2022;55(3): 556–7. https://doi.org/10.1016/j.jmii.2021.12.002.

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