The Value of Metagenomic Next-Generation Sequencing in Leishmaniasis Diagnosis: A Case Series and Literature Review

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Background. Leishmaniasis is a zoonotic disease caused by Leishmania spp. and spreads through sandfly bites. Owing to the wide range of nonspecific clinical symptoms, patients with leishmaniasis are frequently misdiagnosed or underdiagnosed.

Methods. The study participants were 7 metagenomic next-generation sequencing (mNGS)–diagnosed patients with leishmaniasis who could not be diagnosed using conventional methods. Clinical data were retrospectively collected and analyzed. When searching PubMed for mNGS and leishmaniasis, 8 peer-reviewed case reports in English were retrieved.

Results. A total of 7 patients with recurrent fever, pancytopenia, and significant splenomegaly were included in this study. Only 3 individuals tested positive for rK39. Two individuals, 1 of whom was HIV-positive, had Leishmania amastigotes identified in their bone marrow. However, all patients’ blood mNGS findings pointed to Leishmania infection, and they were finally diagnosed with leishmaniasis. Sodium stibogluconate therapy with a short course of amphotericin B was administered to all patients. The prognosis for the remaining patients was good, except for 1 who died of multiple organ failure.

Conclusions. mNGS could be used to identify leishmaniasis, particularly in patients who are difficult to diagnose using conventional approaches.

Keywords. case series; diagnosis; Leishmania; leishmaniasis; mNGS.

Leishmaniasis is a parasitic disease caused by the parasite Leishmania spp. and transmitted by sandflies. Visceral leishmaniasis (VL), cutaneous leishmaniasis, and mucocutaneous leishmaniasis are the 3 forms of leishmaniasis; they differ in infection locations and clinical symptoms [1, 2]. Leishmania donovani and Leishmania infantum are the most common causes of VL, primarily attacking the spleen, liver, bone marrow, and lymph nodes [3]. VL has rapidly spread worldwide, and the Chinese government and individuals have made tremendous headway in containing it [4]. As the economy has evolved in China, the number of travelers visiting epidemic areas has dramatically increased, and sporadic instances have resurfaced in some provinces, including Xinjiang, Sichuan, and Gansu [5, 6]. Patients with VL are easy to ignore as they possess several clinical symptoms with no distinctive presentation. Patients not appropriately treated may die of various complications over the course of a few years.

Metagenomic next-generation sequencing (mNGS) is a method for simultaneously detecting all nucleic acids (DNA and RNA) in a sample and obtaining the genomic information to identify disease-causing microorganisms and guide clinical diagnosis and treatment [7, 8]. mNGS can identify infectious pathogens, including viruses, bacteria, fungi, parasites, mycoplasmas, leptospires, and new microorganisms, such as the SARS-CoV-2 virus. A growing number of studies, particularly in the postepidemic era, have proved the potential of mNGS in the infection field [9–13]. However, there are currently few studies on the application of mNGS in leishmaniasis diagnosis. Therefore, this study aimed to summarize the experience of health care providers using mNGS in diagnosing patients with VL in a hospital and reviewed the literature regarding mNGS and leishmaniasis to clarify the value of mNGS in leishmaniasis diagnosis.

METHODS

Study Design and Participants

We conducted a retrospective case series study involving 7 patients diagnosed with Leishmania infection via blood mNGS and treated at the Center of Infectious Diseases, West China...
Hospital, Sichuan University, since 2020. Demographic data, clinical symptoms, laboratory examinations, imaging results, pathogenic examinations, and treatment regimens were extracted from the electronic medical record system. Additionally, we collected prognosis results. This study was approved by the Ethics Committee of West China Hospital.

mNGS
After informed consent was obtained, peripheral blood was collected from patients, and genomic DNA was extracted using the QIAamp whole-blood DNA extraction kit (Qiagen, Germany) following the kit’s instructions. A single-end library was prepared using the Vazyme TruePrepTM DNA Library Prep Kit (Vazyme) and subsequently sequenced using the NextSeq 550 system (Illumina). Microbial identification was performed after removing low-quality data to obtain clean reads. To date, 6350 bacteria, 1798 DNA viruses, 1064 fungi, and 234 parasites with known genome sequences can be detected and classified.

Literature Review
PubMed searches for mNGS and leishmaniasis yielded 8 peer-reviewed case reports in English. For this study, the search time was set from the establishment of databases to March 31, 2022.

RESULTS
Demographics and Clinical Presentations
There were 7 male patients, whose ages ranged from 16 to 57 years (Table 1). They all presented with irregular fever for >15 days, accompanied by fatigue or cold-like symptoms such as cough and chills. Their temperatures fluctuated around ∼38°C; case 5 reached 42°C. The remaining patients had undergone splenomegaly and hepatomegaly, except for cases 3 and 7. Furthermore, case 4 presented with superficial lymph node enlargement, case 5 had a severe left nasal hemorrhage, and case 7 had a rash on the back. Of these patients, most were farmers with a history of fieldwork, case 2 was a cook who had worked in Africa for 10 years and once had malaria, and case 3 was a student with rural travel experience. Furthermore, all patients had weight loss ranging from 2 to 20 kg. All 7 patients presented with prolonged irregular fever, malaise, weight loss, and hepatosplenomegaly.

Laboratory Tests and Imaging Results
Laboratory tests and imaging results are shown in Table 2. Results of peripheral blood testing suggested pancytopenia with moderate to severe anemia. Simultaneously, the indicators of inflammation, including C-reactive protein, procalcitonin, and interleukin-6, showed a significant increase. Albumin levels in the blood were significantly decreased in all patients; moreover, albumin-to-globulin ratios were significantly decreased. Additionally, most patients showed elevated liver enzymes. In case 2, the serum creatinine level was significantly
Table 2. Laboratory Tests and Imaging Results of 7 Patients

| Parameters                        | Case 1       | Case 2       | Case 3       | Case 4       | Case 5       | Case 6       | Case 7       | Abnormal Rate, No. % | Reference Value |
|----------------------------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|----------------------|-----------------|
| Peripheral blood test            | ...          | ...          | ...          | ...          | ...          | ...          | ...          | ...                  | ...             |
| Erythrocytes                     | 2.51 ↓       | 2.80 ↓       | 2.51 ↓       | 2.1 ↓        | 2.62 ↓       | 3.92 ↓       | 3.237 ↓      | 7100%               | 4.3–5.8 × 10^12/L |
| Hemoglobin                       | 60 ↓         | 83 ↓         | 81 ↓         | 54 ↓         | 72 ↓         | 105 ↓        | 90↓          | 7100%               | 130–175 g/L      |
| Leukocytes                       | 1.9 ↓        | 2.82 ↓       | 3.3 ↓        | 0.87 ↓       | 1.47 ↓       | 3.44 ↓       | 2.65 ↓       | 7, 100%             | 3.5–9.5 × 10^9/L  |
| Platelets                        | 47 ↓         | 10 ↓         | 47 ↓         | 10 ↓         | 22 ↓         | 187          | 79↓          | 6/7, 86%            | 100–300×10^9/L   |
| Inflammatory markers             | ...          | ...          | ...          | ...          | ...          | ...          | ...          | ...                  | ...             |
| Creatine protein                 | 113↓         | 53.5↓        | 25.5↓        | 111↓         | 10.2↓        | 5.71%        | 0.046 ng/mL   | <5 mg/L              | <50 IU/L         |
| Procalcitonin                    | 1.18↑        | 2.11↑        | 5.63↑        | 0.98↑        | 0.21↑        | 5.71%        | 5,71%        | <0.46 ng/mL         | <40 IU/L         |
| Interleukin-6                    | 151↑         | 31.58↑       | 45.73↑       | 21.44↑       | 44.90↑       | 5.71%        | 5.71%        | <7 pg/mL             | <60 IU/L         |
| Liver function                   | ...          | ...          | ...          | ...          | ...          | ...          | ...          | ...                  | ...             |
| ALT                              | 10           | 19           | 204↑         | 17           | 74↑          | 145↑         | 66↑          | 4,57%               | <50 IU/L         |
| AST                              | 25           | 47↑          | 176↑         | 39           | 132↑         | 172↑         | 74↑          | 5.71%               | <40 IU/L         |
| ALP                              | 156↑         | 463↑         | 272↑         | 190↑         | 346↑         | 465↑         | 127↑         | 7, 100%             | 51–160 IU/L      |
| GGT                              | 39           | 234↑         | 153↑         | 37           | 172↑         | 184↑         | 181↑         | 5, 71%              | <60 IU/L         |
| Total protein                    | 72.1         | 54.9↓        | 78.8         | 74.9         | 78.3         | 89.7         | 80.6         | 1, 14%              | 65–90–85 g/L     |
| Albumin                          | 30.6↓        | 26.5↓        | 39↓          | 26.9↓        | 34.5↓        | 25.9↓        | 39.2↓        | 7, 100%             | 40–50–55 g/L     |
| Globulin                         | 41.5↑        | 29.4         | 39.8         | 48↑          | 43.8↑        | 63.8↑        | 41.4↑        | 6, 96%              | 20–40–40 g/L     |
| Albumin/globulin ratio           | 0.74↓        | 0.93↓        | 0.98↓        | 0.56↓        | 0.79↓        | 0.41↓        | 1.00↑        | 7, 100%             | 1.20–2.40        |
| Renal function                   | ...          | ...          | ...          | ...          | ...          | ...          | ...          | ...                  | ...             |
| Creatinine                       | 87           | 212↑         | 70           | 68           | 72           | 68           | 101          | 1, 14%              | 68–108 umol/L    |
| eGFR                             | 64.8         | 30.99↓       | 117.03       | 119.58       | 114.13       | 114.36       | 82.22        | 1, 14%              | 56–122 ml/min/173 |
| Blood lipid levels               | ...          | ...          | ...          | ...          | ...          | ...          | ...          | ...                  | ...             |
| Triglyceride                     | 1.45         | 1.36         | 1.80         | 0.84         | 2.68         | 1.89         | 3.14↑        | 2, 29%              | 0.29–1.83 mmol/L |
| Cholesterol                      | 1.83↑        | 2.09↑        | 3.36         | 1.66↑        | 3.89         | 2.10↑        | 3.73         | 4, 57%              | 2.80–6.70 mmol/L |
| HDL cholesterol                  | 0.4↑         | 0.38↑        | 0.30↑        | 0.32↑        | 0.61↑        | 0.40↑        | 0.46↑        | 7, 100%             | >0.90 mmol/L     |
| Viral load, copies/mL            | ...          | ...          | ...          | ...          | ...          | ...          | ...          | ...                  | ...             |
| EBV DNA                          | Negative      | Negative     | 1.09 × 10^4 | 2.60 × 10^4 | Negative     | 1.56 × 10^4 | Negative     | 4, 57%              | copies/mL        |
| CMV DNA                          | Negative      | Positive, but <50 | 1.29 × 10^4 | Negative     | Negative     | Negative     | 2, 29%       | copies/mL           | ...             |
| HIV RNA                          | ...          | ...          | ...          | ...          | ...          | ...          | 1, 14%       | copies/mL           | ...             |
| Imaging results                  | ...          | ...          | ...          | ...          | ...          | ...          | ...          | ...                  | ...             |
| Computed tomography              | The liver torticollis was 14.7 cm, and the spleen thickness was 7.1 cm | Signs of cirrhotic decompensation | The spleen thickness was 6.6 cm, with no notable liver alterations | Hepatosplenomegaly; a soft tissue shadow at the tip of the right upper lobe lung (3.1 x 2.3 cm) | The liver torticollis was 17.0 cm, and the spleen thickness was 6.4 cm | The liver torticollis was 15.4 cm, and the spleen thickness was 6.9 cm | The spleen thickness was 6.9 cm | ... | ...

Abbreviations: ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; CMV, cytomegalovirus; EBV, Epstein-Barr virus; eGFR, estimated glomerular filtration rate; GGT, gamma-glutamyl transferase; HDL, high-density lipoprotein.
elevated and the estimated glomerular filtration rate was significantly decreased, at 212 µmol/L and 30.99 mL/min/1.73 m², respectively, suggesting severe renal function impairment. Serum high-density lipoprotein levels decreased in all patients, whereas serum triglyceride levels increased in cases 5 and 7, at 2.88 and 3.14 mmol/L, respectively. Cases 3, 4, 5, and 7 had Epstein-Barr virus infection, cases 3 and 4 had cytomegalovirus infection, and case 4 had HIV. Computed tomography findings in all patients suggested the presence of spleen and liver enlargement, with signs of cirrhotic decompensation present in case 2. Furthermore, a soft tissue shadow (3.1 x 2.3 cm) was noted at the tip of the right lung in case 5.

Pathogenetic Diagnosis and Treatment
Bone marrow cytology was performed in all patients except case 2, for whom no bone marrow aspiration was performed owing to extreme weakness; however, only cases 4 and 6 had Leishmania amastigotes (Figure 1A); the bone marrow smear of case 5 was suggestive of hemophagocytic signs (Figure 1B). The positive rate of pathogens detected by bone marrow cytology was 33% in this report. Due to the unavailability of rK39 test strips, only cases 2, 3, 5, 6, and 7 were tested, and cases 3 and 7 were negative, with a total positivity rate of 60%. In all cases, Leishmania-specific sequences were matched by blood mNGS, with an identification rate of 100% (Table 3). Except for case 2, who died of multiple organ failure, all patients received sodium stibogluconate (SSG) therapy (coupled with a brief course of amphotericin B), and all responded well (Table 3). In case 4, relapse occurred after 6 months of follow-up due to simultaneous HIV infection, whereas the remaining 3 patients did not relapse.

Literature Review
In aggregate, the literature to date provides promising and convincing evidence of the diagnostic value of mNGS in Leishmania infection (collected in Table 4). Published studies have highlighted that blood and bone marrow tissues can be used as samples for mNGS testing, with a 100% detection rate of Leishmania reads. Additionally, mNGS testing is not limited by age or gender and shows consistent specificity. In contrast, neither bone marrow cytology nor the rK39 test has a detection rate as high as that of mNGS (82% and 80%, respectively).

DISCUSSION
When sandflies feed on the blood of patients with VL or on sick canines, the protozoa enter the sandflies and multiply into flagellates, which subsequently enter the body and cause infection when the sandflies bite healthy humans [14]. Long working hours, improper prevention measures, and exposure to wild sandflies put male field laborers, particularly those aged 30–60 years, in danger [15]. Except for case 3, all patients were between the ages of 40 and 60 and had a field activity history. The incubation period for the 7 cases reported here ranges from months to years. As the initial infection dates were unknown, the incubation time cannot be estimated for everyone here. Long-term irregular fever, enlarged liver, spleen, and lymph nodes, and pancytopenia are frequent symptoms of VL [3]. All participants in our study had the abovementioned symptoms. Early-stage cases are easily misdiagnosed as colds, whereas advanced cases resemble leukemia and lymphoma [16, 17]. Initially, case 3 showed subtle symptoms and was misdiagnosed as having a cold at the local hospital, receiving only symptomatic treatment (antibiotics, antipyretics, and cough suppressants). Conversely, case 2 was at a late stage of VL and had been treated in different hospitals with little improvement. The patient subsequently developed multiple organ failure as well as a serious infection. His clinical prognosis may have improved if the etiology had been discovered and treated earlier.

The number of HIV-positive individuals is rising, as is the number of patients with HIV–Leishmania coinfection [18]. Both Leishmania and HIV attack macrophages and dendritic

**Figure 1.** A, Bone marrow smear shows Leishmania amastigotes (arrow). B, Bone marrow smear shows hemophagocytosis.
Table 3. Pathogenetic Diagnosis and Treatment Regimen of all Patients

|                     | Case 1       | Case 2       | Case 3       | Case 4       | Case 5       | Case 6       | Case 7       | Pathogen Detection Rate (Positive), No., % |
|---------------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|-------------------------------------------|
| Pathogenetic diagnosis | ...          | ...          | ...          | ...          | ...          | ...          | ...          | ...                                       |
| Bone marrow smears  | No pathogen  | Not done     | No pathogen  | Scattered    | Hemophagocytosis, but no pathogen was found | Leishmania amastigotes | No pathogen | 2/6, 33%                                  |
| rK39 test           | Not done     | Positive     | Negative     | Not done     | Positive     | Positive     | Negative     | 3/5, 60%                                  |
| Blood mNGS          | ...          | ...          | ...          | ...          | ...          | ...          | ...          | 7/7, 100%                                 |
| Parasite-specific sequences | ...          | ...          | ...          | ...          | ...          | ...          | ...          | ...                                       |
| Leishmania, No.     | 38 682       | 33 379       | 38753        | 860613       | 22 468       | 56 362       | 18 433       | ...                                       |
| Leishmania infantum, No. | 1599         | 199          | 750          | 6233         | 507          | 1259         | 822          | ...                                       |
| Leishmania donovani, No. | 228          | 558          | 0            | 9569         | 83           | 65           | 45           | ...                                       |
| Other pathogen-specific sequences | ...          | ...          | ...          | ...          | ...          | ...          | ...          | ...                                       |
| EBV, No.            | ...          | ...          | ...          | 12           | 11           | ...          | 128          | ...                                       |
| CMV, No.            | ...          | ...          | ...          | 10           | ...          | ...          | ...          | ...                                       |
| Treatment regimen  | ...          | ...          | ...          | ...          | ...          | ...          | ...          | ...                                       |
| SSG                 | 0.6 g/d for 10 d | 0.6 g/d for 7 d | 0.6 g/d for 10 d | 0.6 g/d for 10 d | then with ART and the second phase of SSG | 0.6 g/d for 10 d | 0.6 g/d for 10 d | ...                                       |
| Amphotericin B      | Initial 5 mg for 5 d (increasing by 5 mg/d), ceased due to a significant increase in blood creatinine | ... | ... | ... | Initial 5 mg for 3 d (increasing by 5 mg/d), ceased due to a significant increase in blood creatinine | ... | ... | ... |
| Outcome             | Survival     | Death        | Survival     | Survival     | Survival     | Survival     | Survival     | ...                                       |
| Follow-up           | No relapse   | No relapse   | Relapse      | No relapse   | No relapse   | No relapse   | No relapse   | ...                                       |

Abbreviations: ART, antiretroviral therapy; CMV, cytomegalovirus; EBV, Epstein-Barr virus; mNGS, metagenomic next-generation sequencing; SSG, sodium stibogluconate.
cells, and the disease processes interact when both pathogens are present [19]. Conversely, HIV-induced immunodeficiency may cause parasitic blooms in patients with coinfection, worsening their clinical symptoms and leading to worse treatment response and easy relapse after treatment. Case 4 had a relapse of VL and was treated in the hospital again. Conversely, *Leishmania* can accelerate viral replication, causing problems if antiviral medicines are not administered immediately and regularly. Furthermore, immune reconstitution syndrome after antiviral therapy should be separated from the clinical signs of leishmaniasis based on HIV infection. Case 4 can be ruled out because he was diagnosed with HIV for the first time and has yet to begin antiretroviral therapy.

VL is currently diagnosed by detecting *L. amastigotes* in the bone marrow, lymph nodes, spleen, and other tissues [20]. Bone marrow aspiration is the preferred clinical approach due to its simplicity. A minimally invasive bone marrow aspiration may not be tolerated in patients with terminal VL due to extreme malnutrition and poor coagulation, as in case 2. Furthermore, most patients are farmers or migrant workers and are first seen in primary care institutions. However, primary care institutions lack experience identifying *L. amastigotes* microscopically, making it easier to miss or misdiagnose the disease. Moreover, the microscopic characteristics of *Leishmania* are difficult to identify even in advanced medical institutes. Only case 4, who had more *Leishmania*-specific sequences and HIV coinfection, showed *L. amastigotes* in his bone marrow smears. Moreover, microscopic and pathological stains may not be informative when parasite levels are extremely low, as in post-treatment patients. Consequently, the rK39 test is widely employed in China [21]. A previous study in our center showed that its diagnostic sensitivity was 96.2%. However, the rK39 test is completed at the Centers for Disease Control and Prevention. Owing to the epidemic, the rK39 test cannot be performed at any time and is not the best option for patients who require urgent diagnosis. Additionally, most patients with VL presented with unexplained fever, and these patients were in poor condition with severe symptoms. Therefore, conventional methods for some individuals are not timely, thereby prompting the development of more convenient and precise methods.

Recently, mNGS has become increasingly popular in infectious disease diagnosis [7]. Chen et al. isolated RNA from the bronchoalveolar lavage fluid of patients with acute respiratory syndrome and identified the novel coronavirus using mNGS [22]. This indicates that mNGS analysis can swiftly uncover unknown microorganisms, identify emerging infectious diseases, and assist public health organizations in responding to disease outbreaks and epidemics. In 2018, Wilson et al. used mNGS to examine the brain fluid of 7 patients with chronic encephalitis and found *Taenia solium* infection [23]. Seven individuals hospitalized with unexplained fever were subsequently diagnosed with *Leishmania* infection using mNGS. This is the first report using mNGS for diagnosing VL in Southwest China. The specific reads of *L. infantum* spanned from 199 to 6233, whereas those of *L. donovani* ranged from 0 to 9569. Except for case 1, the remaining patients were analyzed at the Precision Diagnosis Centre of our hospital. Due to coinfection with HIV, case 4 exhibited much more specific reads of *Leishmania* than the other patients. Better treatment response may be attributed to his younger age and shorter disease duration of patient 3 at diagnosis; additionally, case 3 was solely infected with *L. infantum*.

Various guidelines propose amphotericin B liposomes as the first-line treatment for VL, with alternative treatment regimens including amphotericin B (1 mg/kg per day for 15–20 days with a total dose of 15–20 mg/kg) or SSG (20 mg/kg per day for

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Table 4. Summary of Case Reports Related to mNGS in Leishmaniasis Diagnosis

| Author | Time | No. of Patients | Age, Gender | No. of Specific Sequences | *Leishmania amastigotes* in Bone Marrow | rK39 Test |
|--------|------|----------------|-------------|--------------------------|----------------------------------------|----------|
| Zhang [27] | 2019 | Case 1 | 38-y-old, male | 1262 in blood | Not done | Positive |
| | | Case 2 | 38-y-old, female | 8 in bone marrow, 114 in blood | Negative | Positive |
| | | Case 3 | 32-y-old, male | 341 427 in bone marrow, 306 657 in blood | Positive | Positive |
| Williams [28] | 2020 | 1 | 61-y-old, male | 294 673 in bone marrow | Positive | Not done |
| Guo [29] | 2020 | 1 | 9.5-mo-old, female | 2144 in blood, 4159 in sputum | Negative | Negative |
| Chen [30] | 2020 | Case 1 | 53-y-old, male | 6915 in bone marrow, 7 in plasma | Positive | Not mentioned |
| | | Case 2 | 80-y-old, male | 18 070 in bone marrow | Positive | Not mentioned |
| | | Case 3 | 65-y-old, male | 438 in bone marrow | Positive | Not mentioned |
| Wang [31] | 2021 | 1 | 65-y-old, male | Prediagnosis: 1791 in blood Post-treatment: 1226 in blood | Positive | Positive |
| Lin [32] | 2021 | 1 | 25-y-old, male | 102 in blood | Positive | Not mentioned |
| Ren [33] | 2021 | 1 | 60-y-old, male | 5074 in blood | Positive | Not mentioned |
| Song [25] | 2021 | 1 | 54-y-old, female | First detection: 1076 in bone marrow After relapse: 941 in blood plasma | Positive | Not mentioned |
| Positive rate | … | … | … | 12/12, 100% | 9/11, 82% | 4/5, 80% |

Abbreviation: mNGS, metagenomic next-generation sequencing.
Considering the high cost and difficulty of obtaining amphotericin B liposomes, as well as the serious side effects of amphotericin B, which must be gradually increased to the target therapeutic dose, most Chinese hospitals, including ours, still use SSG as the first-line drug for treating VL and generally follow a short course of therapy. Except for case 2, who died of multiple organ failure, the other patients had a rapid remission of their fever and lower parasite levels after a short course of SSG (or a short-term combination with amphotericin B), and none relapsed during the 6-month follow-up. A new case study reveals that mNGS can be utilized to monitor treatment efficacy and relapse risk [25]. Owing to economic considerations, none of the participants in this study had their mNGS analysis performed again following treatment. With the improvement and simplification of mNGS, its cost would decrease over time, and it will undoubtedly become more frequently employed in diagnosing and treating infectious diseases, particularly rare or challenging cases.

To date, mNGS has been applied in diagnosing infectious diseases, particularly for critically ill infected patients whose definitive diagnosis is difficult to interpret clearly [26]. mNGS has several advantages over conventional diagnostic techniques. First, mNGS has no special requirements for samples, can detect any part of the genome, can widely identify known pathogens, and can even discover new microorganisms. Second, mNGS has high sensitivity and specificity. A retrospective study showed that its sensitivity and specificity for diagnosing infectious diseases were 50.7% and 85.7%, respectively. Third, based on the number of specific sequences it matches, mNGS can be used to monitor treatment effects. Fourth, the detection period is short. In our hospital, mNGS can be completed in only 2 days, which provides conditions for early and rapid diagnosis.

However, there remain some limitations in the clinical application of mNGS. Combined with this study, the following are the main points: (1) The sensitivity and specificity of mNGS for diagnosing VL still require verification using large-scale clinical trials, and etiological detection remains the standard for diagnosing VL. (2) The cost of mNGS is significantly higher than that of conventional diagnostic methods. Currently, the cost of mNGS in our hospital is ~4000 Yuan, limiting its large-scale clinical application. (3) The pathogens detected using mNGS also should rule out the possibility of contamination and colonization. Therefore, combining clinical manifestations, other conventional detection methods, and treatment effects in clinical applications remain necessary to comprehensively judge whether to perform mNGS.

CONCLUSIONS
This study and previous work suggest that mNGS has great potential value for diagnosing VL. Considering the cost-effectiveness, we believe that mNGS can be used as a supplement to conventional methods for diagnosing VL. For patients with emergency and severe cases, it is necessary to use peripheral blood mNGS to perform comprehensive infection screening at an early stage.

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Author contributions. Libo Yan and Hong Tang had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study design: Libo Yan and Hong Tang. Acquisition, analysis, or interpretation of data: Ning Han and Libo Yan. Drafting of the manuscript: Ning Han. Critical revision of the article: Libo Yan and Hong Tang. Laboratory analyses: Jiang Yu. Provision of study materials or patient: Ming Wang and Yuanji Ma. All authors read and approved the final manuscript.

Patient consent. This study was approved by the Ethics Committee on Biomedical Research, West China Hospital of Sichuan University (No. 2021-1528). Written informed consent was obtained from the patients or their families for all content involved in this study.

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