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CASE REPORT

Bronchoalveolar Lavage Fluid Metagenomic Second-Generation Sequencing Assists in Guiding the Treatment of Visceral Leishmaniasis: A Case Report

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Purpose: The incidence of visceral leishmaniasis (VL), a global infectious disease, has been on the rise in China's Hebei province. When patients achieve clinical cure, they often do not reach an etiological cure, which may lead to recurrence of the disease. Here, we report a case of visceral leishmaniasis with a negative blood smear and bone marrow cytology.

Patients and Methods: A 65-year-old man and bronchoalveolar lavage fluid mNGS.

Results: A 65-year-old man developed a chronic fever, anorexia, splenomegaly, and pancytopenia. The blood metagenomic second-generation sequencing (mNGS) revealed *Leishmania* sequence readings, which led to the diagnosis of VL. After sodium antimony gluconate treatment, the blood smear and bone marrow cytology revealed no *Leishmania* bodies. However, pancytopenia and respiratory failure did not fully subside, and cardiotoxic damage emerged. The bronchoalveolar lavage fluid (BALF) mNGS was performed to detect the pathogen. Through BALF mNGS, *Leishmania* sequence was still detectable. Therefore, after the ECG returned to normal, antimony sodium gluconate was administered as a next course of treatment.

Conclusion: BALF mNGS may assist in evaluating the therapeutic efficacy of VL with respiratory failure, especially in patients with negative blood and bone marrow cytology.

Plain Language Summary:

- Accurate detection of visceral leishmaniasis is essential for clinical diagnosis.
- It is uncommon to use alveolar lavage fluid mNGS in etiological diagnosis.
- Patient with negative bone marrow cytology may refer to alveolar lavage fluid mNGS.

Keywords: visceral leishmaniasis, metagenomic second-generation sequencing, bronchoalveolar lavage fluid, parasite diseases, case report

Introduction

Visceral leishmaniasis (VL) is the second deadliest parasitic disease worldwide after malaria. The lung, like any other organ, can be affected in VL, and interstitial pneumonitis has been described in past decades.[1] Current methods employed to detect leishmaniasis include skin diagnostic test, microscopy, vitro culture, etc. The false positive or negative problems

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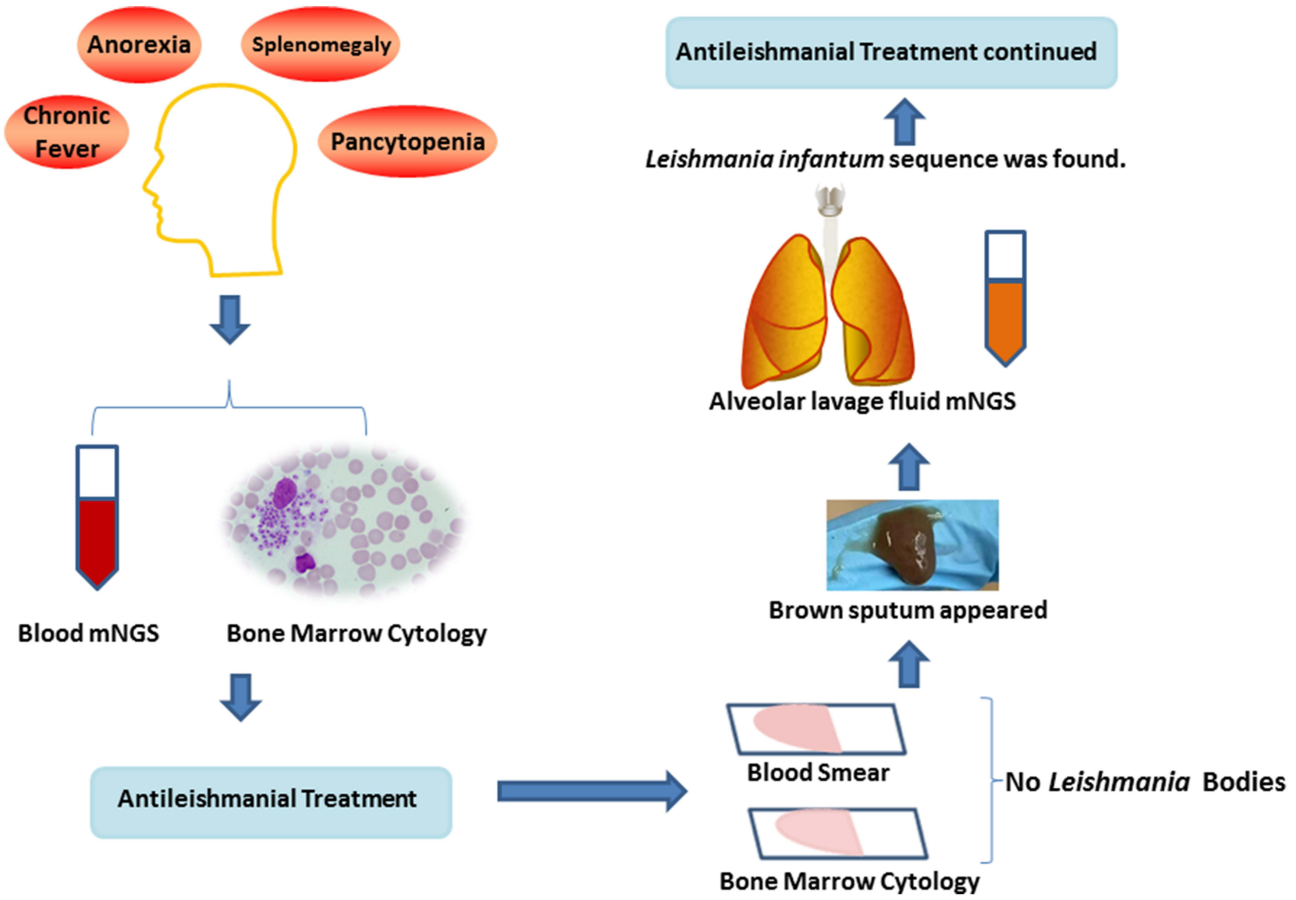
Graphical Abstract



of these methods still perplex clinical practice. Metagenomic second-generation sequencing (mNGS) can effectively detect parasite sequences which are undetectable by traditional methods.[2,3] However, it is uncommon to use mNGS in bronchoalveolar lavage fluid (BALF) as assistance in VL diagnosis.

Case Report

A 65-year-old male from Zanhuang County of Hebei Province, who had a poor appetite and intermittent fever for five months, was admitted to the Infectious Department of the Third Hospital of Hebei Medical University on June 24, 2022.

The patient had experienced intermittent fever, nausea, and vomiting for 5 months. He sought medical consultation at the local hospital. The decrease in whole blood cells and splenomegaly was observed. Dysplasia of megakaryocytes with visible plasma cells and extremely reduced proliferation were observed in bone marrow imaging. But the diagnosis was still unclear. The patient developed fever five days before admission, with the highest temperature of 39.5°C, accompanied by chills, chest tightness, and shortness of breath, without cough, expectoration, nausea, vomiting, abdominal pain, diarrhea, or thamuria. Once again, the patient sought medical care locally. The possibility of fungal infection cannot be excluded due to the patient's long-term fever and repeated use of broad-spectrum antibiotics. So amphotericin B and fluconazole were added for antifungal treatment in local hospital. The dosage of amphotericin B was 5 mg on June 20, 5 mg on June 21, 10 mg on June 22, and 15 mg on June 23. However, the patient's condition had not improved yet.

The laboratory examination revealed white blood cell (0.7×10^9/L), neutrophil (0.3×10^9/L), red blood cell (3.0×10^{12}/L), hemoglobin (72 g/L), platelet (46×10^9/L), albumin (23.2 g/L), blood sodium (128 mmol/L), ESR (101 mm/h), plasma

procalcitonin 1.1 ng/mL, and fungus 1, 3-B-D glucan (814 pg/mL). No obvious abnormality was found in the ENA polypeptide antibody spectrum or Epstein-Barr virus (EB virus) nucleic acid test. Multiple cysts, suspected gallstone and spleen enlargement were revealed by epigastria CT scan. Piperacillintazobactam was given to treat the infection, but the symptoms were not relieved. Considering that it might be an unusual pathogen, the bone marrow aspiration (BMA) was performed. *Leishmania* amastigotes were detected by BMA (Figure 1A). Meanwhile, the bone marrow hyperplasia was extremely reduced, and there was no increase in juvenile cells and lymphocytes. The patient was transferred to another local hospital then, and mNGS blood tests were performed. 40904 *Leishmania infantum*, 17878 *Leishmania donovani*, and 13978 *Leishmania chagasi* sequence readings were detected. Combined with clinical symptoms, the diagnosis of "visceral leishmaniasis" was made.

On June 24, 2022, the patient was referred to the Infectious Disease Department of the Third Hospital of Hebei Medical University, Provincial Infectious Disease Diagnosis and Treatment Center. The patient was admitted as "visceral leishmaniasis". Upon admission, Renal function testing indicated that serum glucose was 16.31 mmol/L, urea was 39.74 mmol/L, creatinine was 286.2 mmol/L, and uric acid was 591 mmol/L. Considering the renal dysfunction of the patient and the side effects of domestic amphotericin B, antimony sodium gluconate was given for antileishmanial treatment.

Two days after antileishmanial treatment, the patient developed dyspnea and telangiectatic breathing. Blood gas analysis showed pH (7.5), pCO_2 (36.3 mmHg), pO_2 (77 mmHg), cLac (3.0 mmol/L) and oxygenation index (128 mmHg). The ECG revealed sinus rhythm, occasional ectopic premature beats, moderate ST depression, and deep inversion of the T wave. B-type natriuretic peptide was 1112 pg/mL. Bilateral pulmonary edema was detected by

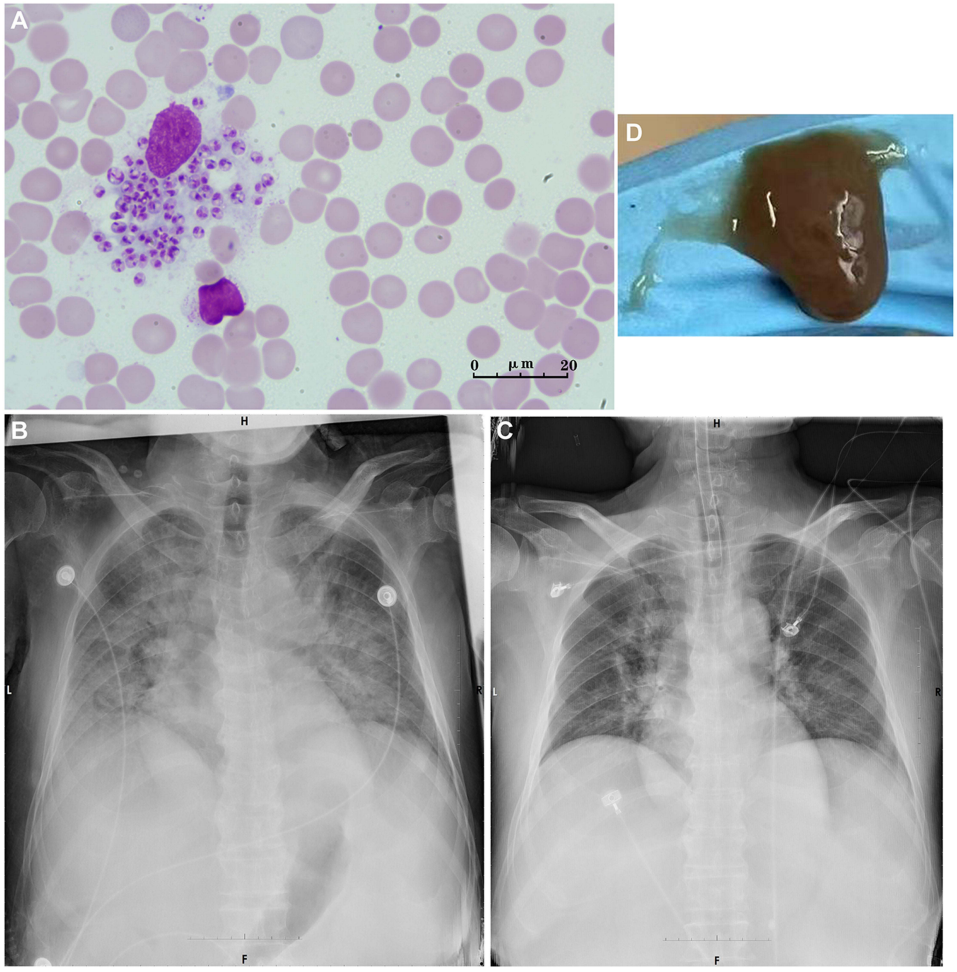


Figure 1 Examination of the case of visceral leishmaniasis: (**A**) *Leishmania* amastigotes were examined by BMA. (Wright-Giemsa, 1000×). (**B**) Chest X-ray disclosed bilateral pulmonary edema. (**C**) A recheck of chest X-ray showed significant resolution of pulmonary edema. (**D**) Brown sputum was aspirated from the patient's endotracheal tube.

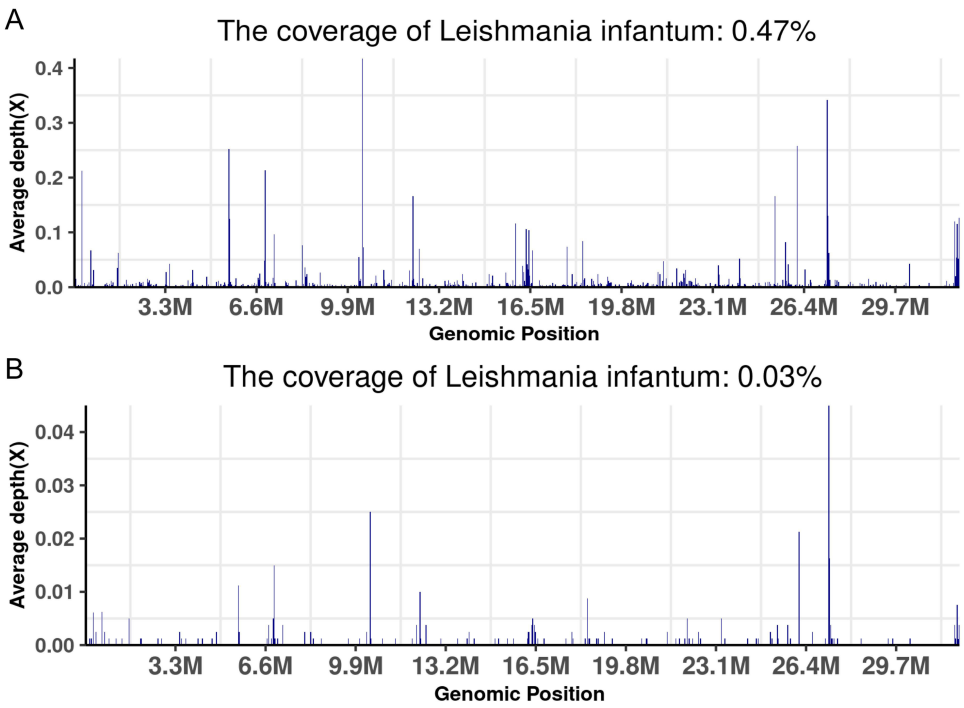


Figure 2 mNGS testing: (**A**) mNGS results of BALF indicate visceral leishmaniasis. BALF mNGS showed 8431 *Leishmania infantum* sequence readings with coverage of 0.47%. (**B**) Reexamination of BALF mNGS reported 806 *Leishmania infantum* sequence readings with coverage of 0.03%.

chest X-ray (Figure 1B). After ventilator-assisted respiration and diuretic treatment, the symptoms of dyspnea were significantly relieved. A recheck of chest X-ray suggested significant resolution of pulmonary oedema (Figure 1C), and the symptoms of asthma were relieved. On July 6, 2022, the blood smear and bone marrow cytology revealed no *Leishmania* bodies.

Nevertheless, pancytopenia and respiratory failure did not relieve completely, and cardiotoxic damage appeared. More seriously, brown sputum can be aspirated from his endotracheal tube (Figure 1D). The patient's pulmonary lesions were the most severe during that period. The intravenous infusion of sodium stibogluconate would reduce the positive rate of blood mNGS. So we chose BALF mNGS to increase the positive rate, while mNGS performed on blood or sputum was not performed. BALF mNGS was performed and reported by Guangzhou Vision Medicals Co. Ltd. Procedure and quality control of bronchoalveolar lavage fluid mNGS is available in Additional file 1.

The BALF mNGS reported 8431 *Leishmania infantum* sequence readings (Figure 2A). We administered sodium antimonyl gluconate for ongoing antileishmaniasis therapy and vancomycin for anticoccal treatment. BMA showed myelodysplasia, and no obvious parasites were detected. Reexamination of BALF mNGS showed 806 *Leishmania infantum* sequence readings (Figure 2B). The ECG showed that the T waves were hypotonic and more shallowly inverted (V1-V6) than before. The spleen was significantly smaller, but the whole blood cell account did not rebound significantly. Considering the incomplete control of VL, a second course of antimony sodium gluconate treatment was started.

However, the patient began to suffer intermittent shortness of breath, a gradual decrease in urine output, and a persistent increase in BNP. The reexamine of ECG suggested the deepened T-wave inversion. Meropenem was used for anti-infective therapy. The patient's cardiac, respiratory, and renal functions continued to decline, despite undergoing active treatment, ultimately resulting in death from multiple organ failure.

Discussion and Conclusions

VL, transmitted by the bite of an infected female sand fly, is a group of zoonotic infectious diseases caused by *Leishmania* in human macrophages.[4,5] This patient lived in a mountainous area, but there were no comparable patients in his living area, and he did not work in the epidemic area. The patient's initial symptoms occurred in January, which is

not the season for sand fly activity. However, it has been documented that kala azar can occur year-round, with an incubation period of several years.[6]

The detection of *Leishmania* in bone marrow, spleen, or lymph node smears is vital for diagnosing leishmaniasis. And we can use the positive detection of specific molecular biology as the basis of pathogenic diagnosis.[7] The patient has previously used amphotericin B and fluconazole for antifungal treatment in the local hospital. Amphotericin B is a second-line treatment for leishmaniasis. So the killing effect of Amphotericin B on *Leishmania* can explain why the initial bone marrow puncture did not detect this parasite. Thus, it is challenging to ascertain if it's a false-negative result.[8] The pathogenicity was detected through blood mNGS, and the presence of *Leishmania* amastigotes in the bone marrow smear provided further confirmation. rk39 screening is the first line diagnostic screening. Unfortunately, rK39 cannot be tested locally. It can only be checked at the Hebei Provincial Center for Disease Control and Prevention. During the initial referral, it was not reported in a timely manner. So it was reported by the Infection Department of our hospital. Subsequent positive results returned from a peripheral blood rK39 test, which was verified in terms of serologically specific antibodies, which enabled the diagnosis to be confirmed.

After the first course of antileishmaniasis therapy in this case, pancytopenia and respiratory failure did not relieve completely, and brown sputum appeared. But the blood smear and bone marrow cytology revealed no *Leishmania* bodies. For suspected VL cases, prior studies always used mNGS of peripheral blood samples.[3] It is uncommon to use BALF mNGS in VL diagnosis.[9,10] However, the detection of causative pathogens of pneumonia has been shown to be highly efficient by mNGS using BALF in recent studies. mNGS is a potential diagnostic tool that can complement conventional methods for pneumonia.[11] Meanwhile, the diagnostic efficiency of mNGS is unaffected by antibiotics. Therefore, mNGS test in BALF can be an important supplement for the diagnosis of VL.

Considering lung injury caused by *Leishmania*, we carried out BALF fluid mNGS test to make a timely diagnosis. *Leishmania* sequence was detected from the lung, which may be related to the abundance of macrophages in the lung. We inferred that the inflammatory reaction induced by the proliferation of *Leishmania* amastigotes may be one of the reasons for the aggravation of lung lesions.

A detailed study of the cardiac effects of sodium stibogluconate (SSG) was reported by Henderson and Jolliffe.[12] A reversible decrease in T wave amplitude was observed on electrocardiography. N. C. Hepburn[13] reported that the regimen did not cause any cardiac side-effects in most young fit patients. Nevertheless, idiosyncratic reactions cannot be excluded. Patients with pre-existing heart disease, impaired renal function, or malnutrition might be more susceptible to any cardiotoxic properties of sodium stibogluconate. Despite its potential cardiotoxicity, sodium stibogluconate is the drug of choice for Kala-azar and cutaneous leishmaniasis caused by *Leishmania braziliensis*. Given that the electrocardiographic and biochemical adverse effects of sodium stibogluconate were transient and reversible, we chose to treat with antimonials. Amphotericin B was withheld in the treatment due to the patient's renal dysfunction.[14]

However, the patient's condition deteriorated during the treatment with the second dose of antimony sodium gluconate. There was no myocardial necrosis of the patient, and there was no obvious abnormality in myocardial enzyme. So it was considered that the final death was related to the underlying disease. However, it is necessary to further study its pathogenesis and mechanisms of death in future clinical studies.

Abbreviations

BALF, bronchoalveolar lavage fluid; VL, visceral leishmaniasis; mNGS, metagenomic second-generation sequencing; BMA, bone marrow aspiration; ECG, electrocardiogram; SSG, sodium stibogluconate; CT, Computed Tomography.

Data Sharing Statement

Raw data of BALF mNGS, the datasets used and/or analyzed during the current study are available from the Zhuojun Deng on reasonable request.

Ethics Statement

The study was conducted in accordance with the Declaration of Helsinki. All procedures performed in the study involving human participants were in conformity to the ethical standards of the Ethics Committee of the Third

Hospital of Hebei Medical University. The ethics committee approved the waiver in this case report, based on the ethical standards to publish the case details.

Consent for Publication

The patient's relative provided written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors have declared no conflicts of interest in this work.

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