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Case Report: Diagnosis and Treatment of Two Clinical Cases of Visceral Leishmaniasis-Related Hemophagocytic Lymphohistiocytosis

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Abstract. Visceral leishmaniasis-related hemophagocytic lymphohistiocytosis (VL-HLH) is a potentially life-threatening secondary hemophagocytic lymphocytic syndrome caused by protozoan parasites of the *Leishmania* species and transmitted by infected sandflies. Therefore, it is important to be highly vigilant of the infection, particularly the visceral subtype, to share information with the public health system, and to improve the early diagnosis rate so that appropriate treatment can be initiated promptly. We report two isolated cases of VL-HLH. The main clinical manifestations were fever, pancytopenia, splenomegaly, hypofibrinogenemia, and hyperferremia, which meet the HLH-2004 diagnostic criteria. In our experience, anti-HLH treatment was not very effective for either case. No *Leishmania* organism was found in the first bone marrow smear of either patient. The first patient was diagnosed after identification of *Leishmania* amastigotes via sternal bone marrow biopsy, rK39 immunochromatography test, and metagenomic next-generation sequencing. The other patient was diagnosed by rK39-rapid diagnostic test and polymerase chain reaction. However, because of the delayed diagnosis in both cases, their conditions continued to deteriorate and both patients eventually died of the disease. Leishmaniasis is a parasitic disease with regional specificity and a low incidence. The occurrence of secondary HLH has a great impact on prognosis. When encountering secondary HLH in clinical practice, leishmaniasis should remain on the list of differential causes. Because of a high mortality rate if diagnosed late, it is crucial to be vigilant of VL-HLH in practice so that early detection, diagnosis, and treatment of the disease can be achieved to reduce adverse patient outcomes.

INTRODUCTION

Visceral leishmaniasis-related hemophagocytic lymphohistiocytosis (VL-HLH) is a hemophagocytic syndrome caused by *Leishmania* infection. The diagnosis of VL-HLH can be challenging even in endemic areas. Delay in the diagnosis of VL-HLH can result in death in up to 90% of patients without appropriate treatment.[1] Visceral leishmaniasis, also known as kala-azar, is a parasitic disease caused by *Leishmania*, which is transmitted by sandflies and hosted by humans and dogs. Long-term irregular fevers, emaciation, hepatosplenomegaly, lymphadenopathy, polyclonal gammopathy, and pancytopenia are the main manifestations of the disease. It is one of the most fatal yet often neglected tropical diseases.[2] Because of the diversity in its clinical manifestations it is often misdiagnosed, even in endemic countries.[3,4] Medical practitioners in nonendemic areas lack experience in VL diagnosis and treatment, thereby leading to misdiagnosis and delayed treatment.

Henan Province used to be one of the main endemic areas of VL in China. After active preventative and control strategies executed after the founding of the People's Republic of China, VL was essentially eradicated from the area in 1958. In subsequent monitoring, there were only sporadic cases that did not cause secondary transmission, indicating that VL was no longer endemic in the area.[5] Most sporadic cases were imported cases, whereas local cases were rare. In this article, we report two cases of VL-HLH in local adult males. No *Leishmania* organism was found in the first set of bone marrow aspirations of either patient. Conventional anti-HLH treatment alone was not very effective, and the patients eventually gave up treatment. The diagnosis and treatment processes of the two cases are reported as follows.

MATERIALS AND METHODS

Case 1. The first patient was a 60-year-old male who lived in Yanshi City, Luoyang City, Henan Province. He presented to the local hospital with diarrhea and thrombocytopenia (platelet count, 9.00×10^9/L), where he received symptomatic treatment with intravenous fluids. He was then transferred to our hospital. After being treated as idiopathic thrombocytopenic purpura, the platelet count increased to 109.00×10^9/L, and his condition improved temporarily. However, the patient developed fever (maximum temperature, 40.6°C) with dizziness and headache 2 weeks later. He re-presented to our hospital, and a physical examination revealed splenomegaly, abdominal tenderness, and ecchymosis of the lower limbs.

His initial laboratory results are shown in Table 1. The patient tested negative to sputum culture, Epstein–Barr virus (EBV), cytomegalovirus (CMV), HIV, and tumor markers including carcinoembryonic antigen, α-fetoprotein, prostate-specific antigen, and cancer antigen (CA) 19-9, CA 15-3, and CA 125.

Imaging results were as follows: positron emission tomography–computed tomography (PET-CT) showed splenomegaly with active spleen metabolism. Bone marrow in the systemic visual field showed increased metabolic activity. There was also bilateral pulmonary inflammation and pleural effusions.

A bone marrow aspiration was done afterward, but the marrow cytology showed no obvious abnormality in morphology.

His temperature remained above 38.5°C for more than 1 week. The other features, including pancytopenia, ferritin $>$ 500 ng/mL, fibrinogen $<$ 1.5 g/L, splenomegaly, and an increased proportion of mature lymphocytes in bone marrow cytology, all suggested a diagnosis of HLH according to the HLH-2004 diagnostic criteria. However, a differential diagnosis of hemophagocytic cell syndrome with pulmonary infection

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TABLE 1
Laboratory data of the patients

Variables	Patient 1	Patient 2
White blood cell count (normal, 3.5–9.5 $\times$ 10^9/L)	0.76	1.4
Neutrophil count (normal, 1.8–6.3 $\times$ 10^9/L)	0.28	0.8
Red blood cell count (normal, 4–5.5 $\times$ 10^{12}/L)	2.81	2.23
Hemoglobin concentration (normal, $>$ 110 g/L)	83	57
Hematocrit (normal, 40–50%)	25.2	18.9
Platelet count (normal, 125–350 $\times$ 10^9/L)	27	27
Partial prothrombin time (normal, 20–40 s)	63.50	91
Fibrinogen (normal, 2–4 g/L)	1.03	1.13
D-dimer (normal, 0.00–0.55 mg/L)	3.22	3.17
Fibrinogen degradation products (normal, 0–5 mg/L)	14.51	41.79
Albumin (normal, 40–55 g/L)	27	22.7
Globulin (normal, 25–35 g/L)	16.9	61
Alanine aminotransferase (normal, 9–50 U/L)	164	31
Aspartate aminotransferase (normal, 15–45 U/L)	430	47
Alkaline phosphatase (normal, 45–125 U/L)	324	247
Lactate dehydrogenase (normal, 90–245 U/L)	2,453	657
Ferritin (normal, 22–322 ng/mL)	$>$ 1,650	$>$ 1,650
Bone marrow hemophagocytosis	Yes	No
Bone marrow amastigotes	Yes	No
rK39	Positive	Positive
PCR	NA	Positive
mNGS	Positive	NA

mNGS = metagenomic next-generation sequencing; NA = not available; PCR = polymerase chain reaction.

was also considered. Splenocentesis was recommended to determine the cause but it was not performed because of the patient's severe thrombocytopenia. Considering possible systemic inflammatory response syndrome caused by HLH, dexamethasone and ruxolitinib were given to control the acute inflammatory response. Following treatment, the patient's fever subsided and he was discharged from the hospital. His platelet count also returned to normal 2 weeks later.

More than 1 month after discharge, the patient was readmitted because of fever and rhinalgia. Repeated chest and abdomen CT showed that the patient's spleen was smaller than before. After treatment as HLH with methylprednisolone combined with etoposide, the patient's temperature returned to normal and he was discharged from the hospital. However, he was hospitalized again because of repeated fever and anorexia after 1 month. We considered the relapse of HLH, and he underwent a sternal bone marrow aspiration. *Leishmania* amastigotes were found in the bone marrow smears (Figure 1). We immediately reported this case to the Luoyang Center for Disease Control and Prevention (Luoyang CDC). The specimen was confirmed positive on rK39-ICT (immunochromatography). At the same time, the metagenomic next-generation sequencing (mNGS) test of the patient's peripheral blood submitted by our hospital showed 191,090 sequence readings of *Leishmania infantum* and 126,425 sequence readings of *Leishmania donovani*. The diagnosis of VL was confirmed.

At this time, the patient's conscious state started to deteriorate with cachexia. Pentavalent antimonial was immediately given intramuscularly, and the patient was transferred to the intensive care unit (ICU) for endotracheal intubation and ventilator-assisted respiration. However, the patient's condition continued to deteriorate, and he died 2 days after being transferred to the ICU.

Case 2. The second patient was a 46-year-old male who lived in Mengjin County, Luoyang City, Henan Province. The patient had a fever and cough of unknown origin for more than 1 month, the maximum temperature was 38.5°C, and a complete blood examination at a different hospital showed a white blood cell count of 2.22 $\times$ 10^9/L, red blood cell count of 2.75 $\times$ 10^{12}/L, hemoglobin of 78 g/L, and platelet count of 128 $\times$ 10^9/L. Bone marrow aspiration showed active myelodysplasia. A peripheral blood smear showed rouleaux formation of red blood cells. Imaging showed hepatomegaly and splenomegaly. Multiple myeloma (MM) was suspected. However, serum protein electrophoresis showed that IgG kappa light chain and lambda light chain were on the high side and that the kappa/lambda ratio was normal, indicating an increase in polyclonal immunoglobulins. Subsequently, the patient was transferred to our hospital for further diagnosis and treatment.

His laboratory results are shown in Table 1. Antimyeloperoxidase and anticitrullinated peptide antibodies were both positive. Rheumatoid factor and hypersensitive C-reactive protein were elevated, whereas EBV, CMV, HIV, phospholipid syndrome antibodies, and tumor markers were all negative.

Imaging showed a probable small amount of subarachnoid hemorrhage in the right frontal region, bilateral lung infection, a small amount of pericardial effusion, cholecystitis with cholestasis, splenomegaly, a right renal cyst, and a small amount of free fluid in the abdominal and pelvic cavity.

The patient had pancytopenia, repeated pyrexia, splenomegaly, ferritin $>$ 500 ng/mL, and fibrinogen $<$ 1.5 g/L, meeting the HLH-2004 criteria, and hence was diagnosed with HLH. The immunoglobulin test found that the patient's polyclonal globulin was elevated, which did not meet the diagnosis of MM. Dexamethasone combined with broad-spectrum antibiotics for anti-infection treatment was ineffective, and the disease continued to progress. Considering that VL infection could not be excluded, a blood sample was sent to the Luoyang CDC for rK39-ICT, which came back positive. A subsequent peripheral blood sample was sent to the Henan CDC immediately, and the polymerase chain reaction (PCR) test result was also positive. As a result, the patient was diagnosed with VL. However, the patient's autoimmune comorbidities (rheumatoid arthritis; not on immune-modulating treatment), multiple hormonal disturbances, as well as the emergence of neuropsychiatric symptoms added many difficulties to the treatment. A bone marrow aspiration was recommended as well as starting pentavalent antimony treatment immediately. However, the patient declined. Five days after being transferred to the Henan Provincial Infectious Diseases Hospital (Zhengzhou Sixth People's Hospital), he and his family gave up treatment because of serious illness.

DISCUSSION

VL is one of the main forms in which leishmaniasis presents clinically. The disease is epidemic in many different regions of China, including Xinjiang, Gansu, Sichuan, Shaanxi, Shanxi, and Inner Mongolia. The source of infection is often infected people and dogs (*L. donovani* is not normally in dogs, but

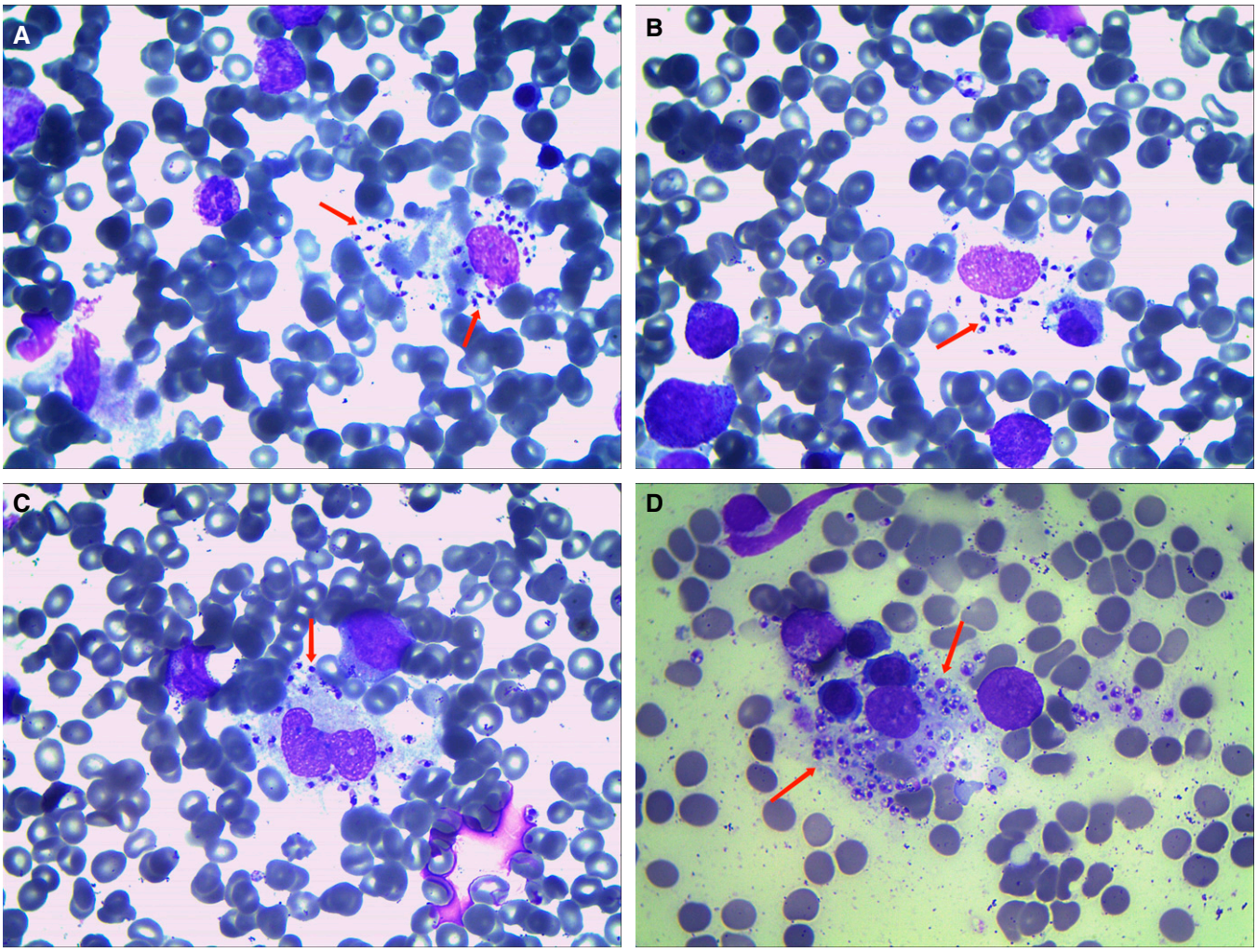

FIGURE 1. Bone marrow smear samples for patient 1 with visceral leishmaniasis. Arrows show amastigotes within macrophages. Wright Giemsa stain, original magnification, ×1,000. The amastigotes are located around broken red blood cells (**A**). The amastigotes are located extracellular (**B**) and intracellular (**C**). The bone marrow smear sample showed hemophagocytosis (**D**).

L. infantum is possible), and the disease is transmitted via blood by sandflies. The peak period of the disease is from March to May, and the second peak is from October to November.[6] Henan Province is located in northern China, and the main terrain is plains. In 1958, VL was basically eradicated from Henan. There had been no local cases for many years.[5] Therefore, many frontline clinical doctors lack insight into the disease, which often leads to misdiagnosis. In 2016, a case of local infection occurred in Linzhou City, Anyang City, Henan Province.[7] Since then, the endemic area has gradually expanded and the number of cases has gradually increased. The Henan CDC has issued an early warning against the increasing numbers of VL cases. When encountering patients with long-term irregular pyrexia, emaciation, enlargement of liver, spleen, and lymph nodes, an increase of polyclonal immunoglobulins, and pancytopenia, the diagnosis of VL needs to be excluded.

It is not difficult to diagnose VL because there are a variety of methods to detect it along with its clinical characteristics. Traditionally, the gold standard method for diagnosing VL is to find *Leishmania* amastigotes by microscopic examination of smears (spleen, bone marrow, lymph node, or liver biopsy).[8] Although this method is highly specific, its sensitivity is not strong except in splenic aspirates, and it also has high requirements for the experience and technique of the pathologist. It should be emphasized that splenocentesis or splenic needle biopsy is a very dangerous procedure and is associated with a high rate of splenic rupture in VL. Both patients had a bone marrow biopsy at an early stage of the disease, but no parasitic infection was revealed at that time. In the first patient, we found *Leishmania* amastigotes in the bone marrow after multiple bone marrow biopsies. Considering that the rK39-rapid diagnostic test was positive and mNGS clearly revealed a large amount of *Leishmania* DNA in blood samples, VL was confirmed. Recently, Guo et al.[9] reported that a child was eventually diagnosed with VL via mNGS after all other tests were negative, suggesting that it might become a more sensitive and specific method to increase the detection of VL.

At present, molecular tools are highly sensitive for *Leishmania* detection, which may be helpful as an additional test in diagnosis. rK39-ICT has been validated in VL endemic regions of North America, eastern Africa, India, and Spain.[10,11] The rK39-ICT of both patients was positive, which shows that the test has decent sensitivity and practicability. In a recent systematic review, sensitivity of the rK39-ICT in India was estimated to be 97%, whereas it was only 85% in eastern

Africa.[12] Another limitation is that rK39-ICT may be falsely positive in apparently healthy individuals and remain positive for a long time after VL is cured in epidemic areas.[13] Consequently, it is recommended for rK39-ICT to be used as a rapid screening test for VL. For those who are positive, further investigations with bone marrow aspiration to look for amastigotes and PCR and mNGS should be conducted to confirm the diagnosis.

The prognosis of secondary HLH depends on the control of primary disease. Therefore, early identification of the etiology is of great significance.[14] Leishmaniasis is considered to be the most common protozoan infection associated with HLH.[9,15,16] Research in Germany found that about 0.77% of adult patients develop secondary HLH after being infected with *Leishmania*.[17] One study from China suggested that compared with VL alone, VL-HLH patients had higher rates of bleeding, hepatomegaly, thrombocytopenia, hypertriglyceridemia, hyperferremia, and hypofibrinogenemia, increased secretion of soluble interleukin 2 receptor, and lower natural killer cell activity. In addition, the level of cytokines in the serum of patients with VL-HLH and the parasite load in bone marrow smears were also higher. In magnetic resonance imaging, more VL-HLH patients have hepatosplenomegaly with iron overload. In addition to anti-*Leishmania* treatment, anti-inflammatory treatment can reduce cytokine storms and excessive immune response.[18] It has been reported that the treatment of patients with secondary HLH with a JAK2 inhibitor—ruxolitinib—is effective.[16] Recently, Cui et al.[19] reported a case of refractory leishmaniasis-associated HLH, and the symptoms were relieved after treatment with ruxolitinib and amphotericin B. In the first patient, we successfully suppressed the systemic inflammatory reaction caused by secondary HLH with ruxolitinib. His condition was in remission temporarily but recurred in the end, indicating that it is extremely important to find the initiating factors of HLH.

Timely and effective use of anti-VL drugs is key to the treatment of VL-HLH. Mottaghipisheh et al.[20] conducted a single-center study involving 40 children with primary HLH and 20 children with HLH secondary to VL. It was found that all VL-HLH patients were successfully treated with amphotericin liposomes without starting etoposide-based chemotherapy, and there was no report of death or recurrence of HLH during the 40-month follow-up period. Amphotericin B is currently found to be safe and effective in most epidemic areas in the world and is recommended as a first-line anti-VL drug. However, pentavalent antimonials are still effective in most VL epidemic areas except for India.[8] Shi et al.[21] reported that two children with VL-HLH were diagnosed and promptly treated with pentavalent antimonials for 3 weeks. No *Leishmania* was found in the repeated bone marrow smears of these patients, and both recovered fully and were discharged. Guo et al.[9] treated children with VL-HLH with amphotericin B for one cycle. As a result, the symptoms of VL-HLH improved significantly, and many laboratory parameters returned to normal.

CONCLUSION

Through the diagnosis and treatment of two VL-HLH patients at our hospital, it can be concluded that VL has typical early presentation but is difficult to diagnose in nonepidemic areas because of a poor awareness of the disease. It is often confused with other infectious and hematological diseases because of features such as long-term irregular pyrexia, hepatosplenomegaly, and pancytopenia. Untreated VL can lead to death. When encountering secondary HLH in clinical practice, leishmaniasis should remain on the list of differential causes. The treatment of secondary HLH requires not only timely inhibition of the excessive systemic inflammatory response but also active searching for the causative factor. rK39-ICT can be used as a rapid screening test for VL, and mNGS may become a new and more efficient method for detecting *Leishmania* infection. For frontline clinicians, it is necessary to maintain vigilance against the disease so that early detection, diagnosis, and treatment can be achieved and the future occurrence of adverse outcomes can be prevented.

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