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### Diagnostic value of bone marrow cell morphology in visceral leishmaniasis-associated hemophagocytic syndrome of two cases

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**Abstract**

**BACKGROUND**

Visceral leishmaniasis related-hemophagocytic lymphohistiocytosis (VL-HLH) is a hemophagocytic syndrome caused by *Leishmania* infection. VL-HLH is rare, especially in nonendemic areas where the disease is severe, and mortality rates are high. The key to diagnosing VL-HLH is to find the pathogen; therefore, the *Leishmania* must be accurately identified for timely clinical treatment.

**CASE SUMMARY**

Two cases suspected of having malignant tumors at other hospitals and who were unresponsive to treatment were transferred to Kunming Children’s Hospital. Both children underwent chemotherapy as per the HLH-2004 chemotherapy regimen, but it was ineffective and accompanied by serious infections. We found *Leishmania* amastigotes in their bone marrow via morphological examination of their bone marrow cells, which showed hemophagocytic cells; thus, the children were diagnosed with VL-HLH.
CONCLUSION

Morphological examination of the bone marrow cells played an important role in diagnosing VL-HLH. When clinically diagnosing secondary HLH, VL-HLH should be considered in addition to common pathogens, especially in patients for whom HLH-2004 chemotherapy regimens are ineffective. For infants and young children, bone marrow cytology examinations should be performed several times and as early as possible to find the pathogens to reduce potential misdiagnoses.

Key Words: bone marrow cell morphology; visceral leishmaniasis; hemophagocytic syndrome; infant

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Core Tip: This study started with the morphology of bone marrow cells, finding the pathogen from the cells, and successfully diagnosed two cases of Visceral leishmaniasis related-hemophagocytic lymphohistiocytosis, and was compared and analyzed with hemophagocytic lymphohistiocytosis. Summarized the key criteria for differential diagnosis of visceral leishmaniasis-related hemophagocytic lymphohistiocytosis is to find the pathogen in the bone marrow cells. This has great guiding significance for clinical laboratory diagnosis and clinical treatment.

INTRODUCTION

Hemophagocytic syndrome (HPS), also known as hemophagocytic lymphohistiocytosis (HLH), is divided into primary and secondary forms. The etiology of secondary HLH is complex; it can be caused by infection, malignant tumors, and autoimmune diseases. Visceral leishmaniasi-related hemophagocytic
lymphohistiocytosis (VL-HLH) is very rare in childhood, especially in nonendemic areas. The disease is severe with high mortality rates of up to 100% without early diagnosis and treatment \(^1\). Therefore, the leishmaniasis-associated pathogen must be rapidly and accurately identified for clinical and timely treatment. Here, we report two young patients with VL-HLH diagnosed \textit{via} bone marrow cell morphology at the Children's Hospital of Kunming, Yunnan, China in the past 5 years.

**CASE PRESENTATION**

\textit{Chief complaints}

We retrospectively analyzed the clinical data, laboratory examination results and bone marrow cell morphology of two children with VL-HLH diagnosed \textit{via} bone marrow cell morphology at Kunming Children's Hospital of Yunnan, China. Two cases suspected of having malignant tumors at other hospitals and who were unresponsive to treatment were transferred to Kunming Children's Hospital. They are girls, their ethnic group is Han. One is two years old and the other one is nine months old. They had repeated fevers, pancytopenia, hepatosplenomegaly, hypertriglyceridemia, and hypofibrinogenemia over a long period and met the HLH-2004 criteria. Their HLH genetic test results were negative, Both children underwent chemotherapy as per the HLH-2004 chemotherapy regimen, but it was ineffective and accompanied by serious infections. We found \textit{Leishmania} amastigotes in their bone marrow \textit{via} morphological examination of their bone marrow cells, which showed hemophagocytic cells; thus, the children were diagnosed with VL-HLH. After being transferred to a specialty hospital for treatment, the condition was well-controlled.

\textit{History of present illness}

**Case 1:** A 2 years old girl from Weining, Guizhou, China, presented in July 2017 with a recurrent and irregular fever lasting 3 mo and reaching 39°C–40°C.
Case 2: A 9 mo old girl from Zhaotong City, Yunnan Province, she had diarrhea for half of December 2020, with a subsequent irregular fever lasting 1 mo and reaching 39°C–40°C and decreased blood cells in the peripheral blood.

History of past illness
Case 1: She continued to have repeated fevers, coughing with sputum, abdominal distension, anorexia, and fatigue. She had a history of mosquito bites and contact with a domestic dog 1 mo before onset as well as a history of Epstein-Barr virus-related hemophagocytic syndrome in April 2017.
Case 2: None

Personal and family history
None

Physical examination
Both children had enlarged livers and sleeps.

Laboratory examinations
Blood routine and biochemical tests were performed.

Imaging examinations
The pathogens revealed the kala-azar pathogen/Leishmania amastigotes in the bone marrow of both patients were found.

FINAL DIAGNOSIS
The children were diagnosed with Visceral leishmaniasis related-hemophagocytic lymphohistiocytosis.

TREATMENT
Both children were treated with sodium stibogluconate (SSG).
OUTCOME AND FOLLOW-UP
Both children recovered and were discharged.

DISCUSSION
HLH is a life-threatening disease caused by excessive inflammation and multiple organ dysfunction, resulting in uncontrollable lymphocyte and macrophage activation and proliferation. HLH is divided into primary and secondary forms. Infection is the most common cause of secondary HLH. VL-HLH is very rare in childhood and has a high mortality rate if not diagnosed and treated early.

Visceral leishmaniasis (VL) is caused by Leishmania. Common pathogens include Leishmania donovani, Leishmania infantum, and Leishmania tropica and phlebotomine sandflies are the main transmission vector. The infectious agents of this disease are mainly the patients and sick dogs. The disease is transmitted between humans and animals via blood sucking by phlebotomine sandflies. The disease has obvious regional characteristics. VL is scattered throughout six western provinces in China and Xinjiang, Gansu, Sichuan, and Shaanxi. The two cases reported herein were from Weining County, Guizhou, and Yunnan after moving from Zhouqu, Gansu. Both children had lived in epidemic areas and had histories of phlebotomine sandflies bites from June to September (the sandfly breeding season) before disease onset. Weining County in Guizhou and Zhouqu County in Gansu Province are both areas where leishmaniasis is spreading. The epidemiological histories of both children were clear.

Both children had long-term irregular fevers, with the highest body temperature exceeding 40°C pancytopenia, and hepatosplenomegaly. Because of these clinical manifestations, the diseases were initially misdiagnosed as malignant hematological diseases. Neither child recovered after long-term treatment with drugs at other hospitals, and both had severe infections. VL-HLH is easily misdiagnosed in nonendemic areas because VL manifestations are very similar to those of hematological malignancies. VL symptoms also include a long-term irregular fever,
hepatosplenomegaly, and pancytopenia. Additionally, VL has a rapid onset and progression. Early symptoms are atypical with many complications; thus, it is easily misdiagnosed, especially when combined with Epstein-Barr viral infections, leading clinicians to think that it is Epstein-Barr virus-associated HLH. Many clinicians have insufficient knowledge and no clinical experience with VL, especially in nonendemic areas. Furthermore, laboratory physicians often lack knowledge of the Leishmania. Due to the morphology of Leishmania amastigote and platelets is very similar, laboratory physicians may mistake them as platelets, they are also easily engulfed by phagocytes. At same time, these phagocytes may also contain platelets, red blood cells, and white blood cells, and if the laboratory technicians are unfamiliar with Leishmania amastigotes or do not read the results carefully, they may mistake them for platelets. Many reports have found that kala-azar is often misdiagnosed owing to clinicians’ and technicians’ lack of knowledge of Leishmania amastigotes. The morphologies of Leishmania, Penicillium marneffei, and histoplasma have many similarities and are easily confused. Clinicians and technicians must be familiar with the morphological characteristics of various pathogens and the differences between them. No Leishmania amastigotes were found in either child via bone marrow cell morphology at the previous hospital; thus, the children were misdiagnosed with hematological malignancies. The key to diagnosing these children is to detect the Leishmania amastigotes via bone marrow cell morphology combined with their epidemiological histories. To diagnose HLH secondary to kala-azar, finding the pathogen in the bone marrow is the most reliable diagnostic criterion.

VL-HLH-associated mortality is relatively high. If HLH treatment is ineffective, clinicians should consider whether the HLH is secondary to VL. Both patients in this study underwent 14 days of chemotherapy according to the HLH-2004 chemotherapy regimen, but the chemotherapeutic effect was unsatisfactory, their clinical symptoms did not significantly improve, and their liver and kidney functions did not recover as per the related infection indicators. After the disease was clearly diagnosed, they received the recommended treatment, and the disease was quickly controlled. Both
patients recovered and were discharged from the hospital. When clinically diagnosing HLH, clinicians should actively search for the cause. If standard treatment for HLH is ineffective, detailed epidemiological histories should be taken, bone marrow cytology examinations should be performed quickly, and HLH secondary to VL should be ruled out.

**CONCLUSION**

In summary, bone marrow cell morphological examinations play a vital role in diagnosing VL-HLH. When secondary HLH is diagnosed clinically, common pathogens and VL-HLH should both be considered, especially in infants and young children who could not be treated satisfactorily as per the HLH-2004 regimen. Detailed epidemiological histories should be taken, and their bone marrow cytology should be re-examined multiple times as soon as possible to find the pathogen and reduce misdiagnoses. Clinicians and technicians should be familiar with the morphological characteristics of *Leishmania* to provide timely and accurate diagnoses.