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CASE REPORT

Case Report: Visceral Leishmaniasis Falsely Diagnosed as Viral Hepatitis C Without Febrile Symptoms

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Background: Visceral leishmaniasis (VL), also known as kala-azar, is caused by an intracellular parasite transmitted to humans by the bite of a sand fly, and with the source of the infection mainly being dogs. The main features of the disease are irregular fever, weight loss, hepatosplenomegaly and anaemia. Diagnosis relies mainly on bone marrow aspiration tests to find Leishman-Donovan (LD) bodies. And we report the case without febrile symptoms and hepatitis C virus antibody was probably false positive.

Case Presentation: The case was a 74-year-old male residing in Yangquan City, Shanxi Province, a VL endemic area. He presented with generalised malaise, hepatosplenomegaly and scarring pigmentation on the skin as a result of scratching. Laboratory tests showed pancytopenia, positive hepatitis C virus antibody (HCV-Ab), positive direct anti-human globulin test (DAT), positive anti-cardiolipin antibody IgG, IgM (+), and increased immunoglobulin IgG. Symptomatic treatments such as hepatoprotection and blood transfusion were given, but the patient's symptoms still persisted and his spleen and liver further enlarged. Further repeat tests were performed and found to be negative for hepatitis C virus antibodies and antigens. The patient was eventually found to be infected with *Leishmania protozoa* by rk39 rapid diagnostic test and metagenomic next-generation sequencing(mNGS). And the patient quickly relieved after one course of treatment with sodium stibogluconate.

Conclusion: Patients with VL may cause abnormalities in the immune system, leading to false positives for various antibodies without clear febrile symptoms, resulting in misdiagnosis or delayed diagnosis. It is important to consider VL in cases where there is a significant hepatosplenomegaly with a relevant epidemiological history. If the diagnosis cannot be confirmed through bone marrow aspiration and the patient is not suitable for splenic aspiration, the rk39 test can be used for initial exclusion and further verified through mNGS.

Keywords: visceral leishmaniasis, symptoms, false-positive antibodies, metagenomic next-generation sequencing, case report

Background

Leishmania parasites are transmitted through the bites of infected female phlebotomine sandflies that require blood to produce eggs. It was estimated that 700,000 to 1 million new cases occur annually worldwide.[1] A survey shows that the number of visceral leishmaniasis(VL) cases in China decreased from 498 in 2015 to 166 in 2019.[2] However, there has been an increase in the annual incidence rate of VL cases reported from 2019 to 2021, with a total of 608 cases reported during this period. Specifically, 158, 213, and 237 cases were reported in 2019, 2020, and 2021 respectively.[3] This indicates that leishmaniasis is still a public health issue that should not be overlooked.

Visceral leishmaniasis typically presents with wasting symptoms such as irregular fever, anemia, darkening of the skin, and enlarged hepatic and splenic lymph nodes. While splenic aspiration is the most accurate diagnostic method with specificity and sensitivity both over 90%,[4] it is invasive and not recommended as the first choice for critically ill patients with active bleeding, thrombocytopenia, severe anemia, or jaundice.[5] Clinical diagnosis commonly relies on bone marrow aspiration to detect Leishman-Donovan(LD) bodies, but this method has low sensitivity and can result in missed

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or delayed diagnosis. Recently, the rk39 dipstick and metagenomic next-generation sequencing(mNGS) have been found to have high diagnostic value in identifying the etiology of infectious diseases.[6]

A case of visceral leishmaniasis was reported without obvious febrile symptoms. The case showed the possibility of having multiple false-positive antibodies, especially to hepatitis C virus. The diagnosis of VL was initially based on the results of bone marrow examination in the early stages, but it failed to reveal a basis for the diagnosis. Finally, the diagnosis was confirmed by the results of the rk39 dipstick and mNGS.

Case Presentation

The patient was a 74-year-old farmer from Yangquan City, Shanxi Province, with a history of cerebral infarction in 2000, without sequelae of cerebral infarction. He was admitted to the hospital on 10 July 2023 with the main cause of "generalised fatigue with pancytopenia for more than half a year". In January 2023, he was infected with the 2019 novel coronavirus (2019-nCOV) and was discharged from the local hospital after treatment. However, he still felt weak and tired. On February 8, 2023, he visited a doctor again and was diagnosed with pancytopenia. For the next months, he had intermittent visits and bone marrow aspirations, but no special abnormalities were found. In June 2023, he was treated at a hematology hospital, and the bone marrow aspiration showed that granulocyte, red, and giant trilogy of cells were still proliferating, and 4% plasma cells could be seen. The bone marrow biopsy showed that the ratio of granulocytes to red cells was approximately normal. Additionally, the abdominal ultrasound showed that his liver and spleen were enlarged, and his peritoneal cavity was fluid-filled. Despite hospitalization and symptomatic treatment, he still experienced general fatigue, dizziness, and pancytopenia. He also lost 10kg in weight over the past six months. While on admission, physical examinations revealed normal body temperature but with features of anaemia. Scattered hyperpigmentation was visible on the whole body after scratching, while enlarged lymph nodes were not palpable throughout the body. The liver was located 5 cm below the ribs with moderate texture, and the spleen was located 8 cm below the ribs with moderate texture as well.

The patient, infected with novel coronavirus in January 2023, continued to have symptoms of malaise, suggesting Long-COVID. According to the patient's diagnosis and treatment history from other hospitals, he was diagnosed with pancytopenia on February 8th, 2023. The bone marrow aspiration tests conducted at that time did not show any significant abnormality. And upon admission to our hospital, the tests indicated that he had pancytopenia (as seen in Table 1) and A/G<1 (A/G=0.33). The abdominal ultrasound revealed that the maximum oblique diameter of the right lobe of the liver was approximately 16.5 cm with a glossy envelope. Moreover, the spleen had a full morphology, with a thickness of about 6.3 cm and a longitudinal diameter of about 19.1 cm. The abdominal and pelvic CT scans revealed cirrhosis of the liver and splenomegaly. The infectious disease series only showed positive HCV-Ab, while the results for

Table 1 Laboratory Result of the Patient

	2023-7-10	2023-7-15	2023-7-21	2023-7-26	2023-7-29	Reference Ranges
White blood cells (WBC)	2.4×10 ^{9}/L	2.0×10 ^{9}/L	2.6×10 ^{9}/L	2.2×10 ^{9}/L	1.8×10 ^{9}/L	3.5–9.5×10 ^{9}/L
Red blood cells (RBC)	2.09×10 ^{12}/L	1.92×10 ^{12}/L	2.28×10 ^{12}/L	1.93×10 ^{12}/L	2.08×10 ^{12}/L	4.3–5.8×10 ^{12}/L
Hemoglobin (Hb)	62.0g/L	58g/L	67.0g/L	58.0g/L	63.0g/L	130–175g/L
Hematocrit (HCT)	19.30%	17.80%	20.80%	17.30%	19.00%	40–50%
Platelet count (PLT)	94×10 ^{9}/L	93×10 ^{9}/L	113×10 ^{9}/L	87×10 ^{9}/L	70×10 ^{9}/L	125–350×10 ^{9}/L
Neutrophil granulocyte (NEUT)	1.34×10 ^{9}/L	0.92×10 ^{9}/L	2.00×10 ^{9}/L	1.40×10 ^{9}/L	1.00×10 ^{9}/L	1.8–6.3×10 ^{9}/L
Alanine aminotransferase (ALT)	20U/L	17U/L	17U/L	14U/L	13U/L	5–40U/L
Aspartate amino transferase (AST)	40U/L	27U/L	27U/L	30U/L	29U/L	8–40U/L
Albumin (ALB)	23.1g/L	20.7g/L	20.9g/L	21.70g/L	24.9g/L	40–55g/L
Globulin (GLB)	69.5g/L	59.5g/L	56.6g/L	50.90g/L	55.4g/L	20–40g/L
A/G	0.33	0.35	0.37	0.43	0.45	1.0–2.5
Alkaline phosphatase (ALP)	156U/L	166U/L	179U/L	159U/L	145U/L	53–140U/L
γ -Gluyltransferase (GGT)	103U/L	85U/L	92U/L	77U/L	64U/L	8–58U/L
Calcitonin (PCT)	0.56ng/mL		0.445ng/mL		0.435ng/mL	0–0.05ng/mL
Hypersensitivity C-reactive protein (hsPCR)			6.9mg/L	10.03mg/L	5.48mg/L	0–6mg/L

the rest of the hepatitis B five, syphilis spirochete antibody, human immunodeficiency virus antigen antibody were negative. The laboratory tumour markers also showed negative results, and therefore, we suspected that the patient might be suffering from viral hepatitis C. The patient has been diagnosed with anemia and has received multiple blood transfusions in external hospitals. Unfortunately, the transfusions did not have the desired effect. In order to rule out haemolytic anemia, several tests were performed including the CD55 and CD59 test, direct and indirect anti-human globulin test. The results showed that the direct antiglobulin test (DAT) was positive, while other tests showed no abnormalities. Additionally, related laboratory tests for rheumatology and immunology showed that the patient had an increased level of immunoglobulin IgG and positive anti-cardiolipin antibody IgG and IgM. These results suggest that the patient's immune system is abnormal and may indicate an underlying autoimmune liver disease. The patient was provisionally given symptomatic treatment such as hepatoprotection (Magnesium Isoglycyrrhizinate Injection) and blood transfusion (red blood cells in additive solution with reduced leukocytes). After 5 days of treatment, the patient's condition did not show significant improvement. A review of routine blood and liver function (as shown in Table 1) suggested that hemoglobin had further decreased. The anaemia did not show significant improvement after blood transfusion, and abdominal ultrasound reported that: the liver's periphery was still glossy, with rough internal echoes and uneven distribution, and the ductal structure was not clear enough, and the lower edge of the right lobe of the liver was almost flat to the umbilicus when the liver was probed at the axillary line during deep inhalation; The spleen had a long diameter of 20.76 cm, a thick diameter of 8.26 cm, and an anatomical long diameter of 16.66 cm, and the lower edge of the spleen was almost flat to the anterior superior iliac spine when probed in the left anterior axillary line during deep inspiration. The patient's liver and spleen have increased in size and his ascites has worsened since admission. The repeat viral hepatitis C antigen and antibody tests came back negative. The peripheral blood smear test showed no abnormalities, and the bone marrow findings indicated that some mature granulocytes had toxic granules in their cytoplasm, while the rest showed no abnormalities, including no parasites. After examining the patient, the haematologist suspected a high possibility of infection due to the progressive enlargement of the spleen and the fact that the patient lived in Yangquan, an area known for visceral leishmaniasis. Further laboratory tests were required to rule out leishmaniasis. The patient's serum and bone marrow samples were sent to the Shanxi Provincial Center for Disease Control and Prevention for the rk39 rapid diagnostic test. The test results indicated that the Leishmania antibody was positive. The mNGS examination was also conducted. The total number of nucleic acid sequences detected by high-throughput sequencing method in peripheral blood samples of the patient was collected, and the obtained sequences were compared and annotated with the human genome sequence database and pathogen microbial genome sequence database, to realize the identification of pathogenic microorganisms. The results showed that 4101 sequence readings were detected for the *Leishmania* genus, and 98 sequence readings were detected for the *Leishmania infantum* species.

The clinical diagnosis of visceral leishmaniasis was confirmed. On July 20th, 2023, the patient received an injection of antimony Sodium Stibogluconate. However, the following day, the patient developed a high temperature with chills, with a maximum temperature of 39°C. Laboratory tests showed that the inflammatory indexes were higher than before. We suspected that it might be drug fever or infectious fever, so we adjusted the interval between medications and added levofloxacin anti-infective treatment. Gradually, the patient's temperature returned to normal. After completing one course of treatment, the results of an abdominal ultrasound showed that the maximum oblique diameter of the right lobe of the liver was 13.26cm, and the long diameter of the spleen was 17.47cm with a thick diameter of 7.19cm. This suggests that the volume of the liver and spleen had reduced compared to the time of admission. And the patient's general condition has improved from that on admission to the hospital.

Discussion

Between 2019 and 2021, a total of 608 cases of VL were reported in China. The number of cases reported each year were 158, 213, and 237, respectively. The majority of cases were found in Shanxi (299 cases), Shaanxi (118 cases), and Gansu (106 cases) provinces, which accounted for 86.02% (523/608) of the total reported cases in China. In China, VL is classified into three types based on the epidemiological characteristics of the disease: anthroponotic type (AVL), zoonotic mountain type (MT-ZVL), and zoonotic desert type (DT-ZVL). Out of the 608 cases, 10 were AVL, 20 were DT-ZVL, and 578 were MT-ZVL. MT-ZVL is usually caused by *Leishmania infantum*, which is highly prevalent in dogs.[3] Therefore, dogs are considered

to be the main reservoirs of *L. infantum*. As the pathogens identified in the cases in this study were *L. infantum*, it is likely that the source of infection for the patient was dogs.

There were four potential pitfalls in diagnosing VL of our case. Firstly, the clinical symptoms of VL are wide-ranging and atypical. Irregular fever, weight loss, hepatosplenomegaly and anaemia are the most common clinical manifestations of VL.[4] Since the onset of the disease, the patient's body temperature was not obviously abnormal. Still, there were obvious hepatosplenomegaly and pancytopenia, which made it easy for clinicians to suspect that the patient was suffering from a haematological disease based on their experience, resulting in the patient's treatment in different hospitals without any obvious improvement in his condition. The repeated bone marrow aspirations did not reveal any evidence of hematological disease. Therefore, clinicians need to expand their thinking and think from different aspects, especially for patients with obvious splenomegaly and a history of traveling in infected areas, they should be alert to VL.

Secondly, our judgement was influenced by the fact that the patient had been infected with a 2019 novel coronavious virus. The patient had been infected with 2019 novel coronavious virus in January 2023, and after treatment, she still had symptoms of generalised malaise, which was considered suspicious for Long COVID. The patient's positive direct anti-human globulin test made it difficult to make a definitive diagnosis, because Long COVID can also affect the patient's immune system, causing symptoms similar to this patient's, such as cold agglutinin syndrome (CAS) and Autoimmune Hemolytic Anemia (AIHA).[7,8] However, it cannot be ruled out that the infection with 2019 novel coronavious virus during the period of Leishmania protozoa infection, and the symptoms such as generalised malaise remaining after treatment of the 2019-COVID infection, were caused by VL. After the diagnosis of VL was clarified and symptomatic treatment was given, the patient's symptoms of general malaise improved, and the diagnosis of Long COVID could thus be excluded.

Thirdly, the patient's laboratory tests conducted on admission revealed positive HCV-Ab. However, the initial results of the hepatitis C virus serology were most likely false-positive due to the following reasons: the later re-test of HCV serology showed negative HCV-Ab and HCV-Ag, and the patient had no history of chronic hepatitis C. Although the patient had undergone a blood transfusion in the six months leading up to the admission, and there was a possibility of transfusion infection with HCV, the patient had marked hepatosplenomegaly and mildly abnormal liver function at the time of admission, which did not align with the clinical presentation of hepatitis C. It has been reported that VL patients infected with leptospirosis can have serological false positives for leptospirosis and trypanosomiasis.[9] However, false positives for HCV-Ab due to leishmaniasis have not been reported. Clinical studies have found instances of false positives for HCV-Ab caused by other factors such as fungal infections and implantation of a left ventricular assist device.[10,11] Therefore, clinicians should be aware of these possibilities, especially if the patient's symptoms do not match the diagnostic guidelines. Repeat tests for antibodies such as HCV-Ab, anticardiolipin IgG, and IgM can assist in identifying visceral leishmaniasis-associated false-positive antibodies, and we will follow up with the patient accordingly.

Fourthly, In this case, the patient's pre-admission bone marrow aspiration tests at other hospitals did not detect the pathogen, and after admission, another bone marrow aspiration was still undetectable, and the diagnosis was ultimately clarified by relying on the rK39 dipstick and mNGS. The definitive clinical diagnosis of VL relies on patients with *Leishmania protozoa* detected on smears of bone marrow, spleen, and lymph node aspirates, with the highest diagnostic value of the splenic aspiration fluid (>90% specificity and sensitivity), followed by the bone marrow and lymph nodes.[4] However, splenic aspirations need to be performed by skilled and experienced technicians. Although serious bleeding complicating splenic aspiration is rare, it may occur. Therefore, bone marrow and lymph node puncture fluids are commonly used for microscopic examination in clinical practice. However, because there are currently few professionals trained in parasite morphology in most clinical laboratories in China, the rate of false negatives by microscopic examination is very high. A previous study identified only 8 out of 1093 confirmed cases of visceral leishmaniasis in China via microscopic examination.[12] Thus making it easy to miss the diagnosis. A meta-analysis[13] and a multicenter evaluation[14] corroborated earlier findings of high diagnostic accuracy of the rK39 dipstick. mNGS can be used to directly identify potential pathogens such as bacteria, fungi, viruses, and parasites in DNA samples by high-throughput sequencing and database comparison without the need to isolate pathogens.[5,15,16] Compared with traditional pathogen detection methods, mNGS provides obvious advantages for the identification of pathogens that cannot be cultured or are not easily cultured. The results of this case revealed that the use of the rK39 dipstick and mNGS is undoubtedly a very useful tool for the diagnosis of visceral leishmaniasis in clinical laboratories that lack the ability to identify parasites by morphological examination.

Conclusion

This study reports a case of visceral leishmaniasis without obvious febrile symptoms, which presented with the possibility of multiple false-positive antibodies, and ultimately relied on the rK39 dipstick and mNGS for a definitive diagnosis. According to this study, visceral leishmaniasis has symptomatic atypia, and high alert for VL is required when the patient does not have obvious fever but has a history of sojourn in endemic areas and splenomegaly. The high sensitivity of rK39 dipstick can be utilized for screening and mNGS for definite diagnosis.

Abbreviations

VL, Visceral leishmaniasis; LD bodies, Leishman-Donovan bodies; HCV-Ab, positive hepatitis C virus antibody; DAT, direct anti-human globulin test; mNGS, metagenomic next-generation sequencing; 2019-nCOV, 2019 novel coronavirus; CT, computed tomography; AVL, anthroponotic type; MT-ZVL, zoonotic mountain type; DT-ZVL, zoonotic desert type; CAS, cold agglutinin syndrome; AIHA, Autoimmune Hemolytic Anemia.

Consent for Publication

Written informed consent was obtained from the patient for publication of this case report. And the consent form is avail able for reviewing by the editor when needed. Details of the case can be published without institutional approval.

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Disclosure

The authors report no conflicts of interest in this work.

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