Visceral Leishmaniasis presenting with Hemophagocytosis and Myelodysplasia: A Case report and review of the literature

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Abstract
A 62-year-old patient presented with fever persisting despite antibiotic treatment, weight loss and sweating for a period of several weeks. On physical examinations hepatosplenomegaly was noticed. Increasing pancytopenia triggered bone marrow biopsy, showing expanded and dysplastic erythropoiesis, dysmegakaryopoiesis and hypoplastic granulopoiesis. The patient was diagnosed with low-risk myelodysplastic syndrome (MDS). Bone marrow cells displayed a normal karyotype. Infectious disease diagnostics were negative. During the course of the disease liver enzymes, d-dimers, laboratory markers of inflammation, coagulation parameters and pancytopenia worsened. Liver puncture revealed severe hemophagocytic syndrome. Treatment with corticosteroids and etoposide was initiated, but the patient continued to be febrile and failed to improve. After transfer to our department, bone marrow biopsy was repeated and visceral leishmaniasis was detected, further confirmed by serologic testing and PCR. The patient was treated with amphotericin B and fully recovered.

Conclusion: Leishmaniasis should be included in the differential diagnosis of myelodysplastic bone marrow failure accompanied by systemic inflammation, and should be recognized as a possible cause of hemophagocytic syndrome. Since leishmaniasis is not confined to tropical zones, but also occurs in the Mediterranean region, increasing travel activities, migration and climate changes may lead to a rising incidence of this disease in Europe.

Case Presentation
A 62-year old man presented in February 2017 with fever, sweating and weight loss (12 kg over two months). Initial symptoms, mainly fever and weakness, already started two months earlier and were attributed to a suspected pneumonia. Treatment with clarithromycin achieved only temporary improvement and eventually clinical symptoms indicated further deterioration. The patient did not report any comorbidities and negated the use of regular medications, drugs, alcohol or smoking.

As the patient spent most of his time on one of the Balearic Islands, but was also engaged in a lot of business travel, diagnostic tests, including bone marrow biopsy, were performed to rule out infectious diseases. There was no evidence of leishmaniasis and borreliosis. Since the patient had acquired malaria quartana thirty years earlier, a blood film was examined and reported to be suggestive of malaria. Therefore, he received treatment with chloroquine.
However, this medication was soon discontinued when a repeated blood film and a PCR test for malaria yielded negative results. Bone marrow cytomorphology showed dysplastic features of erythropoiesis and megalakaryopoiesis without elevated blast count suggesting a diagnosis of MDS-MLD (myelodysplastic syndrome with multilineage dysplasia).

In March 2017, the patient was admitted to the hematology/oncology department of another hospital, where the diagnostic workup for malignancies and infections did not yield a clear diagnosis. By that time, the patient had developed severe pancytopenia (white blood cell count 400/µl, hemoglobin 7.1 g/dl, platelets 45.000/µl) without evidence of hemolysis (normal haptoglobin). He still suffered from persistent fever, despite treatment with broad-spectrum antibiotics. No pathogens were detected in the blood or urine. Virological tests were negative for HIV, CMV, EBV, HAV, HBV, HCV and parvovirus B19. Serological tests for leishmaniasis were also negative.

Abdominal ultrasonography showed hepatomegaly (19 cm in MCL) and a CT scan of thorax and abdomen also confirmed splenomegaly (18 cm). Lymph nodes were not enlarged and other organs did not show any pathological findings. A CT scan of the neck and paranasal sinuses was inconspicuous. A second bone marrow biopsy showed hyperplastic and dysplastic erythropoiesis, dysmegakaryopoiesis and hypoplastic granulopoiesis with normal maturation. The blast count was not elevated, as assessed by cytomorphology and flow cytometry. On histopathology, only reactive changes were noted. Conventional cytogenetics showed a normal karyotype (46, XY [24]). Next generation sequencing with a myeloid panel covering 54 genes did not show any mutations. In particular, none were found in TP53, ASXL1, EZH2, NRRAS, KRAS, SRSF2, and SF3B1. Therefore, the suspected diagnosis of low-risk MDS remained solely, based on cytomorphological features of dysplasia.

In view of unclear hepatosplenomegaly, elevated liver enzymes, CRP, ferritin and d-dimers as well as an unexplained coagulopathy, a liver biopsy was performed in April 2017. On histopathology, activated sinusoidal macrophages were found, displaying phagocytosis of granulocytes and erythrocytes. Accordingly, hemophagocytosis or macrophage activation syndrome (MAS) was diagnosed.

The patient received high-dose dexamethasone for five days, followed by two doses of etoposide and stimulation of granulopoiesis with G-CSF for five days. After a brief increase the white blood cell counts rapidly decreased again.

Seeking a second opinion, the patient presented to our center in May 2017. His general condition had further deteriorated. On repeat bone marrow biopsy, we confirmed marked dyserythropoiesis (Figure 1) as well as hemophagocytosis. Strikingly, abundant leishmanias (including kinetoplasts) were detected in and outside of macrophages. Besides microscopic detection of leishmania (Figure 2), antibody testing and PCR on bone marrow material was performed in a reference laboratory, yielding was positive results with all methods. PCR of the parasitic cytochrome B-complex showed 99% accordance with Leishmania donovani and Leishmania infantum, confirming the diagnosis of visceral leishmaniasis.
Intravenous amphotericin B was administered for three cycles (five days per cycle, every ten days, at 3 mg/kg body weight per application) [2]. After the second cycle, pancytopenia and fever diminished. After the third cycle, the patient gained weight, was no longer fatigued and fully recovered. Subsequent to the inpatient treatment, he received two more cycles of amphotericin B as an outpatient. After the last round of amphotericin B in August 2017, bone marrow was reevaluated and showed neither hemophagocytosis nor leishmaniasis. Antibody testing remained positive, but PCR on bone marrow cells was negative. Therefore, amphotericin B was discontinued. Cytomorphologically, the bone marrow recovered completely, but mild pancytopenia persisted in the peripheral blood, probably due to lingering splenomegaly.

**Discussion**

Visceral leishmaniasis is a parasitic infectious disease occurring in tropical climate zones, but also endemically in the mediterranean region. About 400.000 people become infected per year, and up to 40.000 die of leishmaniasis every year, especially in regions where appropriate medical treatment is not available. The female phlebotomine sand fly is the vector that transmits the parasites by biting the victims. About 20 leishmania species are known to cause the infection, which can present as cutaneous (CL), mucocutaneous (MCL), or visceral leishmaniasis (VL). After the bite of the sand fly, which causes a dermal lesion, the leishmanias persist in the skin of the mammal and are being incorporated by cells of the mononuclear phagocyte system, where the leishmanias develop into amastigote forms and reproduce. Due to their adherence to macrophages, leishmanias can visceralize via the lymphatic system as well as spleen and liver, provoking severe hepatosplenomegaly. Especially the species of leishmania donovani and leishmania infantum are well known for high rates of visceralization. Visceralization of the bone marrow can lead to pancytopenia resulting in secondary immunosuppression, explaining the appearance of superinfections in patients with VL. Incubation of VL ranges from 2 weeks to 18 months or, in some cases, even years until first symptoms are recognized. The mortality rate of untreated VL is 75-95% within two years [1].

The history of our patient underlines the importance of considering leishmaniasis as a differential diagnosis even in countries where this infection is not endemic. Several factors may contribute to a rising incidence of leishmania infections in the near future, like increasing travel activities, migration, climate changes, HIV infections compromising the outcome, immunosuppression due to cytotoxic treatment and malnutrition [3,4].

Diagnostic tools for leishmania detection include microscopy (e.g., blood smears, lymph node, liver, spleen, or bone marrow), serologic testing and PCR. Leishmania detection on microscopy only succeeds in patients with a high disease burden. Serologic testing is not reliable, as it often remains negative despite an underlying infection and, if positive, does not differentiate between active and former infection. As in our patient, both IgM and IgG antibodies remain elevated for several months, even after successful treatment, and can thus not be used for treatment evaluation. Only the detection of the leishmania kinetoplast or RNA via PCR provides reliable information on disease status and therapeutic success. However, on a worldwide scale, this methodology is not easily accessible [4].

The delay between first symptoms and diagnosis in our patient is not unusual. Several serologic tests did not show leishmania-specific antibodies. The diagnosis was finally made, when leishmania were detected on bone marrow microscopy after the patient had received immunosuppression for hemophagocytic syndrome. Subsequently, the diagnosis was confirmed by PCR.

The MDS-like features in the bone marrow and the histopathological finding of hemophagocytosis in the liver did not help in making the correct diagnosis, since they suggested a hematological malignancy.

Leishmaniasis can present with a variety of symptoms and is therefore difficult to diagnose. To the best of our knowledge, this is the first case report of visceral leishmaniasis associated with hemophagocytosis in the liver. After hemophagocytosis had been detected in the liver of our patient, it was also found in the bone marrow together with cytomorphologic evidence of leishmaniasis. None of the previous case reports or studies described hemophagocytosis in the liver of patients with leishmaniasis [5-15]. There is a case report from Sweden alluding to hemophagocytosis in the spleen of a child with VL [5], without mentioning a liver biopsy. Experiments in mice by Morimoto et al. did not indicate hemophagocytes in the liver of these animals [6]. Therefore, our case report provides unique information regarding the possible occurrence of hemophagocytosis in both liver and bone marrow in patients with visceral leishmaniasis.

**Review of the literature**

The frequency of leishmania infections is increasing worldwide. A long incubation time together with busy international air travel may foster infections even in countries where the disease has not been endemic so far. Accordingly, leishmaniasis should be considered as a differential diagnosis in patients with splenomegaly and myelodysplasia, especially in patients who have travelled to...
tropical zones or mediterranean countries.

In conjunction with our case report, we reviewed the pertinent literature, focusing on the combination of leishmaniasis with hemophagocytosis or myelodysplasia.

**Leishmaniasis and hemophagocytosis:**

Several case reports point out that hemophagocytosis in the bone marrow can be caused by visceral leishmaniasis. Granert et al. described how this finding initially raised suspicion of hemophagocytic lymphohistiocytosis (HLH) in a pediatric patient before reevaluation yielded the diagnosis of visceral leishmaniasis [5]. Physicians in endemic regions, e.g., India, employ hemophagocytosis as a diagnostic clue to visceral leishmaniasis, and efforts have been made to classify the morphologic features in the bone marrow of such patients [7-9].

Chandra et al. and Dhingra et al. [8, 9] looked at both aspirates and biopsies and described increased cellularity, erythroid hyperplasia, and mild to severe hemophagocytosis. Chandra et al. detected uncommon bone marrow manifestations such as granuloma and necrosis, and reported an association with worse prognosis, based on their clinical experience [8]. Both findings were also reported by Bhatia et al. [7]. Experiments by Cotterell et al. [10] showed that stromal macrophages serve as a target for Leishmania donovani. Infection of macrophages then induces secretion of granulocyte macrophage-colony stimulating factor (GM-CSF) and tumor necrosis factor-alpha (TNF-alpha), thereby contributing to increased bone marrow cellularity.

**Leishmaniasis and myelodysplasia:**

Besides hemophagocytosis, nonclonal myelodysplasia may contribute to ineffective hematopoiesis and peripheral cytopenia in visceral leishmaniasis [11]. We found reports of pediatric and adult patients presenting with myelodysplasia secondary to visceral leishmaniasis [12-14]. The case reports describe trilineage dysplasia and emphasize bone marrow hypercellularity and erythroid hyperplasia as the most striking features [13,14]. Megaloblastic changes in erythroblasts have also been reported [13]. Dhingra et al. point out that nonclonal myelodysplasia can affect all three myeloid lineages and might be caused by increased TNF-alpha [9].

Regarding granulopoietic precursor cells both hypo- and hypergranululation were observed. In addition, increased megakaryopoiesis and nuclear dysplasia such as nuclear fragmentation or nonlobated nuclei were reported by several authors [12-14]. Despite distinct signs of myelodysplasia, intra- or extracellular leishmania parasites were not seen in every case, challenging the diagnosis of visceral leishmaniasis. It has been suggested that disease duration has a more profound impact on cytomorphological changes in the bone marrow than the number of infected mononuclear cells [15]. Therefore, Varma et al. recommend repeat bone marrow examination and serologic testing in patients with suspected visceral leishmaniasis. Serologic testing helps to identify patients with early stages of VL, whereas bone marrow microscopy can serve as a diagnostic tool in patients with more advanced infection.

**Conclusion**

Similar to the course of disease in our patient, several reports in the literature describe patients where visceral leishmaniasis was initially misdiagnosed as myelodysplastic syndrome. Such cases were mainly reported from non-endemic regions. In contrast, physicians in endemic areas like India employ myelodysplastic features as a clue to underlying infection such as VL. Both hemophagocytosis and myelodysplasia can be found in association with visceral leishmaniasis. Treatment with amphotericin B is effective and can rapidly cure the patient. Therefore, we recommend to consider visceral leishmaniasis as a possible cause of myelodysplasia and/or hemophagocytosis even in non-endemic regions, and to repeat diagnostic procedures (serologic testing as well as bone marrow examination) if the disease is suspected [16].

**Acknowledgements:** No financial or material support was implied in the preparation of this manuscript.

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DOI: 10.55920/JCRMHS.2023.03.001121


