Severe visceral leishmaniasis and COVID-19 coinfection in an immunosuppressed patient

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ABSTRACT

Visceral leishmaniasis (VL) is an opportunistic disease in immunosuppressed individuals, who may present severe clinical conditions, such as the ones described in this patient. She lived in an endemic region for VL, and was possibly infected with *L. (L.) infantum chagasi* through the bite of a contaminated sand fly. This initial infection has triggered a pemphigus vulgaris condition by immunogenic proteins present in the mosquito’s saliva. The immunosuppression caused by the use of high doses of corticosteroids to control the disease promoted a severe VL condition, with hepatosplenomegaly, thrombocytopenia and hemorrhages, requiring hospitalization and the onset of a subsequent SARS-CoV-2 infection. Due to the intensity of clinical manifestations related to VL, aggravated by COVID-19, she died two days after admission to the Clinical Hospital of Marilia Medical School (HC–Famema).

KEYWORDS: Visceral leishmaniasis. COVID-19. Immunosuppression. Pemphigus vulgaris.

INTRODUCTION

The flagellate protozoan *Leishmania (L.) infantum chagasi* is the etiologic agent of visceral leishmaniasis (VL) in the American continent. The parasite has a heteroxenic cycle, involving female mosquitoes of the *Lu. longipalpis* species belonging to genus *Lutzomyia*, as vectors. The disease is endemic in this continent, being found in 13 countries, of which in 2019, Brazil accounted for 97% of the cases, followed by Paraguay, Venezuela, Colombia and Argentina. Subclinical forms of VL are observed in the vast majority of *L. (L.) infantum chagasi* infected individuals with no signs and symptoms, however, about 20% of them will present with fever, dry cough, hepatosplenomegaly, thrombocytopenia and hemorrhages, requiring hospitalization and the onset of a subsequent SARS-CoV-2 infection. Due to the intensity of clinical manifestations related to VL, aggravated by COVID-19, she died two days after admission to the Clinical Hospital of Marilia Medical School (HC–Famema).

VL is an opportunistic disease in immunosuppressed individuals as much as other medical conditions that require the use of immunosuppressive drugs, such as corticosteroids and TNF-α antagonists. These drugs impair the synthesis of cytokines triggered by TH1 lymphocytes, which are essential for the control of *L. (L.) infantum chagasi* infection.

In VL endemic regions, immunosuppressed individuals are at higher risk of death when they are coinfected with SARS-CoV-2, progressing with aggravation...
of the clinical conditions⁶. In these patients, depression of the immune system hinders the inflammatory response, with poor induction of IFN-γ synthesis in response to the viral infection⁶, as in the case presented here. This study was approved by the Ethics Committee on Research with Humans from Marilia Medical School-Famema (CAAE: 49990321.5.0000.5413).

CASE REPORT

A 41-years-old female patient, was a resident in a city located in the Midwest of Sao Paulo State, an endemic region for VL. She was referred to the HC-Famema presenting with increased abdominal volume, skin, oral and vaginal bleeding. In the past history, she had been suffering from rheumatoid arthritis for 20 years, and made regular used of methotrexate and leflunomide. However, 60 days before the referral to our service, she sought medical care in her city of origin due to abdominal pain, skin and oral mucosa lesions. The diagnostic hypothesis was pemphigus vulgar, and she was prescribed high doses of corticosteroids for two months. Nevertheless, the patient evolved with a decline of general conditions and an increase in the abdominal volume associated with jaundice, requiring hospitalization approximately 50 days after the onset of treatment. The exams showed anemia, thrombocytopenia and alterations in the hepatic profile. She progressed with worsening of clinical conditions and laboratory tests and splenomegaly, mild hepatomegaly and ascites detected by the abdominal ultrasound. On the tenth day of hospitalization, as a result of the increased abdominal volume, she developed skin bleeding and bleeding from the oral and vaginal cavity, and she was transferred to the HC-Famema. On admission, the patient had jaundice, Glasgow 14, ecchymosis on the upper limbs, active bleeding in the oral cavity, pulmonary auscultation with wheezing in the left hemithorax, semiglobular and flaccid abdomen, palpable liver at 4 cm from the right costal margin and Traube’s space occupied. Edema of the lower limbs and circular lesion measuring 6 cm in diameter of healing process on the left knee with delimited borders, fibrinous and shiny bottom. Chest computed tomography (Figure 1) showed peripherally distributed ground-glass attenuation, mostly associated with thickening of the interlobular septa and subpleural lines, with a semiquantitative analysis of 75% of lung parenchyma involvement, being then transferred to the Intensive Care Unit. The Reverse transcription Polymerase chain reaction (RT-PCR) on nasopharyngeal swabs was positive for SARS-CoV-2. Laboratory tests showed changes in the liver function, blood glucose and blood cell count (Table 1).

On the blood smear performed together with the blood cell count, the presence of numerous free and intracellular amastigote forms of Leishmania spp (Figure 2) was observed. The rapid test for visceral leishmaniasis was positive, with confirmation of the species L. (L.) infantum chagasi by PCR and restriction fragment length polymorphism (RFLP)⁷ (Figure 3). Ten hours after the patient’s admission to our service, there was a lowering of the consciousness level condition and a significant drop in the O₂ saturation, requiring orotracheal intubation, with progressive clinical worsening, leading to death after two days of hospitalization.

DISCUSSION

The diagnosis of VL in immunosuppressed patients and residents of an endemic area is of fundamental importance to avoid lethality. According to Galvão-Castro et al.⁸,

![Figure 1 - Computerized tomography (CT) of the chest without contrast. Multislice helical tomography of the chest and mediastinum with 5 mm slices and axial, sagittal, coronal and 3D multiplanar reconstructions, whose images show extensive lung lesions and moderate pleural effusion on the left.](image-url)
Table 1 - Results of laboratory tests performed on the patient's admission to the Clinical Hospital of Marilia Medical School (HC-Famema).

| Laboratory tests                  | Result  | Normal range          |
|-----------------------------------|---------|-----------------------|
| **Liver function test**           |         |                       |
| Direct Bilirubin                  | 6.69 mg/dL | Up to 0.40 mg/dL     |
| Indirect Bilirubin                | 1.44 mg/dL | Up to 0.80 mg/dL     |
| Alkaline phosphatase (ALP)        | 150 U/L | 50 - 150 U/L          |
| Gamma-glutamyltransferase (GGT)   | 127 U/L | 9 - 36 U/L*           |
| Aspartate transaminase (AST)      | 241 U/L | 15 - 37 U/L           |
| Alanine transaminase (ALT)        | 104 U/L | 12 - 78 U/L           |
| **Blood cell count**              |         |                       |
| Red blood cells                   | 2.99 million/mm³ | 4.3 ± 0.5*           |
| Leukocytes                        | 13,610/mm³ | 7,000 ± 3,000*       |
| Myelocytes (%)                    | 1       | 0                     |
| Metamyelocytes (%)                | 1       | 0                     |
| Band neutrophils (%)              | 15      | 2-4                   |
| Segmented neutrophils (%)         | 66      | 36-66                 |
| Eosinophils (%)                   | 0       | 2-4                   |
| Basophils (%)                     | 0       | 0-1                   |
| Lymphocytes (%)                   | 12      | 25-45                 |
| Monocytes (%)                     | 5       | 2-10                  |
| Platelets                         | 19,000/mm³ | 150,000 - 400,000 mm³*|

**Other serum tests**

| Result | Normal range          |
|--------|-----------------------|
| Blood Glucose | 16 mg/dL | 75 - 99 mg/dL       |
| C-Reactive Protein (CRP) | 135.5 mg/L | Less than 5.0 mg/L   |

*Hematological reference values for adult women.

Figure 2 - Amastigote forms of *L. (L.) infantum chagasi* in peripheral blood (red arrows); A) Free amastigote forms; B and C) Amastigote forms phagocytosed by monocytes; D) Amastigote forms phagocytosed by neutrophils.
The etiology of autoimmune diseases is multifactorial and can be influenced by genetic, hormonal, immunological and environmental factors. It seems likely that they were fundamental in the process of development of pemphigus vulgaris in this patient, who was susceptible to autoimmune diseases and had already the diagnosis of rheumatoid arthritis.

In this context, as the patient lived in an endemic area, she was infected with *L. (L.) infantum chagasi* possibly through the bite of a contaminated sand fly, triggering the pemphigus vulgaris condition. Researches by Chiossi and Roselino, Flores *et al.* and Qian *et al.* showed that immunogenic proteins, Maxadilan and LJM11, present in *Lutzomyia longipalpis* saliva would be related to the production of autoantibodies against transmembrane glycoproteins of desmosomes, called desmoglein 1 (Dsg1), leading to the development of this disease.

Thus, immunosuppression caused by the use of high doses of corticosteroids to control the disease, has triggered a severe VL condition. The severity of the disease has been proven by the intense hepatic impairment and thrombocytopenia, resulting in hemorrhage, leading to the hospital admission. Presumably, the immunological response induced by visceral leishmaniasis increased turned patient’s susceptibility to the subsequent SARS-Cov-2 infection acquired during the hospitalization period in her city of origin, since the average incubation time of this disease is 3 to 7 days.

Studies by Viana *et al.* showed that the TH1 response with production of IFN-γ, TNF-α and IL-10 by neutrophils and TNF-α by monocytes is important for effective control of parasitic multiplication. The absence of a cellular immune response was crucial in the aggravation of leishmaniasis, favoring the activation of TH2 cells and the disease progression.

Consequently, the deficient inflammatory response reduced IFN-γ synthesis, and IFN-γ is necessary for an antiviral response, triggering a severe case of COVID-19 in addition to promoting the multiplication and dissemination of the parasite and involvement of multiple organs such as lymph node, bone marrow, spleen, liver and more rarely peripheral blood, as seen in this patient.

The diagnosis of VL was defined late, through the analysis of a peripheral blood smear performed together with blood cell count, what was surprising as peripheral blood is biological material of low sensitivity for the diagnosis of AVL, since parasitemia in immunocompetent individuals is low. However, in this case, numerous free and intracellular amastigote forms were observed inside neutrophils and monocytes, a fact that has not been reported in the literature in the context of the diagnosis of AVL in humans.

On admission of the patient in our service, she had already shown a serious COVID-19, with a high viral load detected by RT-PCR and pulmonary involvement evidenced by CT scan. Miotti *et al.* reported the first case of leishmania and SARS-CoV-2 coinfection, with fatal outcome, like the case presented here. However, due to the short period of hospitalization in our service and the late diagnosis of VL, it was not possible to initiate a treatment.

As a consequence of the serious manifestations triggered by the two infections, the patient died two days after being admitted to our hospital.

**CONFLICT OF INTERESTS**

The authors report that there is no conflict of interests.

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