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Differential diagnosis of visceral leishmaniasis in children: a five-year retrospective study at a pediatric referral hospital

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Abstract

Background Visceral leishmaniasis (VL) is a zoonotic disease caused by protozoa of the genus *Leishmania* and is transmitted by sandflies of the genus *Lutzomyia*. Children under 15 years are disproportionately affected. In pediatric patients, the clinical and laboratory features of VL often overlap with those of other infectious and hematology-oncology diseases, making differential diagnosis challenging. Rapid and accurate identification of VL is critical for effective treatment. This study aimed to evaluate the epidemiological, clinical, and laboratory characteristics of pediatric patients initially suspected of having VL and to compare their final diagnoses upon discharge from a referral hospital.

Methods We retrospectively analyzed medical records of children with suspected VL, admitted between July 2014 and June 2019.

Results Infectious diseases were confirmed in 61% of cases (86 patients), with VL confirmed in 55 cases. Hematology-oncology diseases were the second most common diagnosis, affecting 22.7% of patients (32 cases). Comparisons between the VL-confirmed group and those with other diagnoses revealed no significant age difference ($p = 0.690$). However, female sex, spleen size, and leukopenia were identified as significant predictors of VL.

Conclusions Female sex, spleen size, and leukopenia were key predictors for differentiating VL from other pediatric diseases in a referral center in the Northeast Region of Brazil.

Keywords Visceral leishmaniasis, Children, Neglected diseases, Differential diagnosis

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Background

Visceral leishmaniasis (VL) is a zoonotic disease caused by at least 20 protozoan species of the genus *Leishmania*. In Brazil, the main species responsible for VL is *Leishmania infantum*, which is transmitted by sandflies of the genus *Lutzomyia* [1].

According to Sundar and Chakravarty, more than 90% of global cases of VL occur in the following six countries: India, Bangladesh, Sudan, South Sudan, Brazil, and Ethiopia [2]. In Brazil, more than 70,000 official notifications and more than 3,800 deaths have been recorded over the past 30 years [3]. Of all parasitic diseases, leishmaniasis is second only to malaria in terms of mortality; in terms of disability-adjusted life years, it is the third most common cause of morbidity after malaria and schistosomiasis. Children under 15 years of age are disproportionately affected [4].

VL is a long-lasting febrile disease, and the clinical signs are nonspecific, characterized by fever, pallor, weight loss, increased abdominal volume, hepatosplenomegaly, and edema. Due to the nonspecific signs and symptoms, VL can be diagnosed in a late manner, leading to delayed treatment, which worsens the prognosis of the disease [5].

The most frequent non-specific laboratory abnormalities are anemia, thrombocytopenia, leukopenia, pancytopenia, and neutropenia. Hypergammaglobulinemia is present in the vast majority of cases, and aspartate aminotransferase and alanine aminotransferase may be elevated [6].

The Brazilian Ministry of Health considers patients from endemic areas for VL who have fever associated with splenomegaly as suspected cases of VL. Cases are considered confirmed when the parasite is found in direct exams or bone marrow aspirate culture, when serological tests are positive, or even when a patient with clinical suspicion shows a therapeutic response to antileishmania medications [7].

In the pediatric age group, there are several other pathologies whose clinical and laboratory evolution is similar to VL. Examples include hematology-oncology diseases, such as acute leukemias, hemophagocytic lymphohistiocytosis, and myelodysplastic syndromes; infectious diseases, such as malaria; certain viruses, such as HIV, parvovirus B19, influenza A, and Epstein-Barr virus; and bacterial diseases, such as severe sepsis, liver abscess, and tuberculosis [8].

Performing differential diagnosis between these pathologies within a short time frame is a challenge for pediatricians, given that some of these diseases are potentially fatal if they are not treated during the first weeks. This study aims to analyze the differences between children with confirmed VL and those with initial suspicion of VL upon admission to a pediatric referral hospital in the Northeast Region of Brazil, with the goal of identifying key characteristics that differentiate these two groups.

Methods

This study was conducted retrospectively for a period of 5 years (July 2014 to June 2019), in the pediatric ward of a tertiary hospital in the city of Petrolina, state of Pernambuco, Brazil, which is a referral hospital for the central region of the São Francisco Valley. The present study was approved by the Research Ethics Committee of the Universidade Federal do Vale do São Francisco under protocol CAAE: 00285418.6.0000.5196. This study used secondary data; therefore, the participants' consent form was waived by the ethics committee.

Analysis was conducted of medical records of all children admitted during the study period, who had been notified as cases of VL by the hospital's epidemiology unit. Data such as age, sex, city of origin, total days of fever, hepatomegaly, splenomegaly, anemia (classified as mild: hemoglobin 9.0–10.9 g/dL, moderate: hemoglobin 7.0–8.9 g/dL, and severe: hemoglobin less than 7.0 g/dL), leukopenia (defined as leukocyte count below 5000 per μL), and thrombocytopenia (defined as fewer than 150,000 platelets/μL) were extracted from the medical records. Pancytopenia was diagnosed when all 3 cell lines were reduced. Additionally, blood cultures and serological tests for possible infectious agents associated with cytopenias were performed depending on the patients' clinical suspicion, including tests for antibodies to viruses such as parvovirus B19, enterovirus, Epstein-Barr virus, and cytomegalovirus. Bone marrow aspiration was carried out in patients with clinical suspicion of myeloproliferative diseases, such as acute leukemia, or in order to confirm leishmaniasis. For the diagnosis of VL, an immunochromatographic test (IT-Leish®) was used, which allows the detection of antibodies against Leishmania spp. using the rk39 antigen. The test, supplied by the Brazilian Ministry of Health, has a sensitivity of 93% and specificity of 97% [9]. Statistical analysis was carried out using SPSS-PC for Windows (version 17.0; SPSS Inc, Chicago, IL, USA).

Results

One hundred and forty-one medical records of children with suspected diagnosis of VL from July 2014 to June 2019 were analyzed. Approximately 61% (87 patients) were diagnosed with some infectious disease. VL was confirmed in 55 cases (39%). After infectious diseases, the most diagnosed group of diseases was hematology-oncology diseases, accounting for 33 patients (23.4% of

those notified). The most frequently diagnosed hematology-oncology disease was leukemia, with 15 cases (Fig. 1).

Table 1 shows the main clinical and laboratory characteristics of confirmed and unconfirmed cases of VL. The age of the groups was observed to be similar ($p=0.690$),

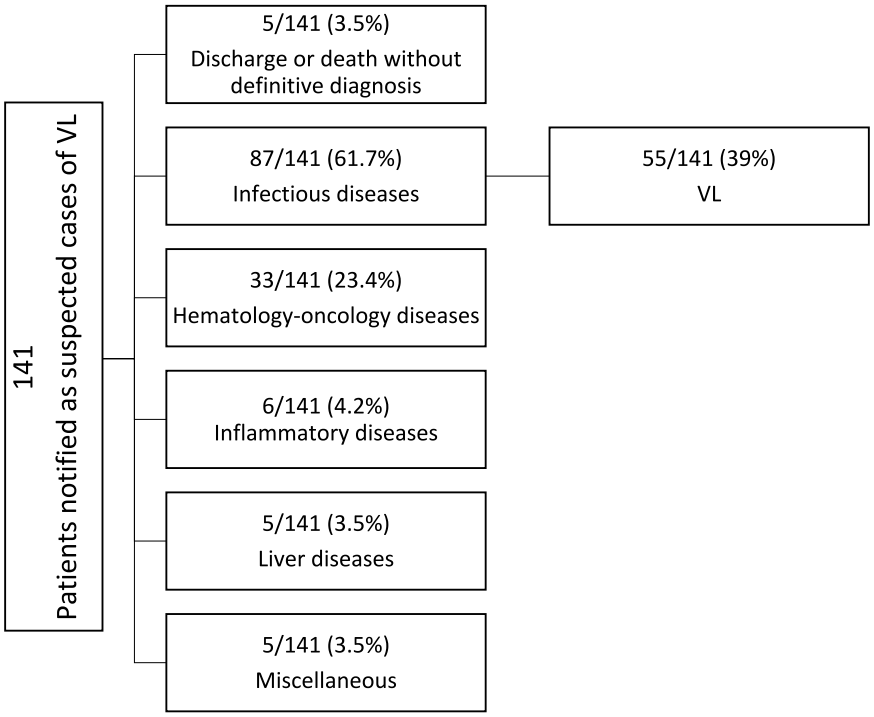

Fig. 1 Flowchart with differential diagnoses of cases included in the study. Note. VL: visceral leishmaniasis

Table 1 Comparison between children with and without visceral leishmaniasis admitted to the Dom Malan/IMIP Hospital between 2015 and 2019

Variables	Non-VL ($N=86$)	VL ($N=55$)	*p*
Age (years)	3.0 (0.0 – 14.0)	2.0 (0.0 – 12.0)	0.690
Sex			
Male	53 (61.6%)	22 (40.0%)	**0.015**
Female	33 (38.4%)	33 (60.0%)	
Fever	72 (83.7%)	53 (96.4%)	**0.028**
Fever (days)	6 (0 – 180)	15 (0 – 120)	**0.000**
Anemia			**0.008**
Absent	11 (12.8%)	2 (3.7%)	
Mild	14 (16.3%)	1 (1.9%)	
Moderate	31 (36.0%)	28 (51.9%)	
Severe	30 (34.9%)	23 (42.6%)	
Hemoglobin (g/dL)	8.0 ± 2.8	7.3 ± 2.1	0.126
Leukopenia	27 (31.8%)	44 (81.5%)	**0.000**
Leukocytes (counts/ml)	7,100 (1,200 – 72,000)	3,290 (216 – 10,600)	**0.000**
Thrombocytopenia	50 (58.8%)	48 (90.6%)	**0.000**
Platelet count (counts/ml)	130,000 (2,700 – 1,117,000)	83,000 (7,000 – 601,000)	**0.005**

Mild anemia (hemoglobin 9.0–10.9 g/dL), moderate anemia (hemoglobin 7.0–8.9 g/dL), and severe anemia (hemoglobin less than 7.0 g/dL); Leukopenia: leukocytes < 5000/ ml; thrombocytopenia: < 150,000 platelets/ ml; *VL* Visceral leishmaniasis

but the female sex was more prevalent in the VL group (60.0% versus 38.4%; $p=0.015$). Fever was the most commonly reported symptom in all groups. This complaint was reported in 83.7% of patients in the non-VL group and in 96.4% of patients with VL ($p=0.028$). The time from onset of fever to arrival at the referral hospital showed an important correlation with the diagnosis of VL; patients with VL had a median fever time of 15 days upon arrival at the hospital, while patients without VL had a median of 6 days ($p=0.000$). Furthermore, patients with VL showed a higher frequency of moderate to severe anemia when compared to the non-VL group ($p=0.008$).

In relation to physical abdominal examination, a higher frequency of hepatomegaly and splenomegaly was observed in the group with VL; this group also showed larger liver and spleen size ($p<0.05$).

With respect to laboratory data, the group with VL showed lower leukocyte and platelet counts ($p<0.05$). Among those with VL, pancytopenia was observed in 37 (67.2%) patients and bicytopenia in 13 (23.6%). Hemoglobin levels did not show any statistically significant differences between groups.

With the aim of identifying variables independently associated with VL, a multivariate logistic regression model, shown in Table 2, was generated by inserting the variables that showed statistical significance in Table 1.

Among the variables included in the model, only sex (female), spleen size, and leukopenia were shown to be predictive factors for differential diagnosis of VL ($p<0.05$). Patients with larger spleen size, female patients, and patients with leukopenia were 1.32, 2.91, and 6.32 times more likely to have VL, respectively.

Discussion

This study analyzed the clinical, epidemiological, and laboratory characteristics and the final diagnoses of patients with suspected VL treated at a referral center in the interior of the Brazilian state of Pernambuco. Of the notified cases, 55 (39%) were confirmed as VL. Other infectious and hematology-oncology diseases represented the other final diagnoses of cases initially suspected as VL.

Table 2 Multivariate logistic regression analysis for identification of predictors of occurrence of visceral leishmaniasis

	Occurrence of VL	
	OR (95% CI)	*p*
Sex (female)	2.91 (1.08–7.83)	**0.034**
Liver size (cm)	1.09 (0.90–1.31)	0.347
Spleen size (cm)	1.32 (1.14–1.54)	**0.000**
Fever (days)	1.00 (0.99–1.02)	0.374
Moderate to severe anemia	2.37 (0.50–11.08)	0.273
Leukopenia	6.32 (2.21–18.11)	**0.001**
Thrombocytopenia	3.56 (0.85–14.81)	0.080

CI Confidence interval, *OR* Odds ratio

The diagnosis of VL in pediatric patients represents a challenge in clinical practice, due to its similarity to other diseases. VL has clinical manifestations and laboratory alterations that largely overlap with other lymphoproliferative diseases and hematological disorders [10]. In a 2012 study carried in Greece out by Alexandropoulou et al., including 112 children with febrile neutropenia, 67% were due to infectious causes, and 9 were diagnosed with VL (8%) [8]. The greater number of confirmed cases of VL in our study reflects the hyperendemic character of the region. Andrade et al. conducted a study in the same region in 2020, showing an incidence of 1.9 cases per 100,000 inhabitants in the state of Pernambuco in 2017 [11]. In the city of Petrolina, this incidence was even higher, according to a 2018 study by Diniz et al., showing 6.1 cases of VL per 100,000 inhabitants [12]. This regional case series is closer to the situation in Sudan, where, in 2016, Ahmed et al. found 32.4% of suspected cases of VL to be positive [13].

In our study, confirmed cases of VL were more frequent in the female sex. This result is divergent from the 2010 study by Sampaio et al., which found an equal frequency of male and female sex in a sample of 546 children with suspected VL in a capital city in the Northeast Region of Brazil [14]. A 2017 study carried out in Tunisia by Helel et al. showed a slightly higher frequency in boys than in girls (50.4% versus 49.6%), in a sample of 230 children with VL [15]. A study conducted in Piauí, another state in the Northeast Region of Brazil, also showed a higher frequency in the male sex, but there was a greater association of deaths related to the female sex [16]. This factor was not found in our study, perhaps due to smaller sampling and less severity of patients upon arrival at the hospital. On the other hand, a recent study carried out in northeastern Italy with pediatric patients with confirmed VL found a higher prevalence of females (56.2%), corroborating our findings [17]. This divergence with the literature in relation to sex may be due to the fact that the hospital is a tertiary referral center in a macroregion of 55 municipalities, not only for infectious diseases, but also for hematology-oncology and inflammatory diseases, thus leading to a reference sample of patients. Additionally, biological or immunological factors or even sociocultural issues, such as greater demand for health services for girls, could also explain this difference. Further studies are needed to confirm this hypothesis.

Patients with VL showed a higher frequency of leukopenia and thrombocytopenia compared to those in the group without VL. In addition, the rates of pancytopenia

and bicytopenia were 67.2% and 23.6%, respectively, in VL-infected individuals. Pancytopenia varies in frequency and severity in VL patients, but it is usually associated with prolonged illness [18]. These cytopenias are typical in patients with VL, given that they have a multiplication of macrophages of the mononuclear phagocytic system, leading to a progressive reduction in the production of red blood cells, granulocytes, and platelets [18]. On the other hand, in other diagnoses, such as viral and bacterial infections, leukocytosis with normal platelets or thrombocytosis were predominant, which contribute to this statistically significant difference.

Fever is an important symptom of VL, cited in all studies that address the clinical characteristics of the disease in children. For example, in the study by Krepis et al., 98% of children reported having fever, with an average duration of 8 days [19]. In our study, fever was the most common clinical sign, both in the VL group (96.4%) and the non-VL group (83.7%). There was a difference between the groups in relation to the duration of the fever, which was more prolonged in patients with confirmed diagnosis of VL (15 days) than in those in the non-VL group (6 days; $p=0.000$).

According to the Visceral Leishmaniasis Control and Surveillance Manual of the Brazilian Ministry of Health, VL, in its illness phase, "is characterized by irregular fever, usually associated with progressive weight loss, cutaneous-mucosal pallor, and increased hepatosplenomegaly" [20]. In our study, fever, moderate to severe anemia, hepatomegaly, and splenomegaly were important findings associated with VL. In the same manner, the Iranian study by Naeem et al. showed fever, pallor, weakness, loss of appetite, splenomegaly, and hepatomegaly among the main clinical and laboratory findings of children with VL, thus confirming a similar evolution of the disease in different parts of the world [21].

Most patients with VL had splenomegaly and hepatomegaly. These clinical findings are frequent in pediatric patients with VL, given that it is a reticuloendothelial system disease, and they are important for establishing differential diagnosis. In the Brazilian study by Pedrosa and Rocha in 2004, the clinical manifestations most frequently found on admission were hepatomegaly, splenomegaly, fever, and pallor, similarly to the current study [22]. The authors also highlighted a correlation between the liver and spleen size and the disease duration; patients with shorter disease duration (< 30 days) showed smaller viscera size than patients who were ill for a longer time (> 360 days), demonstrating the continuous aggression to the organism when initiation of treatment is delayed.

Following multivariate regression analysis, it was observed that only the variables of sex, spleen size, and leukopenia were statistically significant in differential diagnosis of VL. Studies addressing the differential diagnosis of patients with suspected VL are rare in the literature. A study conducted with this approach in Greece provided evidence that, in children with febrile neutropenia, severe thrombocytopenia would be a predictive factor for VL, which is different from the finding of our study, and that leukopenia would be useful to differentiate bacterial diseases from parasitic ones [23]. This difference between studies may be associated with the fact that we found several patients with a final diagnosis of hematology-oncology diseases, such as acute lymphoid leukemia, which also occur with severe thrombocytopenia but not leukopenia. After external causes, neoplasms are the second leading cause of death for young people between 1 and 19 years of age in Brazil, and they should always be considered for differential diagnosis of children suspected of having VL [24].

This study has limitations, including the use of secondary data, the size of the sample, and the fact that it is a single-center study. Nevertheless, it is important to note that we used a time interval of five years and included all suspected patients admitted during this period. Given that it was conducted in a referral hospital, this study makes an important contribution to the differential diagnosis of VL in severe pediatric cases in a hyperendemic region of Northeastern Brazil.

Conclusions

The variables of female sex, spleen size, and leukopenia were shown to be significant predictors for differential diagnosis of VL in a referral hospital in the Northeast Region of Brazil. Further studies are needed to validate these parameters in other cohorts of critically ill pediatric patients suspected of having VL.

Abbreviations

VL Visceral leishmaniasis

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Not applicable.

Authors' contributions

AGRS and RFC conceived the study. JSL and NGF obtained the data. AGRS and JSL wrote the first draft of the manuscript. EBAF and RFC did the statistical analysis. AGRS, EBAF and RFC interpreted the data. All authors read and approved the final manuscript.

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Data availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The present study was approved by the Research Ethics Committee of the Universidade Federal do Vale do São Francisco under protocol CAAE:

00285418.6.0000.5196. This study used secondary data; therefore, the participants' consent form was waived by the ethics committee.

Consent for publication
Not applicable.

Competing interests
RFC is Senior Editor at BMC Series.

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