REVISTA DO INSTITUTO DE MEDICINA TROPICAL DE SÃO PAULO
JOURNAL OF THE SÃO PAULO INSTITUTE OF TROPICAL MEDICINE

CASE REPORT
http://doi.org/10.1590/S1678-9946202365036

What else in times of COVID-19? The role of minimally invasive autopsy for the differential diagnosis of acute respiratory failure in a case of kala-azar

João Carlos Geber-Júnior[1], Renata Aparecida de Almeida Monteiro[2], João Wilson Pedro da Rocha[1], Edson Luiz Társia Duarte[3], Elizabete Nicodemo[3], Olavo Munhoz[3], Edison Ferreira de Paiva[1], Thais Mauad[2], Luiz Fernando Ferraz da Silva[2], Paulo Hilario Nascimento Saldiva[2], Marisa Dolhnikoff[2], Amaro Nunes Duarte-Neto[2]

ABSTRACT

Visceral leishmaniasis (VL) is a chronic vector-borne zoonotic disease caused by trypanosomatids, considered endemic in 98 countries, mainly associated with poverty. About 50,000–90,000 cases of VL occur annually worldwide, and Brazil has the second largest number of cases in the world. The clinical picture of VL is fever, hepatosplenomegaly, and pancytopenia, progressing to death in 90% of cases due to secondary infections and multi-organ failure, if left untreated. We describe the case of a 25-year-old female who lived in the metropolitan area of Sao Paulo, who had recently taken touristic trips to several rural areas in Southeastern Brazil and was diagnosed post-mortem. During the hospitalization in a hospital reference for the treatment of COVID-19, the patient developed acute respiratory failure, with chest radiographic changes, and died due to refractory shock. The ultrasound-guided minimally invasive autopsy diagnosed VL (macrophages containing amastigote forms of Leishmania in the spleen, liver and bone marrow), as well as pneumonia and bloodstream infection by gram-negative bacilli.

KEYWORDS: Visceral leishmaniasis. Kala-azar. Pneumonia. Autopsy. Minimally invasive autopsy.

INTRODUCTION

Visceral leishmaniasis (VL), also known as kala-azar, is a chronic vector-borne zoonotic disease caused by protozoa from the genus *Leishmania* and is considered a neglected tropical disease (NTD), endemic in 98 countries in Africa, America, Asia, and Europe[1]. It is estimated that about 50,000–90,000 cases of VL occur annually worldwide. In 2018, 95% of the cases occurred in only ten countries: Brazil, China, Ethiopia, India, Iraq, Kenya, Nepal, Somalia, Sudan and South Sudan[2]. VL has already been identified in 12 Latin American countries[3].

The etiologic agent of leishmaniasis is *L. donovani* in Asia and Africa, and *L. infantum* in the Mediterranean Basin, the Middle East, Central Asia, South America and Central America[1]. The disease mainly affects populations in poverty and can be fatal (90%) if there is a delay in diagnosis and initiation of treatment.

Since the first description of VL in South America in 1913, it has been considered one of the most important endemic diseases in Brazil[4], where it is also considered a reemerging disease, accounting for 97% of the cases reported

[1]Universidade de São Paulo, Faculdade de Medicina, Departamento de Clínica Médica, São Paulo, São Paulo, Brazil

[2]Universidade de São Paulo, Faculdade de Medicina, Departamento de Patologia, São Paulo, São Paulo, Brazil

[3]Universidade de São Paulo, Faculdade de Medicina, Divisão de Moléstias Infecciosas e Transmissíveis, São Paulo, São Paulo, Brazil

Correspondence to: Amaro Nunes Duarte-Neto
Universidade de São Paulo, Faculdade de Medicina, Departamento de Patologia, Av. Dr. Arnaldo 455, Sala 1161, Cerqueira César, CEP 01246903, São Paulo, SP, Brazil
Tel: +55 11 30617322

E-mail: amaro.ndneto@hc.fm.usp.br

Received: 16 December 2022

Accepted: 20 April 2023

in the Americas[2]. In 2014, a total of 3,453 cases of VL were confirmed in the country, with a lethality rate of 6.7% and related medical costs of almost US$ 2 million for the Brazilian public health system – 40% and 22% of this amount allocated for hospitalizations and treatment, respectively[5]. The clinical hallmark of VL is the classic triad of fever, hepatosplenomegaly and pancytopenia[6]. However, the clinical course can be variable and even challenging for some experienced physicians, as it depends not only on the parasite burden, but also on the host-pathogen interaction. We report a case of a young woman who traveled around rural areas of Southeastern Brazil and acquired visceral leishmaniasis, which took a long time to be diagnosed. She was hospitalized in the Hospital das Clinicas of the Faculdade de Medicina da Universidade de Sao Paulo (HCFMUSP) during the COVID-19 pandemic presenting febrile hepatosplenomegaly and died due to acute respiratory insufficiency. The ultrasound guided-minimally invasive autopsy (MIA-US) diagnosed kala-azar and suppurative bacterial pneumonia.

CASE REPORT

A 25-year-old female who was referred to a rheumatologic outpatient clinic, complaining of fever for 8 months, initially daily but changing to an irregular pattern along the course, predominantly in the afternoon, and associated with arthralgia in small and large joints, malaise, pallor, increased abdominal volume, and involuntary weight loss (20 kilograms during the period). The patient lived in the metropolitan area of Sao Paulo. Laboratory tests showed pancytopenia associated with increased erythrocyte sedimentation rate and C-reactive protein, polyclonal gammopathy, and a weakly positive rheumatoid factor. The abdominal tomography showed great splenomegaly (reaching > 26 cm in greater diameter) and hepatomegaly. A bone marrow aspirate was performed and did not show any hematological diseases or etiological agents. The initial clinical diagnosis was Still's disease, and she was treated with a 2-month course of prednisone 1 mg/kg/day (tapered to 0.6 mg/kg/day) and methotrexate (MTX) 15 mg/week.

The patient progressed with significant worsening of symptoms and required hospitalization due to poor general condition, presenting cachexia, persistent fever, abdominal distension, and pancytopenia. She was admitted to HCFMUSP, in a ward reserved for cases without clinical suspicion of COVID-19 ('non-COVID-19 area'). Upon admission, we retrieved a detailed clinical history, showing that she toured to many places, from the north and coastal areas of Sao Paulo State to the south region of Minas Gerais State, all considered endemic areas for leishmaniasis in Southeastern Brazil (Figure 1), from September 2018 to September 2019. A new laboratory work-up showed no helminthic diseases in stool samples,

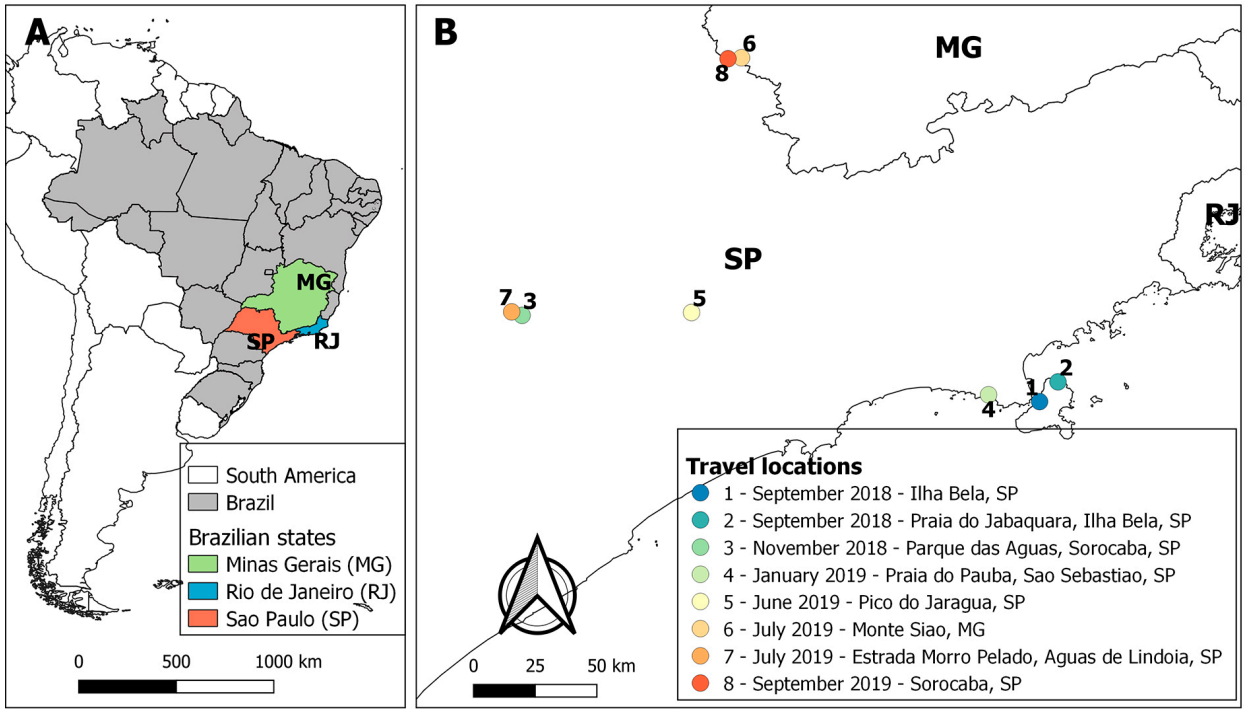

Figure 1 - A) Schematic representation of Latin America, with Brazil and the states of Sao Paulo, Minas Gerais and Rio de Janeiro in colors, corresponding to endemic regions for visceral Leishmaniasis in Brazil; B) Areas visited by the patient in the period from September 2018 to September 2019.

and serologies for systemic mycoses were non-reactive. The initial blood sample taken for Polymerase Chain Reaction of KDNA for *Leishmania* was negative on the third day of hospitalization (DH). On the eighth DH, the patient progressed with dyspnea associated with ground-glass opacity and consolidations on the chest tomography. In this context, SARS-CoV-2, influenza A and influenza B infections were ruled out by molecular tests, and piperacillin-tazobactam was started. Blood cultures were negative, and the patient presented an improvement in respiratory status after the antibiotic treatment. On the thirteenth DH, serologies to detect anti-*Leishmania* antibodies were collected (rapid immunochromatographic test for qualitative detection of anti-recombinant antigen K39 and indirect immunofluorescence), showing positive results. At this time, the patient was taking 10 mg/day of prednisone, without MTX for 45 days. Treatment with liposomal amphotericin 300 mg/day was started, but on the second day of treatment, the patient developed hypoglycemia, torpor, dyspnea, and shock, evolving within a few hours to cardiac arrest, unresponsive to cardiopulmonary resuscitation maneuvers. An autopsy was requested to clarify the cause of the acute respiratory failure, with pulmonary thromboembolism, nosocomial infection or COVID-19 as the main clinical hypotheses.

The MIA-US was performed after written consent from the next-of-kin, using a portable SonoSite M-Turbo R (Fujifilm, Bothell, WA, USA) ultrasound, with C60x (5–2 MHz Convex) multifrequency broadband transducers and DICOM standard images, to guide the tissue sampling from the liver, spleen, lungs, heart and kidneys, with Tru-Cut semi-automatic coaxial needles (14G; 20 cm in length). Additional sampling of skeletal muscle (*quadriceps femoris*) and skin (left thigh) was performed with a 5-mm punch needle; the brain was sampled through transsphenoidal puncture, and the bone marrow through sternal puncture. This autopsy protocol was approved by the HCFMUSP Ethical Committee (protocol Nº 3951.904) and was previously described in detail[7].

The US showed hepatomegaly, splenomegaly and lungs with irregular and thickened pleura, B lines, and subpleural hyperechogenicity, indicative of pneumonia (Figure 2A). Microscopic analysis showed: hypocellular bone marrow aspirate with hemophagocytosis and several macrophages containing amastigote forms of *Leishmania* spp. (Figure 2B); spleen with necrotic red pulp and hyperplastic sinusoidal cells containing amastigotes (Figure 2C); liver with hyperplastic Kupffer cells containing amastigotes, hepatic steatosis, and gram-negative bacilli in vessels. The immunohistochemistry labeled amastigotes forms, using as the primary antibody a polyclonal anti-*Leishmania* in the bone marrow, liver and spleen samples (Figure 2D). The lungs exhibited pulmonary edema and foci of suppurative pneumonia (Figure 2E), with numerous gram-negative bacilli in the alveoli and vessels (Figure 2F). Other findings included: acute tubular necrosis, hyperplasia of mesangial cells, and reactive microglia.

DISCUSSION

This case shows two important aspects: first, that the delayed diagnosis of VL affected the patient's outcome, and second, that MIA-US is useful for clarifying the immediate and underlying cause of death in patients with febrile splenomegaly and pancytopenia, when the conventional autopsy is not available.

Despite the old paradigm that leishmaniasis is strongly associated with rural areas and poverty, the transmission of the disease has become increasingly frequent at the interfaces between urban and rural areas in Brazil, throughout the 20th century. The case exemplifies a real problem in the practice of travel and tropical medicine in Brazil: the delay in the diagnosis of certain endemic and neglected diseases in individuals living in large urban centers, not considering them as susceptible to those diseases, even if the past history points to visiting transmission areas. The false premise that VL affects only those living in rural and poor areas can underestimate the value of displacement data in the clinical history, leading to a low clinical suspicion and diagnostic and therapeutic delay, resulting in unfavorable outcomes[8]. This issue was recently addressed, showing that a prolonged time between the onset of symptoms and the definitive diagnosis of VL occurs in rural and peri-urban areas, among adult and elderly patients, associated with several visits to primary care services, and jeopardizes the definitive diagnosis, mainly in referral hospitals[9]. This diagnostic delay explains why the VL mortality rate has not declined in recent years, although modern diagnostic and treatment techniques are available on the Brazilian public system. In this specific case, additional confounding factors delayed the final definitive diagnosis, which certainly influenced the negative outcome of our case: the negativity of the first laboratory tests for leishmaniasis, the initial misdiagnosis of Still's disease and the immunosuppressive therapy.

The clinical presentation of VL results from factors such as virulence, genetic background, and immune status of the host. The triad fever–hepatosplenomegaly–pancytopenia is the classic presentation of VL, however it is only present in the minority of cases[6]. For instance, the asymptomatic infection can be common in endemic areas, reaching up to 63% for *L. donovani*[10] and 34% for *L. infantum*[11]. In individuals with clinical suspicion and

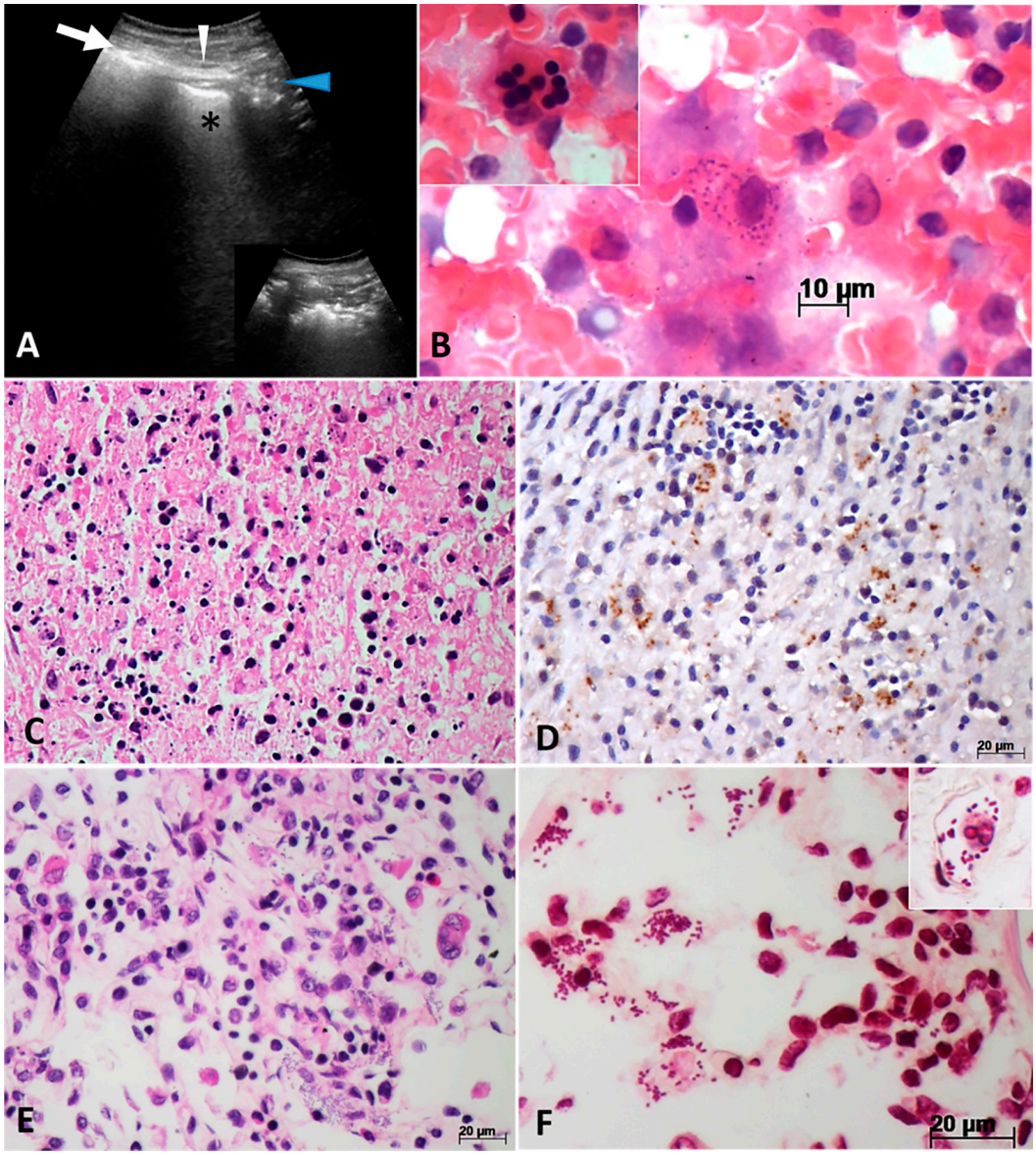

Figure 2 - Ultrasound-guided minimally invasive autopsy findings of a fatal case of kala-azar, from the metropolitan area of Sao Paulo, Brazil: A) Post-mortem lung ultrasound shows thickened pleura (arrow), lines B (asterisk), irregular pleural line (white arrowhead), and hyperechogenic subpleural area (blue arrowhead and inset), corresponding to pulmonary condensation (pneumonia); B) Bone marrow fine needle aspirate shows, in the center, numerous amastigote forms within the macrophage cytoplasm. The inset shows a figure of hemophagocytosis; C and D) Necrotic red pulp of the spleen with debris and mononuclear cells phagocytosing amastigote forms (C, H&E), labeled by immunohistochemistry reaction, using an in-house primary antibody (D, peroxidase); E and F) Foci of suppurative pneumonia (E, H&E), by gram-positive bacilli, present in the alveolar space, with inflammatory cells surrounding the bacillary colonies (F, Brown–Brenn). The inset shows various bacilli within a small vessel and a neutrophil attached to the endothelial line.

epidemiological criteria, tissue collection by aspiration or biopsy is recommended for histopathological study, cultures, and molecular tests to demonstrate the presence of the parasite[12]. Although splenic aspirate is the most sensitive method, lymph node, liver and marrow aspiration samples can be performed accordingly, although there is wide variability in sensitivity[13,14]. The first choice is usually the marrow or splenic aspirate[12]. The decision will depend on the patient's clinical conditions as potential complications can be lethal[15,16]. In our case, two marrow aspirates were negative. Accordingly, the bone marrow aspirate sensitivity for diagnosing VL is correlated with the duration of the microscopic exam by the pathologist, the sensitivity being 40.2%, 65.5%, 89.7%, 92%, and 95.4% at 1, 5, 20, 30, and 60 min, respectively[16].

As VL causes a breakdown in the reticuloendothelial system, the disease may mimic autoimmune diseases, leading to a missed diagnosis. Moreover, it has been

shown that in immunosuppressive status, especially related to HIV infection, the accuracy of the ELISA and blood sample PCR for the diagnosis of VL can be decreased[17]. As seen in the case described here (sepsis by gram-negative bacilli), infections (mainly pneumonia) are the main causes of death among patients with VL (41%), followed by bleeding (38%) and respiratory failure (5%)[6]. Pancytopenia, hemophagocytosis and malnutrition are involved in the predisposition to infections in these cases.

Regarding the autopsy procedure, during the current epidemic of SARS-CoV-2 infection in Brazil, a conventional autopsy (CA) was not recommended due to the lack of autopsy rooms with biosafety level III in the country. To overcome this problem, we employed a safe alternative for professionals – the MIA-US approach, in order to diagnose the *causa mortis* in suspected fatal cases to minimize excesses of mortality falsely attributed to COVID-19 and to investigate the pathology of this novel disease, in various organs[12]. Our group had previously used this autopsy methodology during the sylvatic yellow fever (YF) epidemic that affected the peri-urban region of Sao Paulo city in 2018. We obtained 100% of agreement between MIA-US and the gold-standard CA for determining the underlying cause of death (YF and other diseases) and the immediate cause of death in 20 cases[18]. During the COVID-19 pandemic, an acute respiratory infectious disease with systemic repercussion, we used the MIA-US method to diagnose the *causa mortis* and to study the pathogenesis of this novel disease[7]. Given those successful results, it was possible to infer that MIA-US would have good accuracy for *post-mortem* diagnosis in future epidemics by other respiratory agents (such as measles, influenza, hantavirus, and others) or hemorrhagic fever.

VL is one of the main etiologies of another relevant clinical-pathological syndrome in tropical regions: febrile splenomegaly, associated or not with hepatomegaly and anemia, which also includes schistosomiasis, malaria, brucellosis, typhoid, and leukosis as differential diagnoses. In the case of our study, MIA-US was able to identify both the underlying (VL) and the immediate cause of death (gram-negative bacilli pneumonia and sepsis) of the patient.

MIA can replace CA in low-resource settings, obtaining satisfactory diagnostic samples for determining the cause of death. Nevertheless, in a comparative study between MIA and CA, both methods had an overall agreement of 75.9%, with higher concordance for neoplasia (81.3%) and infectious diseases (78.8%), and lesser for other diseases (56.2%)[19]. Finally, MIA presents a better performance when it is associated with ultrasound to guide the tissue sampling, rather than collection using only anatomical reference points[20].

CONCLUSION

To conclude, the delay in the diagnosis and treatment of VL is a serious problem in Brazil. A detailed medical history, including a history of travel to areas of VL transmission, can help strengthen clinical suspicion and reduce the mortality rate from this disease. The MIA-US can allow the *post-mortem* diagnosis of diseases that progress with febrile splenomegaly and respiratory failure, such as VL, in scenarios with limited resources to perform a conventional autopsy.

ACKNOWLEDGMENTS

The authors acknowledge all healthcare providers involved in the care of the patients with coronavirus disease 2019 (COVID-19); the hospitals and the Sao Paulo Autopsy Service staff involved in the Coronavirus Crisis Task Force during the epidemic season; and the Nucleo de Vigilancia Epidemiologica for providing the hospital data during the epidemic period. The authors also acknowledge and are deeply thankful to all relatives and legal representatives who consented to the post-mortem examinations of their beloved relatives who died during the COVID-19 pandemic. The authors are thankful to Prof. Francisco Chiaravalloti-Neto for assistance in the geopositioning chart of Figure 1.

AUTHORS' CONTRIBUTIONS

JCGJ: provided medical care, conceptualization, methodology, formal analysis, and writing (review and editing); RAMA: minimally invasive autopsy analysis, data curation, formal analysis, investigation, figures, and writing (review and editing); JWPR: provided medical care and writing (review and editing); ELTD: provided medical care, review and editing; EN: provided medical care, review and editing; OM: provided medical care, review and editing; EFP: provided medical care, review and editing; TM: visualization, data curation and writing (review and editing); LFFS: supervision, project administration, and writing (review and editing); PHNS: supervision, project administration, autopsy analysis, and writing (review and editing); MD: supervision, project administration, autopsy analysis, and writing (review and editing); ANDN: conceptualization, methodology, autopsy analysis, figures, and writing (review and editing).

CONFLICT OF INTERESTS

The authors declare that they have no competing financial interests or personal relationships that influence the work reported in this article.

FUNDING

Fundacao de Amparo a Pesquisa do Estado de Sao Paulo, grant Nº 2020/10281-0, recipient PHNS; Bill and Melinda Gates Foundation, grant Nº INV002396, recipient PHNS; Conselho Nacional de Desenvolvimento Cientifico e Tecnologico, grant Nº 401825/2020-5, recipient PHNS; Conselho Nacional de Desenvolvimento Cientifico e Tecnologico, grant Nº 316485/2021-7, recipient MD.

REFERENCES

1. Burza S, Croft SL, Boelaert M. Leishmaniasis. Lancet. 2018;392:951-70.
2. World Health Organization. Leishmaniasis. [cited 2023 Apr 24]. Available from: https://www.who.int/en/news-room/fact-sheets/detail/leishmaniasis
3. Brasil. Ministério da Saúde. Secretaria de Vigilância em Saúde. Departamento de Vigilância Epidemiológica. Manual de vigilância e controle da leishmaniose visceral. Brasília: Ministério da Saúde; 2014. [cited 2023 Apr 24]. Available from: https://bvsms.saude.gov.br/bvs/publicacoes/manual_vigilancia_controle_leishmaniose_visceral_1edicao.pdf
4. Penna HA. Leishmaniose visceral no Brasil. Bras Med. 1934;48:949-50.
5. Carvalho IP, Peixoto HM, Romero GA, Oliveira MR. Cost of visceral leishmaniasis care in Brazil. Trop Med Int Health. 2017;22:1579-89.
6. Costa CH, Werneck GL, Costa DL, Holanda TA, Aguiar GB, Carvalho AS, et al. Is severe visceral leishmaniasis a systemic inflammatory response syndrome? A case control study. Rev Soc Bras Med Trop. 2010;43:386-92.
7. Monteiro RA, Duarte-Neto AN, Silva LF, Oliveira EP, Theodoro Filho J, Santos GA, et al. Ultrasound-guided minimally invasive autopsies: a protocol for the study of pulmonary and systemic involvement of COVID-19. Clinics. 2020;75:e1972.
8. Conti RV, Lane VF, Montebello L, Pinto Junior VL. Visceral leishmaniasis epidemiologic evolution in timeframes, based on demographic changes and scientific achievements in Brazil. J Vector Borne Dis. 2016;53:99-104.
9. Luz JG, Carvalho AG, Naves DB, Dias JV, Fontes CJ. Where, when, and how the diagnosis of human visceral leishmaniasis is defined: answers from the Brazilian control program. Mem Inst Oswaldo Cruz. 2019;28;e190253.
10. Srivastava P, Gidwani K, Picado A, Van der Auwera G, Tiwary P, Ostyn B, et al. Molecular and serological markers of Leishmania donovani infection in healthy individuals from endemic areas of Bihar, India. Trop Med Int Health. 2013;18:548-54.
11. Marques LH, Rocha IC, Reis IA, Cunha GM, Oliveira E, Pfeilsticker TR, et al. Leishmania infantum : illness, transmission profile and risk factors for asymptomatic infection in an endemic metropolis in Brazil. Parasitology. 2017;144:546-56.
12. Aronson N, Herwaldt BL, Libman M, Pearson R, Lopez-Velez R, Weina P, et al. Diagnosis and treatment of leishmaniasis: clinical practice guidelines by the Infectious Diseases Society of America (IDSA) and the American Society of Tropical Medicine and Hygiene (ASTMH). Am J Trop Med Hyg. 2017;96:24-45.
13. Ho EA, Soong TH, Li Y. Comparative merits of sternum, spleen and liver punctures in the study of human visceral leishmaniasis. Trans R Soc Trop Med Hyg. 1948;41:629-36.
14. Zijlstra EE, Ali MS, El-Hassan AM, El-Toum IA, Satti M, Ghalib HW, et al. Kala-azar: a comparative study of parasitological methods and the direct agglutination test in diagnosis. Trans R Soc Trop Med Hyg. 1992;86:505-7.
15. Sundar S, Rai M. Laboratory diagnosis of visceral leishmaniasis. Clin Diagn Lab Immunol. 2002;9:951-8.
16. Silva MR, Stewart JM, Costa CH. Sensitivity of bone marrow aspirates in the diagnosis of visceral leishmaniasis. Am J Trop Med Hyg. 2005;72:811-4.
17. van Griensven J, Carrillo E, López-Vélez R, Lynen L, Moreno J. Leishmaniasis in immunosuppressed individuals. Clin Microbiol Infect. 2014;20:286-99.
18. Duarte-Neto AN, Monteiro RA, Johnsson J, Cunha MP, Pour SZ, Saraiva AC, et al. Ultrasound-guided minimally invasive autopsy as a tool for rapid post-mortem diagnosis in the 2018 Sao Paulo yellow fever epidemic: correlation with conventional autopsy. PLoS Negl Trop Dis. 2019;13:e0007625.
19. Castillo P, Martínez MJ, Ussene E, Jordao D, Lovane L, Ismail MR, et al. Validity of a minimally invasive autopsy for cause of death determination in adults in Mozambique: an observational study. PLoS Med. 2016;13:e1002171.
20. Cox JA, Lukande RL, Kalungi S, Van de Vijver K, Van Marck E, Nelson AM, et al. Practice of percutaneous needle autopsy; a descriptive study reporting experiences from Uganda. BMC Clin Pathol. 2014;14:44.