Analysis of the diagnostic flow of cutaneous leishmaniasis in the state of Minas Gerais-Brazil

Fernanda Alvarenga Cardoso Medeiros
Pós-Graduação em Ciências da Saúde: Infectologia e Medicina Tropical da Faculdade de Medicina da Universidade Federal de Minas Gerais

Job Alves Souza Filho
Serviço de Doenças Parasitárias da Divisão de Epidemiologia e Controle de Doenças da Diretoria do Instituto Octávio Magalhães da Fundação Ezequiel Dias, Belo Horizonte-MG, Brasil;

Ilka Afonso Reis
Instituto de Ciências Exatas da Universidade Federal de Minas Gerais, Belo Horizonte-MG

Daniel Menezes-Souza
Departamento de Patologia Clínica do Colégio Técnico da Universidade Federal de Minas Gerais, Belo Horizonte-MG

Aline Fagundes da Silva
LaPClinVigiLeish-Instituto Nacional de Infectologia Evandro Chagas da Fundação Oswaldo Cruz, Rio de Janeiro-RJ

Andreza Pain Marcelino
andrezapmarcelino@ini.fiocruz.br

LaPClinVigiLeish-Instituto Nacional de Infectologia Evandro Chagas da Fundação Oswaldo Cruz, Rio de Janeiro-RJ

Research Article

Keywords: Leishmaniasis, Cutaneous, Laboratory diagnosis, Public Health laboratories, Diagnostic flow

Posted Date: December 21st, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3711058/v1

License: © This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License

Additional Declarations: No competing interests reported.
Abstract

Objective

To assess the performance of the proposed new diagnostic pathway in patients with suggestive clinical symptoms of CL within MG’s public laboratory network.

Methods

This is a descriptive study where the results of CL tests were analyzed in patients with clinical suspicion of the disease who had their tests conducted within MG’s public laboratory network and the results were registered in the Laboratory Management System of the Ezequiel Dias Foundation, during the period from 2017 to 2020.

Results

Out of a total of 1,369 individuals analyzed, the diagnosis of CL was confirmed in 704 (51.4%), with 610 (86.7%) through DPE and 94 (13.4%) through PCR. Additionally, 53 (25.3%) patients with negative DPE results showed positive PCR results.

Conclusion

The proposed CL diagnostic pathway in MG proved to be effective as it ensures that the initial test is conducted on-site, allowing for greater access and efficiency in the treatment of confirmed cases. PCR demonstrated to be an effective confirmatory test.

1. INTRODUCTION

According to the World Health Organization (WHO), leishmaniasis ranks among the top six infectious diseases worldwide. Leishmaniasis is endemic in 98 countries, with 72 of them being developing nations, where 220,000 cases of cutaneous leishmaniasis (CL) are reported annually (ALVAR; VELEZ; BERN; HERRERO et al., 2012; DESJEUX, 2004). In Brazil alone, in 2020, 16,432 cases of CL were registered, with approximately half of them occurring in four states: Pará, Amazonas, Mato Grosso, and Minas Gerais (OPAS, 2022).

The diagnosis of CL relies on both clinical and laboratory methods, supported by epidemiological data. Traditionally, the disease manifests in two forms: cutaneous leishmaniasis and mucosal leishmaniasis, each with different clinical presentations (BRASIL, 2017). Due to the wide spectrum of lesions, the diagnosis is not always straightforward and may require differentiation from other diseases such as leprosy, ulcers, syphilis, sporotrichosis, tuberculosis, and câncer (CHAPPUIS; SUNDAR; HAILU; GHALIB et
In 2018, approximately 83.6% (38,511) of CL cases in the Americas were confirmed through laboratory tests, while 9.96% (4,587) relied on clinical-epidemiological criteria (OPAS, 2022).

Until 2016, the diagnosis of CL in Brazil was primarily conducted using the Montenegro Skin Test (MST), parasitological examinations, and molecular methods (polymerase chain reaction - PCR). Direct demonstration of the parasite can be achieved through microscopic examination of stained smears obtained through procedures like scarification, impression biopsy, and fine needle aspiration. The direct parasitological examination is considered the first-choice procedure due to its speed, affordability, and ease of execution compared to parasite isolation and culture (GOTO; LINDOSO, 2010) (BENSOUSAN; NASEREDDIN; JONAS; SCHNUR et al., 2006).

PCR is a useful method for detecting low parasite loads due to its high sensitivity (DE VRIES; REEDIJK; SCHALLIG, 2015). Currently, there is no PCR kit registered in Brazil for CL diagnosis, and the method is used with significant methodological variation and performance for case confirmation (DE VRIES; REEDIJK; SCHALLIG, 2015; GOMES; ARMELIN; MENON; PEREIRA-CHIOCCOLA, 2008).

MST assesses the delayed cellular immune response by subcutaneously applying *Leishmania* antigen (BRASIL, 2017; DE VRIES; REEDIJK; SCHALLIG, 2015). MST is a cost-effective and easily executable method that does not require sophisticated equipment, making it suitable for on-site testing. Unfortunately, this test is not available in the country due to the discontinuation of its production in 2016 (BRAZ, 2019).

Due to the discontinuation of MST production and distribution in Brazil in 2016, the Ezequiel Dias Foundation (FUNED), Oswaldo Cruz Foundation, and the State Health Department of Minas Gerais jointly issued Technical Note CZFRB/DVA/SVEAST/Sub.VPS-Nº11 /2017 (MG, 2019) which established the guidelines for CL diagnosis in the state of Minas Gerais (MG). This note mandated that laboratory diagnosis in Minas Gerais would be conducted through direct parasitological examinations followed by a PCR test. After the officialization of this Technical Note, a network of laboratory diagnosis was established in the state of Minas Gerais, where 50 healthcare professionals were trained to conduct direct parasitological examination in their respective municipalities, while PCR testing was centralized at FUNED. All laboratory results obtained within this network are entered into the computerized biological sample registry system of the state of Minas Gerais, in the Laboratory Environment Management (Gerenciamento de Ambiente Laboratorial)/Ezequiel Dias Foundation (GAL/FUNED), enabling quality control of the network (FUNED, 2021).

Considering the available diagnostic tests for CL, this study aimed to evaluate the performance of the diagnostic workflow used in patients with suspected CL, utilizing retrospective data from examinations conducted in the public laboratories network of the state of Minas Gerais. The outcome of this study allows for the proposal of improvements in the laboratory diagnostic workflow, based on the performance, practicality, and invasiveness of the analyzed tests.
2. MATERIALS AND METHODS

2.1. Study design

A descriptive study in which the results of diagnostic tests for CL from the network of public laboratories in Minas Gerais registered with the Management of Laboratory Environment (Gerenciamento de Ambiente Laboratorial)/Ezequiel Dias Foundation (GAL/FUNED) were verifed in the period between 2017 and 2020.

2.2. Study Location

The study area was the state of Minas Gerais. The state is located in the southeast region of Brazil with a territorial area of 586,513,993 km², a population of 21,411,923 inhabitants, and 853 municipalities (IBGE, 2021). These municipalities are divided into 14 health macro-regions, which were grouped into 8 Health Regions registered with GAL/FUNED: 1- Macroregional of Belo Horizonte and state health network of Belo Horizonte, 2- North, Northeast, Northwest and Jequitinhonha, 3 - Southeast and Center-South, 4- East, Southeast and Steel Valley, 5- West, 6- South, 7- North Triangle and South Triangle, and 8- Belo Horizonte and metropolitan region (IBGE, 2021; SES-MG, 2020).

2.3. LT diagnostic ow of the MG public laboratory network

The standardization of the CT diagnostic ow in the state established in Technical Note CZFRB/DVA/SVEAST/Sub.VPS-N°11/2017(MG, 2019) is as follows: direct parasitological examination and in case of a negative result, PCR. Laboratory tests carried out in the public network of MG follow the following protocols:

Direct parasitological examination (DPE): the slides are fixed with methanol, stained with Giemsa and the presence of parasites is investigated using optical microscopy (BARCIA, 2007), carried out in municipal/regional laboratories.

Polymerase chain reaction (PCR): conventional, qualitative PCR, through the revelation of DNA by agarose gel electrophoresis (PASSOS; FERNANDES; LACERDA; VOLPINI et al., 1999; SHEPPARD; DWYER, 1986) carried out in the FUNED laboratory.

2.4. Variables analyzed

The main variable analyzed was the result (positive or negative) of the DPE, and PCR tests/exams performed on patients with signs of CL symptoms registered in GAL/FUNED. The other variables analyzed were: lesion characteristics, biological sample, gender, patient age, and health units in the state where the exams were recorded.

The results of patients who presented a clinical syndrome suggestive of the disease (single, localized skin ulcers, diffuse cutaneous leishmaniasis and/or mucosal disease), and who underwent at least one direct parasitological examination (DPE), and/or Polymerase Chain Reaction (PCR) registered with GAL/FUNED were eligible for the study.
Results of patients confirmed with CL by other methods (indirect immunofluorescence reaction and/or indirect immunoenzymatic assay), patients who had incorrect biological sample registration, outpatients, treated in other health units and/or in other states, in addition to results that presented discrepancies in the database, due to limited information or any other reason not specified here that made its safe use impossible, were excluded from the study.

2.5. Data analysis

Data from categorical variables were summarized using absolute values and percentages. The results were presented through frequency tables or graphs.

3. RESULTS

The CL MG public laboratory network carried out, from January 2017 to December 2020, a total of 1,685 exams, of which 1,311 (77.8%) DPE and 374 (22.2%) PCR, according to data from the GAL/FUNED. 1,369 individuals with suspected CL were evaluated, of which 704 (51.4%) were confirmed cases, an average of 172.4 cases/year. The DPE methodology was used to confirm 610 (86.7%) of the CL cases, the remaining 94 cases (13.4%) were confirmed by PCR. Furthermore, 53 (25.3%) patients with negative DPE had positive PCR results. Figure 1 shows the diagnosis followed by health professionals from the MG public laboratory network during this period.

In the analysis of the biological material used to perform the DPE examination, 812 (65.8%) are from lesion fragment biopsy material, 384 (31.2%) from lesion scarification procedures and the remaining 38 (3.0%) from unidentified material. Of these, 52.8% (429/812) of lesion fragment biopsies and 43.3% (167/384) of scarifications were positive.

Of the samples sent for PCR after a negative DPE result, 24.4% (44/180) of the EPD performed with biopsy material and 30% (9/30) of the DPE performed with scarifications were positive in the PCR. Regarding negative DPE samples that were not sent for PCR, 45.7% (189) were from biopsies and 47.8% (198) from scarifications. Figure 2 represents the location of lesions in positive samples.

Among patients confirmed with CL, 423 (60.1%) were male and 281 (39.9%) were female. The patients’ ages ranged from 1 to 99 years, with a mean of 42 ± 19.9 years (mean ± standard error). Regarding children, 36 (5.1%) are < 10 years old. Figure 3 shows the distribution of CL cases by age group and sex. The majority of patients with TL symptoms ranged from 20 to 50 years old, and the male gender was the most common in almost all age groups.

Analyzing the GAL/FUNED registration regions and the number of exams performed (DPE and PCR), a large variation in the number of exams between regions was observed. Regarding PCR, not all health regions sent samples for examination at FUNED. Table 1 shows the number of tests performed in each health region, the positivity rate and the number of negative DPEs in which PCR was performed and the positivity rate.
Table 1 Association between cutaneous leishmaniasis diagnostic method, positivity and Health Region according to GAL/Funed in the period 2017–2020.

<table>
<thead>
<tr>
<th>Health Regions</th>
<th>DPE</th>
<th>PCR</th>
<th>DPE negative and PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Positive (%)</td>
<td>N</td>
</tr>
<tr>
<td>1</td>
<td>19</td>
<td>4 (21.1)</td>
<td>16</td>
</tr>
<tr>
<td>2</td>
<td>727</td>
<td>382 (52.5)</td>
<td>280</td>
</tr>
<tr>
<td>3</td>
<td>14</td>
<td>3 (21.4)</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>414</td>
<td>203 (49.0)</td>
<td>31</td>
</tr>
<tr>
<td>5</td>
<td>34</td>
<td>15 (44.1)</td>
<td>15</td>
</tr>
<tr>
<td>6</td>
<td>8</td>
<td>1 (12.5)</td>
<td>8</td>
</tr>
<tr>
<td>7</td>
<td>13</td>
<td>1 (7.7)</td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td>5</td>
<td>1 (20.0)</td>
<td>4</td>
</tr>
</tbody>
</table>

DPE (direct parasitological examination), PCR (polymerase chain reaction), N (number of examinations).
1- Macro-regional health network of Belo Horizonte and state of Belo Horizonte, 2- North, Northeast, Northwest and Jequitinhonha, 3- Southeast and Center-South, 4 - East, Southeast and Steel Valley, 5- West, 6- South, 7- North Triangle and South Triangle, and 8- Belo Horizonte and metropolitan region (GAL/FUNED registration regions).

In evaluating the number of tests carried out after the training of the Technical Note for CL (2017) in the health regions in MG, there was an increasing increase in tests carried out from 2017 to 2019. In 2020, despite the number of tests still being higher than that in 2017, there was a drop in the number of exams compared to 2019. The number of exams performed and registered with GAL/FUNED was 21 exams (DPE + PCR) in 2017 and 499 in 2020, a number 23.8 times higher. This assessment is even greater in registration regions with a greater number of CL cases, such as health units two and four. Figure 4 shows the temporal evolution of the number of CL cases due to DPE and PCR.

4. DISCUSSION

In the scenario of high incidence of CL in Brazil, lack of immunological tests for the disease, and lack of laboratory resources in many regions of the country, it is necessary to establish a diagnostic flow that would favor an effective identification of CL cases. The proposal for a diagnostic flow in the state of Minas Gerais is premised on the identification, and early treatment of CL cases, reducing common and serious morbidities caused by the disease. (BRASIL, 2017; 2021). As Minas Gerais is the second most populous state in the country, with more than 20 million inhabitants, endemic for CL and has a well-structured health network, the assessment of its diagnostic network for CL can guide the diagnosis flow of the disease in different countries.
The analysis of a Na information system (GAL/FUNED) to verify the effectiveness of a diagnostic flow for cutaneous leishmaniasis is extremely important since confirmed cases of the disease can be determined by the diagnostic results of DPE and PCR (BRASIL, 2022b). The proposal to carry out direct parasitological examination as the first diagnostic method after clinical suspicion is mainly due to the speed of the result, as it is carried out at the patient's place of care (MURBACK; HANS FILHO; NASCIMENTO; NAKAZATO et al., 2011; PASSOS; FERNANDES; LACERDA; VOLPINI et al., 1999) and the high specificity of the test (ESPIR; GUERREIRO; NAIFF MDE; FIGUEIRA LDE et al., 2016; GOTO; LINDOSO, 2010). Furthermore, the collection of the material can be carried out by any properly trained healthcare professional and the exam does not require expensive equipment to perform (BRASIL, 2017). In the GAL/FUNED analysis, it was demonstrated that the laboratories in the CL diagnostic network followed the flow after its implementation, as 90% of tests after suspicion of the disease were DPE and only 9.9% were PCR.

The positivity of the direct parasitological examination found was 49.4%, which may be related to the sensitivity of the technique, in which the probability of finding the parasite is inversely proportional to the time of evolution of the skin lesion, and the type of lesion, varying from 15 to 74.4% (BENSOUSSAN; NASEREDDIN; JONAS; SCHNUR et al., 2006; GOTO; LINDOSO, 2010). Furthermore, it cannot be said that all suspected patients were cases of CL, due to the difficulty of clinical diagnosis in differentiating it from other diseases, such as leprosy, ulcers, syphilis, sporotrichosis, tuberculosis and cancer. (BENSOUSSAN; NASEREDDIN; JONAS; SCHNUR et al., 2006; SINGH, 2006).

Parasitological diagnosis is considered the gold standard in the diagnosis of CL due to its high specificity (DE VRIES; REEDIJK; SCHALLIG, 2015; GOMES; ARMELIN; MENON; PEREIRA-CHIOCCOLA, 2008). Despite this, 18 biological samples (2.9%) positive in the DPE were performed by PCR. Of these samples, two (0.3%) had discordant results. This result may have occurred due to errors in the pre-analytical phase (sample exchange, sample degradation, poor conservation, among others) and in the analytical phase (diagnostic errors) or due to the inherent sensitivity and specificity of each technique (PLEBANI, 2006). Showing that despite the PCR technique being more sensitive, negative results may occur with positive parasitological tests.

In the proposed CL diagnostic flow, performing PCR would increase diagnostic sensitivity, as in parasitological tests with negative results, a second test would be performed, reducing false-negative diagnoses (BRASIL, 2017; REITHINGER; Dujardin; Louzir; Pirmez et al., 2007). PCR technique has high specificity (84.0% and 100%) and is considered an excellent confirmatory test. (BENSOUSSAN; NASEREDDIN; JONAS; SCHNUR et al., 2006; PENA; BELO; XAVIER-JUNIOR; TEIXEIRA-NETO et al., 2020). In the study, only 41 patients (30.4%) were confirmed cases of CL using the PCR technique in the first examination, which may reflect the fact that the majority of patients with suspected CL are not cases of the disease. However, 51 (25.3%) patients were confirmed by PCR after a negative direct parasitological exam, confirming the importance of a second diagnostic exam. These data are in agreement with Reithinger and collaborators who estimate that with the use of PCR, an increase in sensitivity in the diagnosis of cutaneous leishmaniasis is around 20 to 30% and, for mucosal leishmaniasis, between 55
and 70%, compared to parasitological diagnosis (REITHINGER; DUJARDIN; LOUZIR; PIRMEZ et al., 2007). Furthermore, the PCR technique allows the identification of Leishmania species in infected patients, which is important to obtain a correct prognosis (AREVALO; RAMIREZ; ADAUI; ZIMIC et al., 2007) and promote disease surveillance actions (BENSOUSSAN; NASEREDDIN; JONAS; SCHNUR et al., 2006; OPAS, 2022).

In analyzing the biological sample used, the study showed that, although biopsy is a minimally invasive procedure for patients and an exclusively medical technique (GOTO; LINDOSO, 2010) the majority of biological samples collected were from biopsy procedures (65.8%). Thomaz (2021) demonstrated that for PCR the parasitic loads are greater on the inner edge compared to the outer edge of the lesions, however for DPE, there is no significant difference in the parasite load between scarification or biopsy imprint (THOMAZ; DE MELLO; ESPINDOLA; SHUBACH et al., 2021).

The majority of samples sent 482 (69%) for the diagnosis of CL were from the upper and lower limbs, the most uncovered and exposed extremities of the body, as described in other studies (GONTIJO; DE CARVALHO MDE, 2003).

A higher rate of involvement was found in males compared to females (ratio of 3:2) and in the age group of 20 to 50 years. This suggests an exposure profile related to work activities. In rural areas, men are more exposed to the vectors responsible for extra-household transmission The occurrence in women > 40% and children under 10 years old > 10% indicates the occurrence of per-household and intra-household transmission (BRASIL, 2017; OPAS, 2022). In the GAL/FUNED analysis, this profile was not observed, which may be due to the growing number of women in the rural labor market, allowing the disproportion between men and women affected by CL to decrease over the years. Despite the low number of cases registered in GAL/FUNED, these data are representative of the data registered in the Notifiable Diseases Information System/SINAN-MG, where in 2019 1,172 (62.1%) men and 716 (37, 9%) women (3:2 ratio) and 94 (5.0%) cases registered in < 10 years (BRASIL, 2022a; c).

Considering the number of CL cases confirmed by GAL/FUNED, health region 2 (North, Northeast, Northwest, and Jequitinhonha) is the one with the highest number of positive tests (DPE and PCR-450), followed by region 4 (East, Southeast and Vale do Aço) with 2011 exams in the period analyzed. The same epidemiological profile can be observed in the Notifiable Diseases Information System -SINAN evaluation. In 2019, the health regions that reported the most cases of CL were: health region 2 with 874 (46.3%) cases and health region 4 with 549 (29.1%) (BRASIL, 2022c). This showed that the places with the highest number of suspected patients are the ones that report the most cases and use the GAL/FUNED system, demonstrating that the GAL is representative of the state's reality.

The low number of exams registered in all health regions about data from the - SINAN demonstrates the deficiency in the recording of CL exams in the system of the State of Minas Gerais (BRASIL, 2022c). The main problems can be attributed to the difficulty of carrying out direct parasitological examination, which requires experienced professionals to suspect, carry out the examination and report the case.
Furthermore, the exams are not performed with a specific diagnostic kit, which makes it difficult to control the exams performed, and the quality of these exams (CORREA, 2019).

Despite the low number of exam registrations at GAL/FUNED, it is clear that this number increased 23.8 times from 2017 to 2020, which can be justified by the creation of a diagnostic flow by state health institutions and the constant training of professionals (CORREA, 2019). There was a reduction in records of CL exams in 2020, which may have been caused by the Coronavirus Pandemic, which made it difficult for patients with various diseases to access the healthcare system (OPAS, 2022).

5. CONCLUSION

The CL diagnostic flow implemented by health services in the state of Minas Gerais proved to be effective in ensuring that the first examination carried out on the suspected patient is carried out at the place of care, allowing greater access and agility in the treatment of cases of the disease. Additionally, PCR proved to be a good confirmatory test. The diagnostic flow established in MG proved to be an excellent model that can be followed by other states. However, there are still many challenges to be overcome to improve the diagnosis of CL, such as development of a PCR kit for standardized diagnosis, greater control of the diagnostic network through the implementation of adequate quality controls, improved records, and the number of qualified professionals, which will effectively contribute to the identification of cases and the entire surveillance program in the country.

Declarations

Ethical Approval: The study was conducted with the Declaration of Helsinki, Declaration of Helsinki of 1964, revised in 1975, 1983, 1989, 1996, and 2000, and approved by the Ethics Committee of Fundação Ezequiel Dias (protocol code CAAE: 44042721.5.0000.9507).

Informed Consent Statement: Patient consent was waived due to the retrospective nature of the study and because it involves data collection from the Laboratory Environment Manager (GAL) database, which will be kept confidential, by with the terms of the Resolution 466/12 of the National Health Council and the Term of Prior Consent authorized by the institution Fundação Ezequiel Dias, where the research was carried out.

Competing interests: The authors declare that they have no conflict of interest.

Authors' contributions: FACM, JASF, and APM: conceptualized the study, developed the methodology, conducted the formal analysis, carried out the investigation, wrote the original version of the manuscript and took care of project administration. IAR, DMS, and AFS: conducted the investigation and participated in reviewing and editing the manuscript.

Funding: This research received no external funding.
Availability of data and materials: The authors confirm that the data supporting the findings of this study are available within the article. Other data that support the findings of this study are available from the corresponding author, F.A.C.M., upon reasonable request.

Acknowledgments: We would like to thank the technicians and analysts of the Brazilian Laboratory of National Reference for the diagnosis of visceral Leishmaniasis, at Fundação Ezequiel Dias, who passed through the laboratory over the ten years covered in this study and collaborated in carrying out the diagnostic tests evaluated here.

References


Figures

![Diagram showing diagnostic follow-up for cutaneous leishmaniasis]  

**Figure 1:** Diagnostic followed by health professionals for the diagnosis of cutaneous leishmaniasis (data from GAL/FUNED date, 2017-2020. DPE (direct parasitological exam); PCR (polymerase chain reaction).
See image above for figure legend

**Figure 2**

Location of collection of lesions from cases of cutaneous leishmaniasis according to GAL/Funed in the period 2017-2020

**Figure 2**

See image above for figure legend
**Figure 3**: Association between age group and sex of cases of cutaneous leishmaniasis according to GAL/Funed in the period 2017-2020.

**Figure 3**

See image above for figure legend
Figure 4: Temporal evolution of the number of cases of cutaneous leishmaniasis diagnosed by direct parasitological examination and polymerase chain reaction in Minas Gerais according to GAL/Funed in the period 2017-2020. DPE (direct parasitological examination); PCR (polymerase chain reaction).

See image above for figure legend