RESEARCH ARTICLE

Comparison of serum cytokine levels in symptomatic and asymptomatic HIV-
*Leishmania* coinfect ed individuals from a Brazilian visceral leishmaniasis endemic area

Diego Lins Guedes¹,²*, Elis Dionísio da Silva³, Maria Carolina Accioly Brelaz Castro³,⁴, Walter Lins Barbosa Júnior¹, Ana Victoria Ibarra-Meneses⁵,⁶, Achilleas Tsoumanis⁷, Wim Adriaensen⁷, Johan van Griensven⁷, Valéria Rêgo Alves Pereira³, Zulma Maria de Medeiros¹

¹ Department of Parasitology, Aggeu Magalhães Institute–Oswaldo Cruz Foundation (Fiocruz), Recife, Pernambuco, Brazil, ² Curso de medicina, Núcleo de Ciências da Vida, Centro Acadêmico do Agreste, Universidade Federal de Pernambuco, Caruaru, Pernambuco, Brazil, ³ Department of Immunology, Aggeu Magalhães Institute–Oswaldo Cruz Foundation (Fiocruz), Recife, Pernambuco, Brazil, ⁴ Parasitology Laboratory, Federal University of Pernambuco, Vitoria de Santo Antônio, Pernambuco, Brazil, ⁵ Département de pathologie et microbiologie, Faculté de médecine vétérinaire, Université de Montréal, Saint-Hyacinthe, QC, Canada, ⁶ The Research Group on Infectious Diseases in Production Animals (GREMIP), Faculty of Veterinary Medicine, Université de Montréal, Montreal, Canada, ⁷ Department of Clinical Sciences, Institute of Tropical Medicine, Antwerp, Belgium

* Current address: Instituto de Saúde e Biotecnologia, Universidade Federal do Amazonas, Coari, Amazonas, Brazil.

* diego.linsguedes@ufpe.br

Abstract

Background

Visceral leishmaniasis (VL) remains an important infectious disease worldwide. VL-HIV coinfected individuals can present with atypical clinical forms of VL and have a high risk of VL relapse. Some cytokines have been described as potential markers to diagnose active VL and to predict the severity of the cases. However, few studies have included VL-HIV coinfected patients. We aimed to characterize the levels of several cytokines among VL-HIV coinfected individuals living in a VL-endemic area in Northeast Brazil.

Methods

This was a retrospective, cross-sectional study, aiming to estimate the levels of various cytokines in symptomatic and asymptomatic VL-HIV coinfected individuals. There were 134 study participants (35 symptomatic VL-HIV, 75 asymptomatic VL-HIV, and 24 healthy controls), all ≥ 18 years-old. Serum cytokine levels (interferon-γ, tumor necrosis factor, and interleukins 2, 4, 6, 10, and 17A) were quantified using the Becton Dickinson-BD’s Cytometric Bead Array (CBA) system.

Results

The population mainly consisted of men (64.9%), with a median age of 35 (27–41) years. Asymptomatic individuals were younger (p = 0.013), with more years of education (p <
0.001), and were more often on antiretroviral therapy (p < 0.001) than those in the symptomatic group. Hemoglobin levels (p < 0.001), lymphocytes (p < 0.001) and CD4 count (p < 0.001) were lower in symptomatic individuals, while HIV viral loads were higher (p < 0.001).

In the symptomatic VL-HIV coinfected group, we observed increased serum levels of IL-17A, IL-6, and IL-10 compared to asymptomatic patients and the healthy controls. There were no differences in the levels of all cytokines between asymptomatic VL-HIV coinfected individuals and the healthy controls.

Conclusions
Higher serum levels of IL-17A, IL-6, and IL-10 cytokines were observed in symptomatic coinfected individuals but not in asymptotically infected individuals. More studies among HIV-positive persons are needed to better understand the role of serum cytokines for prognosis, to define cure and predict VL relapses in VL-HIV coinfected individuals.

Author summary
Visceral leishmaniasis (VL) is a parasitic disease, classified as a neglected disease by the World Health Organization. It is present in more than 60 countries in four continents, with most tropical and subtropical countries affected. Without treatment, the disease is fatal. HIV-positive persons are especially affected by VL, with a worst prognosis. One of the main complications is the frequent reactivation of the disease even after successful treatment (VL relapse). Up to now, it is not clear why and when these reactivations occur, and many researches are trying to find some biological markers to answer this. Cytokines are one of these markers that may explain the progression of the disease. In this study, we compared the level of several key cytokines in symptomatic VL-HIV coinfected patients, asymptomatic VL-HIV coinfected individuals, and healthy controls living in a VL-endemic region in Northeastern Brazil. The serum cytokine levels were higher in symptomatic patients when compared with asymptomatic and healthy controls. More studies following VL-HIV patients are necessary to understand how these cytokines and the other biomarkers vary over time and whether they can predict VL relapse, and also the progression and the prognosis of the disease.

Introduction
In spite of all the efforts to control visceral leishmaniasis (VL), it remains an important and prevalent infectious disease worldwide. Affecting mainly neglected people in tropical and subtropical nations [1, 2], VL is present in more than 60 countries in four continents [3]. In South America, Brazil carries the highest burden [4]. While most Leishmania infections are self-limiting and asymptomatic, symptomatic VL is inevitably lethal without treatment. Despite receiving anti-Leishmania drugs and appropriate health care, death is not rare, particularly in HIV coinfected individuals [5]. In addition, VL-HIV coinfected individuals also display higher parasite loads and frequent relapses, compared with VL cases without HIV coinfection [6, 7].

Immunotherapy for VL has been proposed as a potential way to support the treatment with anti-Leishmanial drugs [8, 9]. In fact, despite the disease being known for such a long time, and its considerable lethality [10], only two drugs are available in Brazil—pentavalent...
antimonials and amphotericin B (in different preparations). These two drugs have significant adverse effects [11], and for some specific patient groups—such as persons living with HIV—the traditional chemotherapy is less effective with higher rates of treatment failure, mortality and relapse [12, 13].

Some cytokines have been described as potential markers for VL. For example, interleukin (IL)-2 could be used for detecting asymptomatic *Leishmania* infection [14–16], IL-6 for predicting the severity of the disease [17–19], interferon (IFN)-γ to define cure after treatment [20, 21], and IL-10 and IFN-γ could be helpful as markers of active VL [22, 23].

However, few studies have included VL-HIV coinfected patients. Consequently, the impact of HIV co-infection on the cytokine profile among patients infected with *Leishmania* is not well understood. In this study, we aimed to compare the levels of several key cytokines between symptomatic and asymptomatic VL-HIV coinfected individuals, and healthy controls living in Pernambuco, a VL-endemic area, in Northeast Brazil.

**Methods**

**Ethics statement**

All subjects were adults and provided written, informed consent. The study was approved by the research ethics committee of Instituto Aggeu Magalhães, Fiocruz Pernambuco (approval number 51235815.0.0000.5190). The samples were collected partly in 2014 and partly in 2018, and were stored in the freezer (−80°C). At the time of consent, the participants had agreed that the samples could be used by the Instituto Aggeu Magalhães in other future studies.

**Study design and sample**

This was a retrospective, cross-sectional study. The main objective of this study was to estimate the levels of different cytokines in symptomatic and asymptomatic VL-HIV coinfected individuals, and healthy controls (negative for VL and HIV). We 1) compared cytokine levels amongst the three groups; 2) assessed the correlation among the levels of cytokines within each group; 3) assessed the correlation between the serum levels of these cytokines and general laboratory data; and 4) examined the association between the CD4 count, HIV viral load and the cytokine levels.

A total of 134 individuals were included (35 symptomatic VL-HIV, 75 asymptomatic VL-HIV, and 24 healthy controls). All patients were at least 18 years-old. Except for the group of healthy controls, all the participants had already been diagnosed with HIV and they were tested for *Leishmania*, presenting at least one positive result on four VL test done: the rK39 rapid test (InBios International, Seattle, USA), and the Direct Agglutination Test (DAT) (Biomedical Research, AD Amsterdam), both done on serum; the KAtex *Leishmania* antigen test on urine (Kalon Biological Ltd, Guildford, UK); and the kDNA Polymerase Chain Reaction (PCR) test on peripheral blood, according to Souza et al [24] and Gualda et al [25]. For the symptomatic group, we used samples of the 35 VL-HIV coinfected patients diagnosed in a previous study in 2014 in three referral hospitals from Recife, Brazil [26]. For the asymptomatic group, we randomly selected samples of asymptomatic VL-HIV cases in 2018 attending an HIV outpatient service in Petrolina, Brazil [27]. All samples were stored in freezer (−80°C). The healthy controls were tested in 2018 for HIV and VL and were found negative for both.

**Data collection and laboratory procedures**

The following data were collected from the medical records for the symptomatic and asymptomatic VL-HIV patients: gender, age, education; levels of hemoglobin, leucocytes,
neutrophils, lymphocytes, platelets, aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, creatinine, CD4 and HIV viral load. For the healthy controls, we only collected demographic data and quantified cytokine levels.

Using serum samples, we used the Becton Dickinson-BD’s Cytometric Bead Array (CBA) system to determine the following cytokines: IFN-γ, tumor necrosis factor (TNF), IL-2, IL-4, IL-6, IL-10, and IL-17A, according to the manufacturer’s recommendation. The limits of detection of these cytokines, according to the manufacturer, are: IL-2—2.6 pg/ml; IL-4—4.9 pg/ml; IL-6—2.4 pg/ml; IL-10—4.5 pg/ml; TNF—3.8 pg/ml; IFN-γ—3.7 pg/ml; IL-17A—18.9 pg/ml. The flow cytometer FACSCalibur (BD, San Jose, California, USA) was used, with subsequent analysis using the FACAPArray (BD, San Jose, California, USA).

**Statistical analysis**

Continuous variables were summarized as medians and interquartile range, while categorical variables were as counts and proportions. Differences in proportions of categorical variables by group were compared using Chi-square or Fisher’s exact test as appropriate. Differences in medians by group were compared using the Mann-Whitney U test. Multiple comparisons of cytokine levels among the three groups were performed using the Dunn’s multiple comparisons test. The Spearman’s correlation coefficient was calculated to assess the correlation between the different cytokines and to assess the correlation between cytokine levels and hematological and biochemical parameters. Data analysis was done with Stata SE 12.0 software (StataCorp, College Station, TX, USA) and GraphPad Prism version 8 (GraphPad Software, San Diego, California, USA).

**Results**

**Clinical characteristics**

The study population was composed mainly of men (64.9%), with a median age of 35 (27–41) years, and with eight or fewer years of education. Demographic and laboratory characteristics by group are shown in Table 1. Asymptomatic individuals were younger (p = 0.013), with more years of education (p < 0.001), and were more often on antiretroviral therapy (p < 0.001) than those in the symptomatic group. The levels of hemoglobin (p < 0.001), lymphocytes count (p < 0.001) and CD4 count (p < 0.001) were lower in symptomatic individuals, while HIV viral load were higher (p < 0.001) in the latter (Table 2).

**Table 1. Comparison of demographic characteristics between groups (univariate analysis) from Pernambuco, Brazil.**

| Characteristic | Symptomatic (N = 35) | Asymptomatic (N = 75) | Healthy controls (N = 24) | p-value |
|---------------|---------------------|----------------------|---------------------------|---------|
| Gender (n (%))| Male | 22 (62.86) | 54 (72) | 11 (45.83) | 0.062 |
| Female | 13 (37.14) | 21 (28) | 13 (54.17) | |
| Age (years)  (median and IQR) | 38 (31–48) | 33 (26–41) | 24 (23–25) | < 0.001 |
| Education (years) (n (%)) | 0–8 | 30 (85.71) | 35 (46.67) | 0 (-) | < 0.001 |
| > 8 | 5 (14.29) | 40 (53.33) | 24 (100) | |
| On ART (n (%)) | 22 (62.86) | 75 (100) | - | < 0.001 |

IQR, interquartile range. ART, antiretroviral therapy. All percentages are column percentages.

https://doi.org/10.1371/journal.pntd.0010542.t001
**Determination of serum cytokine levels**

When compared with the asymptomatic VL-HIV group and with the healthy controls, we observed among the symptomatic VL-HIV coinfected group increased levels of all cytokines tested, mainly of IL-17A, IL-6 and IL-10 (Fig 1). There were no differences in the levels of all cytokines evaluated between asymptomatic VL-HIV coinfected individuals and the healthy controls. We also evaluated whether the lymphocyte T CD4 count was associated with the levels of the various cytokine analyzed. For this, we categorized the CD4 count (< 200, 200–349, and ≥ 350) for each group of coinfected individuals (symptomatic and asymptomatic), and we compared the levels of each cytokine in each CD4 subgroup. We did not observe any statistically significant difference in the analysis (Fig 2).

**Correlations between cytokine levels and laboratory data**

The correlations between cytokine levels and hematological and biochemical parameters for the general sample, including CD4 count and HIV viral load, are shown in Table 3. By group, we did not see any statistically significant correlation between cytokines levels and hematological and biochemical parameters among asymptomatic VL-HIV patients. In the symptomatic group, IL-4 was positively correlated with lymphocytes count (rho = 0.5, p = 0.002), and IL-10 was negatively correlated with hemoglobin levels (rho = -0.4, p = 0.020) and positively correlated with AST levels (rho = 0.36, p = 0.049). We did not observe a statistically significant correlation when we compared each cytokine level with the CD4 count and with the HIV viral load in both groups of VL-HIV coinfected individuals.

Table 2. Comparison of laboratory characteristics between symptomatic and asymptomatic VL-HIV coinfected individuals (univariate analysis) from Pernambuco, Brazil.

| Characteristic (median (IQR)) | Symptomatic (N = 35) | Asymptomatic (N = 75) | p-value |
|-------------------------------|----------------------|-----------------------|---------|
| Hemoglobin (g/dL)             | 10.45 (8.9–11.4)     | 13.1 (12.2–14.7)     | < 0.001 |
| Leucocytes (/mm³)             | 4695 (3030–7360)     | 5350 (4600–6300)     | 0.558   |
| Neutrophils (/mm³)            | 2790 (1400–5535)     | 2576 (2064–3358)     | 0.348   |
| Lymphocytes (/mm³)            | 880 (538–1300)       | 1782 (1551–2310)     | < 0.001 |
| Platelets x10⁵ (/mm³)         | 227.5 (156–333)      | 253.5 (207.5–299.5)  | 0.180   |
| AST (U/L)                     | 31 (20.7–72.4)       | 25 (21–37)           | 0.239   |
| ALT (U/L)                     | 30 (20–53.6)         | 24 (18–38)           | 0.150   |
| Urea (mg/dL)                  | 27.3 (22.67–39.75)   | 23 (19–34)           | 0.065   |
| Creatinine (mg/dL)            | 0.71 (0.51–0.9)      | 0.8 (0.7–1)          | 0.159   |
| LTCD4+ count (cells/μL)       | 197 (16–414)         | 587 (422–765)        | < 0.001 |
| Characteristic (n (%))        |                      |                      |         |
| LTCD4+ count (cells/μL)       |                      |                      |         |
| < 200                         | 16 (51.6)            | 8 (11.6)             | < 0.001 |
| 200 - 349                     | 6 (19.4)             | 6 (8.7)              |         |
| ≥ 350                         | 9 (29.0)             | 55 (79.7)            |         |
| HIV viral load (copies/mL)    |                      |                      |         |
| Undetectable (< 50)           | 5 (16.67)            | 52 (75.36)           |         |
| 50–100,000                    | 14 (46.67)           | 15 (21.74)           | < 0.001 |
| ≥ 100,000                     | 11 (36.67)           | 2 (2.90)             |         |

IQR, interquartile range; AST, aspartate aminotransferase; ALT, alanine aminotransferase. LTCD4+, lymphocyte T CD4+.

All percentages are column percentages.

https://doi.org/10.1371/journal.pntd.0010542.t002
Serum cytokine levels in HIV-Leishmania coinfected Brazilian patients

**IFN-γ**

- HC: Healthy Control
- Asym: Asymptomatic
- Sym: Symptomatic

*** TNF

- HC: Healthy Control
- Asym: Asymptomatic
- Sym: Symptomatic

** IL-2 **

- HC: Healthy Control
- Asym: Asymptomatic
- Sym: Symptomatic

IL-4

- HC: Healthy Control
- Asym: Asymptomatic
- Sym: Symptomatic

IL-6

- HC: Healthy Control
- Asym: Asymptomatic
- Sym: Symptomatic

IL-10

- HC: Healthy Control
- Asym: Asymptomatic
- Sym: Symptomatic

IL-17A

- HC: Healthy Control
- Asym: Asymptomatic
- Sym: Symptomatic
In this study, we measured the serum levels of different cytokines in symptomatic and asymptomatic VL-HIV coinfected individuals living in a VL-endemic area. We also correlated these cytokine levels with laboratorial characteristics. We observed increased levels of IL-17A, IL-6 and IL-10 in the symptomatic VL-HIV coinfected group. In this same group, low levels of IL-2, IFN-γ and TNF were observed. Low levels of all cytokines analyzed were found in samples from asymptomatic VL-HIV coinfected individuals and from the healthy controls.

Usually, it is expected a predominantly T helper type 2 (mainly IL-4, IL-6, and IL-10) response in symptomatic Leishmania infections [18, 28], however, a mixed T helper type 1 (mainly IL-2, IFN-γ)/T helper type 2 response could also be seen [29, 30]. Probably, this dichotomy is not the only factor involved in the course of infection [28, 31], and other variables such as parasitic load, nutritional status, and genetic factors, might have an important role in the development and outcome of the disease [32, 33].

In our study, IL-6 levels were sixteen times greater in symptomatic individuals when compared with the asymptomatic individuals and with the healthy controls. High levels of IL-6 have already been observed in patients with active VL, and this could be related to more severe cases and death [18, 19]. Increased IL-6 levels are also related to HIV replication [34, 35], which could explain the high levels in our group of symptomatic VL-HIV coinfected patients, as they presented more frequently with detectable HIV viral load. Similarly, a negative correlation between IL-6 and CD4 count was previously reported [34], with high CD4 counts associated with low IL-6 levels. This could have been reflected in the low values observed in the healthy controls and in the asymptomatic VL-HIV coinfected individuals–the last ones presenting high LTCD4+ levels (median of 587 cells/μL) when compared with those who were symptomatic (median of 197 cells/μL).

It is known that IL-6 influences IL-17 production [36], and this could explain why these two cytokines were the highest expressed. IL-17, an interleukin related to the Th17 profile, was described having a protective effect against the Leishmania (L) donovani infection at high levels [37]. In our study, IL-17A presented the highest levels, particularly among the symptomatic patients. This could be due to the fact that the Th17 response is necessary to control infections caused by intracellular pathogens [36]. Higher serum levels of IL-17A have been described in patients with active VL [38]. IL-17A levels started to decrease during the treatment, but remained higher compared with uninfected study participants [39]. Furthermore, IL-17 has been associated with susceptibility to L. donovani infection in an animal model [40], which likewise might explicate our findings.

Regarding IL-10, we detected levels twelve times higher in symptomatic individuals when compared with asymptomatic individuals and with the healthy controls. In the asymptomatic group, we observed low serum concentrations of IL-10, not significantly higher than in the healthy controls. It was previously demonstrated that IL-10 levels are higher during active VL [14, 18, 30]. Further, some studies suggest an association between high IL-10 levels and the severity of the disease [17, 23]. The opposite is also true, where asymptomatic individuals often present low levels of IL-10 [41]. High levels of IL-10 could inhibit the human immune response against Leishmania parasites [31]. In addition, IL-10 has a regulatory role on the Th1 immune response, which in turn, when exacerbated, presents a harmful effect on the host. Therefore, the balance of IL-10 production may be determinant to the progression of the
Fig 2. Serum cytokine levels by CD4 count, by disease group (asymptomatic and symptomatic VL-HIV coinfecte d individuals). ns, not significant.

https://doi.org/10.1371/journal.pntd.0010542.g002
disease. We also observed in symptomatic individuals a positive correlation between IL-10 and AST levels, which have previously been found to be associated to active VL [18, 42]. Active VL could also explain the negative correlation we observed between IL-10 and hemoglobin levels, since anemia is a frequent finding in patients with VL.

In this study we used stored serum samples, which could be seen as a potential limitation since the levels of the cytokines might decrease overtime and could be affected by a freeze-thaw cycle. As a cross-sectional study, we cannot infer about the clinical outcomes of the participants and their correlation with the various cytokine levels. Moreover, since we did not explore the presence of other opportunistic infections, their role on the cytokine levels of the patients evaluated could act as a confounder factor. Further cohort studies could clarify the potential influence of other opportunistic and latent coinfections (e.g., toxoplasmosis or tuberculosis) on the immune profile of the VL-HIV coinfected cases.

Conclusions

We observed among the symptomatic VL-HIV coinfected participants high levels of IL-17A, IL-6 and IL-10, compared with the asymptomatic coinfected individuals and the healthy controls. The LTCD4+ count showed no relation with the cytokine levels. The cytokines IL-6 and IL-10 seems to be related with active VL even in cases of coinfection with HIV. More studies among HIV-positive patients are needed to better understand the role of these cytokines in terms of prognosis, prediction of cure or relapses in these coinfected individuals. Such information could better guide prophylaxis and treatment—and consequently might reduce lethality and unnecessary medical interventions. Cohort studies, and studies based on cell stimulation with Leishmania antigens, and with a more wide-ranging group of cytokines and other biomarkers, can be useful to fill these gaps.

Author Contributions

Conceptualization: Diego Lins Guedes, Elis Dionísio da Silva, Valéria Rêgo Alves Pereira, Zulma Maria de Medeiros.

Formal analysis: Diego Lins Guedes, Elis Dionísio da Silva, Achilleas Tsoumanis.

Funding acquisition: Zulma Maria de Medeiros.

Table 3. Correlation coefficients between serum cytokine levels and general laboratory data of all patients.

|                         | IFN-γ | TNF  | IL-2 | IL-4 | IL-6 | IL-10 | IL-17A |
|-------------------------|-------|------|------|------|------|-------|--------|
| LTCD4 (cells/μL)        | -0.17 | -0.17| -0.23 | -0.22 | -0.33 | -0.26 | -0.28  |
| HIV viral load (copies/mL) | 0.22  | 0.10 | 0.24  | 0.34  | 0.40  | 0.39  | 0.28   |
| Hemoglobin (g/dL)       | -0.35 | -0.38| -0.29 | -0.35 | -0.38 | -0.44 | -0.39  |
| Neutrophils (/mm³)      | 0.04  | 0.01 | 0.11  | 0.14  | 0.04  | 0.04  | -0.01  |
| Lymphocytes (/mm³)      | -0.18 | -0.17| -0.10 | -0.19 | -0.42 | -0.43 | -0.30  |
| Platelets (/mm³)        | -0.09 | -0.13| -0.06 | 0.04  | -0.21 | -0.22 | -0.15  |
| AST (U/L)               | 0.14  | 0.08 | 0.14  | 0.03  | 0.20  | 0.26  | 0.25   |
| ALT (U/L)               | 0.04  | -0.03| 0.09  | 0.09  | 0.12  | 0.19  | 0.15   |

IFN-γ, interferon-gamma; TNF, tumor necrosis factor; IL, interleukin; LTCD4, lymphocyte T CD4+.

* p ≤ 0.05

* * p ≤ 0.005

* * * p ≤ 0.0005.

https://doi.org/10.1371/journal.pntd.0010542.t003
Investigation: Diego Lins Guedes, Elis Dionísio da Silva, Maria Carolina Accioly Brelaz Castro, Walter Lins Barbosa Júnior, Valéria Rêgo Alves Pereira, Zulma Maria de Medeiros.

Methodology: Diego Lins Guedes, Elis Dionísio da Silva, Walter Lins Barbosa Júnior, Valéria Rêgo Alves Pereira, Zulma Maria de Medeiros.

Project administration: Diego Lins Guedes, Valéria Rêgo Alves Pereira, Zulma Maria de Medeiros.

Resources: Elis Dionísio da Silva, Maria Carolina Accioly Brelaz Castro, Walter Lins Barbosa Júnior, Valéria Rêgo Alves Pereira.

Supervision: Valéria Rêgo Alves Pereira, Zulma Maria de Medeiros.

Validation: Valéria Rêgo Alves Pereira.

Visualization: Diego Lins Guedes, Elis Dionísio da Silva, Maria Carolina Accioly Brelaz Castro, Walter Lins Barbosa Júnior, Ana Victoria Ibarra-Meneses, Achilleas Tsoumanis, Wim Adriaensen, Johan van Griensven, Valéria Rêgo Alves Pereira, Zulma Maria de Medeiros.

Writing – original draft: Diego Lins Guedes, Valéria Rêgo Alves Pereira, Zulma Maria de Medeiros.

Writing – review & editing: Diego Lins Guedes, Elis Dionísio da Silva, Maria Carolina Accioly Brelaz Castro, Walter Lins Barbosa Júnior, Ana Victoria Ibarra-Meneses, Achilleas Tsoumanis, Wim Adriaensen, Johan van Griensven, Valéria Rêgo Alves Pereira, Zulma Maria de Medeiros.

References

1. Alvar J, Vélez ID, Bern C, Herrero M, Desjeux P, Cano J, et al. Leishmaniasis Worldwide and Global Estimates of Its Incidence. Kirk M, editor. PLoS One. 2012; 7: e35671. https://doi.org/10.1371/journal.pone.0035671 PMID: 22933548

2. WHO. WHO website—Leishmaniasis: fact sheets. 2019. Available: https://www.who.int/en/news-room/fact-sheets/detail/leishmaniasis.

3. Burza S, Croft SL, Boelaert M, Organizacão Mundial da Saúde. Leishmaniasis. Lancet. 2018; 392: 951–970. https://doi.org/10.1016/S0140-6736(18)31204-2 PMID: 30126638

4. Pan American Health Organization. Leishmaniasis: Epidemiological Report of the Americas. 2019. p. 8. Available: http://iris.paho.org/xmlui/bitstream/handle/123456789/50505/Leish report2019_eng.pdf?sequence=9&isAllowed=y

5. Henn GA de L, Ramos Júnior AN, Colares JKB, Mendes LP, Silveira JGC, Lima AAF, et al. Is Visceral Leishmaniasis the same in HIV-coinfected adults? Brazilian J Infect Dis. 2018; 22: 92–98. https://doi.org/10.1016/j.bjid.2018.03.001 PMID: 29601790

6. Das VN, Siddiqui NA, Verma RB, Topno RK, Singh D, Das S, et al. Asymptomatic infection of visceral leishmaniasis in hyperendemic areas of Vaishali district, Bihar, India: A challenge to kala-azar elimination programmes. Trans R Soc Trop Med Hyg. 2011; 105: 661–666. https://doi.org/10.1016/j.trstmh.2011.08.005 PMID: 21945327

7. Molina R, Jiménez M, García-Martínez J, San Martín JV, Carrillo E, Sánchez C, et al. Role of asymptomatic and symptomatic humans as reservoirs of visceral leishmaniasis in a Mediterranean context. Schönnagl G, editor. PLoS Negl Trop Dis. 2020; 14: e0008253. https://doi.org/10.1371/journal.pntd.0008253 PMID: 32324738

8. Adriaenssen W, Dorlo TPCC, Vanham G, Kestens L, Kaye PM, van Griensven J. Immunomodulatory Therapy of Visceral Leishmaniasis in Human Immunodeficiency Virus-Coinfected Patients. Front Immunol. 2018; 8: 1943. https://doi.org/10.3389/fimmu.2017.01943 PMID: 29375567

9. Taslimi Y, Zahedifard F, Rafati S. Leishmaniasis and various immunotherapeutic approaches. Parasitolology. 2018; 145: 497–507. https://doi.org/10.1017/S003118201600216X PMID: 27974063

10. Ruiz-Postigo JA, Grout L, Jain S. Global leishmaniasis surveillance, 2017–2018, and first report on 5 additional indicators. Wkly J Infect Dis Rec. 2020; 25: 265–280. Available: https://www.who.int/publications/i/item/who-wer9525.
Akbari M, Oryan A, Hatam G. Application of nanotechnology in treatment of leishmaniasis: A Review. Acta Trop. 2017; 172: 86–90. https://doi.org/10.1016/j.actatropica.2017.04.029 PMID: 28460833

van Griensven J, Diro E. Visceral Leishmaniasis: Recent Advances in Diagnostics and Treatment Regimens. Infect Dis Clin North Am. 2019; 33: 79–99. https://doi.org/10.1016/j.idc.2018.10.005 PMID: 30712769

Zijlstra EE. Visceral leishmaniasis: A forgotten epidemic. Arch Dis Child. 2016; 101: 561–567. https://doi.org/10.1136/archdischild-2015-309302 PMID: 26895806

Ibarra-Menezes A V., Carrillo E, Sánchez C, García-Martínez J, López Lacomba D, San Martin J V., et al. Interleukin-2 as a marker for detecting asymptomatic individuals in areas where Leishmania infantum is endemic. Clin Microbiol Infect. 2016; 22: 739.e1–739.e4. https://doi.org/10.1016/j.cmi.2016.05.021 PMID: 27265372

Botana L, Ibarra-Menezes AV, Sánchez C, Castro A, Martin JVS, Molina L, et al. Asymptomatic immune responders to Leishmania among HIV positive patients. PLoS Negl Trop Dis. 2019; 13: 1–14. https://doi.org/10.1371/journal.pntd.0007461 PMID: 31158223

Van den Bogaart E, Talha ABA, Straetemans M, Adams ER, Grobusch MP, et al. Cytokine profiles amongst Sudanese patients with visceral leishmaniasis and malaria co-infections. BMC Immunol. 2014; 15: 1–10. https://doi.org/10.1186/1471-2172-15-16 PMID: 24886212

Gama MEA, Gomes CM de C, Silveira FT, Laurenti MD, Gonçalves E da G, Silva AR da, et al. Severe visceral leishmaniasis in children: The relationship between cytokine patterns and clinical features. Rev Soc Bras Med Trop. 2013; 46: 741–745. https://doi.org/10.1590/0037-8682-2003-2013 PMID: 24474016

dos Santos PL, de Oliveira FA, Santos MLB, Cunha LCS, Lino MTBB, de Oliveira MFSS, et al. The Severity of Visceral Leishmaniasis Correlates with Elevated Levels of Serum IL-6, IL-27 and sCD14. Oliveira SC, editor. PLoS Negl Trop Dis. 2016; 10: e0004375. https://doi.org/10.1371/journal.pntd.0004375 PMID: 26814478

Costa DL, Rocha RL, Carvalho RMA, Lima-Neto AS, Harhay MO, Costa CHN, et al. Serum cytokines associated with severity and complications of kala-azar. Pathog Glob Health. 2013; 107: 78–87. https://doi.org/10.1179/2047773213Y.0000000078 PMID: 23683334

Ibarra-Meneses A V., Ghosh P, Hossain F, Chowdhury R, Mondal D, Alvar J, et al. IFN-γ, IL-2, IP-10, and MIG as Biomarkers of Exposure to Leishmania spp., and of Cure in Human Visceral Leishmaniasis. Front Cell Infect Microbiol. 2017; 7: 1–8. https://doi.org/10.3389/fcimb.2017.00001

Carrillo E, Carrasco-Antón N, López-Medrano F, Salto E, Fernández L, San Martín JV, et al. Cytokine Release Assays as Tests for Exposure to Leishmania, and for Confirming Cure from Leishmaniasis, in Solid Organ Transplant Recipients. PLoS Negl Trop Dis. 2015; 9: 1–14. https://doi.org/10.1371/journal.pntd.0004179 PMID: 26496965

Tadesse D, Abdissa A, Mekonnen M, Belay T, Hailu A. Antibody and cytokine levels in visceral leishmaniasis patients with varied parasitemia before, during, and after treatment in patients admitted to Arba Minch General Hospital, southern Ethiopia. PLoS Negl Trop Dis. 2021; 15. https://doi.org/10.1371/journal.pntd.0009067 PMID: 33476331

Ramos PK, Carvalho KI, Rosa DS, Rodrigues AP, Lima LV, Campos MB, et al. Serum cytokine responses over the entire clinical-immunological spectrum of human Leishmania (L.) infantum chagasi infection. Biomed Res Int. 2016; 2016. https://doi.org/10.1155/2016/6937988 PMID: 27051688

Souza NP, de Almeida A do BPF, de Freitas TPT, da Paz RCR, Dutra V, Nakazato L, et al. Leishmania (Leishmania) infantum chagasi in canídeos silvestres manílhos em catheiro, no Estado de Mato Grosso. Rev Soc Bras Med Trop. 2010; 43: 333–335. https://doi.org/10.1590/s0037-86822010000300024 PMID: 20563507
cytokine profile and disease outcome. Scand J Immunol. 2005; 62: 487–495. https://doi.org/10.1111/j.1365-3083.2005.01686.x PMID: 16305646

30. Costa ASA, Costa GC, de Aquino DMC, de Mendonça VRR, Barral A, Barral-Netto M, et al. Cytokines and visceral leishmaniasis: A comparison of plasma cytokine profiles between the clinical forms of visceral leishmaniasis. Mem Inst Oswaldo Cruz. 2012; 107: 735–739. https://doi.org/10.1590/s0074-02762012000600005 PMID: 22990961

31. Nylen S, Sacks D. Interleukin-10 and the pathogenesis of human visceral leishmaniasis. Trends Immunol. 2007; 28: 378–384. https://doi.org/10.1016/j.it.2007.07.004 PMID: 17689290

32. Dayakar A, Chandrasekaran S, Kuchipudi S V, Kalangi SK. Cytokines: Key Determinants of Resistance or Disease Progression in Visceral Leishmaniasis: Opportunities for Novel Diagnostics and Immunotherapy. Front Immunol. 2019; 10. https://doi.org/10.3389/fimmu.2019.00670 PMID: 31024534

33. Gonçalves-de-Albuquerque S da C, Pessoa-e-Silva R, Trajano-Silva LAM, de Goes TC, de Morais RCS, da C. Oliveira CN, et al. The Equivocal Role of Th17 Cells and Neutrophils on Immunopathogenesis of Leishmaniasis. Front Immunol. 2017; 8. https://doi.org/10.3389/fimmu.2017.01437 PMID: 29163510

34. Borges AH, O’Connor JL, Phillips AN, Rønsholt FF, Pett S, Vjecha MJ, et al. Factors Associated With Plasma IL-6 Levels During HIV Infection. J Infect Dis. 2015; 212: 585–595. https://doi.org/10.1093/infdis/jiv123 PMID: 25722296

35. Tanaka T, Kishimoto T. The Biology and Medical Implications of Interleukin-6. Cancer Immunol Res. 2014; 2: 288–294. https://doi.org/10.1158/2326-6066.CIR-14-0022 PMID: 2474575

36. Khader SA, Gopal R. IL-17 in protective immunity to intracellular pathogens. Virulence. 2010; 1: 423–427. https://doi.org/10.4161/viru.1.5.12862 PMID: 21178483

37. Pitta MGR, Romano A, Cabantous S, Henri S, Hammad A, Kouriba B, et al. IL-17 and IL-22 are associated with protection against human kala azar caused by Leishmania donovani. J Clin Invest. 2009; 119: 2379–2387. https://doi.org/10.1172/JCI38813 PMID: 19620772

38. Babaloo Z, Oskoei MR, Kohansal MH, Barac A, Ahmadpour E. Serum profile of IL-1β and IL-17 cytokines in patients with visceral leishmaniasis. Comp Immunol Microbiol Infect Dis. 2020; 69: 101431. https://doi.org/10.1016/j.cimid.2020.101431 PMID: 32059125

39. Nascimento MSL, Carregaro V, Lima-Júnior DS, Costa DL, Ryffel B, Duthie MS, et al. Interleukin 17A acts synergistically with interferon γ to promote protection against leishmaniasis infantum infection. J Infect Dis. 2015; 211: 1015–1026. https://doi.org/10.1093/infdis/jiu531 PMID: 25274569

40. Terrazas C, Varikutti S, Kimble J, Moretti E, Boyaka PN, Satoskar AR. IL-17A promotes susceptibility during experimental visceral leishmaniasis caused by Leishmania donovani. FASEB J. 2016; 30: 1135–1143. https://doi.org/10.1096/fj.15-277202 PMID: 26581600

41. de Oliveira França A, Soares LS, Pompilio MA, Tozetti IA, Bonif CM, Dorval MEMC. Cytokine profile in Leishmania-positive blood donors. Schallig HDFH, editor. PLoS One. 2020; 15: e0238933. https://doi.org/10.1371/journal.pone.0238933 PMID: 32966326

42. Teles L de F, Viana AG, Cardoso MS, Pinheiro GRG, Bento GA, Lula JF, et al. Evaluation of medullary cytokine expression and clinical and laboratory aspects in severe human visceral leishmaniasis. Parasite Immunol. 2021; 43: 1–10. https://doi.org/10.1111/pim.12880 PMID: 34558674