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ORIGINAL ARTICLE

C-REACTIVE PROTEIN FOR THE DIAGNOSIS AND PROGNOSIS OF VISCERAL LEISHMANIASIS CAUSED BY *Leishmania infantum*

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ABSTRACT

Acute-phase reaction (APR) and its marker C-reactive protein (CRP) are heightened in some infectious diseases. APR may contribute to clinical manifestations of systemic inflammation such as hemorrhages, anemia and edema. These symptoms are shared by visceral leishmaniasis (VL), a disease caused by the protozoa *Leishmania infantum* and *L. donovani*. The former is zoonotic, hitting mostly children and the immunosuppressed, with higher mortality. As APR and CRP have not been studied in VL caused by *L. infantum*, we decided to investigate their role as diagnostic and prognostic tools in Brazil. We measured CRP in 136 patients before the treatment of VL and 128 who survived and returned 30 days later and compared it to the clinical presentation, HIV status, and disease severity. Sensitivity for the disease was 97.8% (95%CI: 93.7 - 99.5) and specificity was 85.9% (95%CI: 78.7 - 91.0) with the cut-off of 10mg/L. There was no association of CRP concentration with demographic, clinical and laboratory data. The correlation between pre-and post-treatment levels existed but was poor. Marginal association with the presence of parasites in the bone marrow and death was noticed. The role of APR in the pathogenesis of VL and disease severity remains to be explored. However, the study reveals the significant role of CRP for VL caused by *L. infantum* and should be routinely required for the diagnosis and follow-up.

KEY WORDS: visceral leishmaniasis, kala-azar, acute phase reaction, C-reactive protein, *Leishmania infantum*, diagnosis, prognosis.

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INTRODUCTION

Visceral leishmaniasis (VL), or kala-azar, is a febrile parasitic illness transmitted by the bites of sandflies. It is endemic in Central Asia, the Middle East, Mediterranean Basin, and Latin America, but the largest numbers arise from the Indian Subcontinent, East Africa and Brazil. The two disease-causing protozoa, *Leishmania infantum,* and *L. donovani*, lead to slightly different diseases: while *L. donovani* is restricted to humans, *L. infantum* is a zoonosis involving canids, rodents and other animals (Alvar et al., 2012; Burza et al., 2018). While post-kala-azar dermal leishmaniasis is the source of *L. donovani* for transmission (Verma et al., 2010), it is rare in infections caused by *L. infantum*, which hits more children and seems to be more severe (Singh et al., 2011; Burza et al., 2018). Both are significant coinfections for immunosuppressed patients (Akuffo et al., 2018). The two infections share most symptoms, signs, and complications: prolonged fever, paleness, wasting, diarrhea, enlarged spleen and liver, and kidney involvement, together with hematological changes such as anemia, low white blood cell and platelet counts (Burza et al., 2018). The two main complications are hemorrhages due to disseminated intravascular coagulation (DIC) and bacterial coinfections (Costa et al., 2010). Disease severity is strongly linked to a concerted activity leading to exaggerated systemic inflammation determined principally by IL-6, γ-INF, and IL-8 (Costa et al., 2013) counterbalanced by the immunosuppressive action of IL-10 (Nylén & Sacks, 2007). The disease may be confounded with unrelated conditions such as systemic lupus erythematosus (SLE), leukemia, lymphoma, portal hypertension due to chronic liver diseases, hemolytic anemia, viral infections and other tropical diseases (Cunha et al., 2015).

Acute-phase reaction (APR) happens when the liver changes its protein synthesis profile after a particular stimulus such as injury, inflammation, and infection. By definition, they change their concentration by more than 25%. Proteins can be down-expressed, such as albumin, transferrin, and retinol-binding protein, or increased such as ferritin, serum amyloid A and C-reactive protein (CRP) (Janciauskiene et al., 2011). The last is a routine marker of infection and cure and inflammation, usually with the erythrocyte sedimentation rate (ESR) (Markanday, 2015). Although typically elevated in most infections and inflammatory diseases and some types of cancer, it is low in illnesses included in the differential diagnosis of VL (Pepys & Hirschfield, 2003; Kilicarslan & Uysal, 2013; Cunha et al, 2015; Markanday, 2015).

Proinflammatory cytokines trigger APR after mononuclear phagocytes are exposed to pattern recognition molecules or danger signals. Upon signal transduction, IL-1, IL-6, and TNF-a are expressed. IL-1 further stimulates IL-6, which is the principal cytokine to trigger APR. Among the systemic spectrum of activities of IL-6 are fever, activation of the coagulation cascade, inappetence, wasting, cachexia and B-cell activation and proliferation (Pepys & Hirschfield, 2003; Janciauskiene et al., 2011; Hunter & Jones, 2015).

APR has significant clinical consequences. Besides being an evolutionary old innate defense helping to control infections at its site, it also leads to systemic effects through the opsonization of microorganisms and healing (Pepys & Hirschfield, 2003; Janciauskiene et al., 2011). The three most visible clinical manifestations of it are coagulation changes [thrombosis or disseminated intravascular coagulation (DIC)], anemia, and edema (Bisoendial, 2005; Johansson et al., 2011; Madu & Ughasoro, 2017; Eckart et al., 2020). DIC results from the additive effect of increased expression of coagulation factors and downregulation of anticoagulant proteins (Davidson, 2013), and the IL-6 induced expression of tissue factor in the surface of mononuclear cells (Singh, 1999). Anemia is generated by the action of the iron-retaining acute phase protein hepcidin (Sangkhae & Nemeth, 2017) and edema by the reduced synthesis of seroalbumin (Soeters et al., 2019).

CRP has been demonstrated to be very high in patients with VL caused by *L. donovani*, leading to high sensitivity for diagnosis. Due to its high sensitivity, it has been used as a marker of response to treatment (Singh, 1999). Also, its association with parasite burden indicates its use as a biomarker of parasitological cure. Although CRP is the most used APR marker, it has not yet been investigated in VL caused by *L. infantum*. Many of the manifestations of APR overlap with those of VL, so we decided to investigate, for the first time, the role of PCR as a diagnostic and prognostic tool for VL caused by *L. infantum.*

MATERIALS AND METHODS

Study design

Study participants were consecutively admitted to the Tropical Medicine Institute "Natan Portella" from mid-2018 to mid-2019 as part of a clinical trial on vitamin A and zinc supplementation for VL treatment. Patients were included in the study after diagnostic confirmation of VL and followed for 30 days after starting the specific treatment. Diagnosis was performed through microscopic direct observation of *Leishmania* amastigotes in a bone marrow smear. Also, the bone marrow smear was routinely sowed in Novy–MacNeal–Nicolle medium (NNN) covered with Schneider's media enriched with 3% human urine. If direct examination was negative, OnSite Leishmania Ab Rapid Test®, CTK, CA immunochromatographic test was performed, albeit also performed under physician's request. Then, if negative and required by the assistant physician, usually a conventional in-house polymerase chain reaction (PCR), amplifying a segment of the conservated region of *Leishmania* kDNA is performed. The sole inclusion criterion was age over six months. Patients with hematological neoplasms submitted to chemotherapy, pregnant women, and patients who presented contraindications to vitamin A and zinc supplementation were excluded.

C-reactive protein, blood tests, and disease severity

For C-reactive protein (PCR), the blood sample (4.0 mL) was transferred to a tube with clot activator, centrifuged at 3,200 rpm for 12 minutes; the serum obtained was stored under refrigeration (-20ºC) and analyzed by a fixed-point immunokinetic method in dry chemistry, considering the reference value below 10.0 mg/dL (Vitros XT 7600 equipment®, Ortho Clinics Diagnostics, Haritan, NJ, USA). Hemoglobin, white blood cells (WBC), and platelets were performed by hospital routine laboratory evaluation. Disease severity was established by using the Kala-Cal prognostic scoring system (available at www.sbmt.org/kalacal/).

Statistical analysis

The statistical package Stata 15 version 15.1, College Station, TX, was used. According to the distribution, variables were tested for normality, and parametric or non-parametric tests were used (for independent or paired variables) and the t-test. The analysis of variance analysis were performed when indicated as well as univariate and multivariate linear regression. Pearson and Spearman correlation tests were also performed, again according to the distribution of variables distribution. 95% confidence intervals (95% CI) and interquartile range (IQR) were calculated accordingly.

Ethical issues

All ethical and legal aspects regarding the research phases were respected following resolution 466/2012 of the National Health Council, which contains guidelines and regulatory standards for research in humans. The study was approved by the Ethics and Research Committee of the Federal University of Piauí under the number 2.445.708. To preserve the participants' anonymity, they were identified only according to the research enrollment number.

RESULTS

A total of 136 patients with VL measured CRP before treatment, and 128 measured it at day 30 due to seven deaths and only one loss of follow-up. Among those who started the follow-up, 34.6% were under five years of age, while 9.6% were over 50. Most were men (73.5%) and 17/136 (12.5%) had HIV coinfection. Most were diagnosed by bone marrow parasitology tests (89/114, 78.1%) rather than by serology although all 57 immunochromatographic tests were reagent. No PCR was requested. Regarding disease severity, the Kala-Cal software indicated that 25.0% had a chance of death above 10%. However, only seven patients died (5.2%) (Table 1).

Table 1. Characteristics of the study population.

Characteristics	Number	Percent (95% CI)
Age group (years)		
<2	26	19.2 (12.9 - 26.7)
2 <5	21	15.4 (9.8 - 22.6)
5 < 18	22	16.2 (10.4 - 23.5)
18 <50	54	39.7 (31.4 - 48.5)
>50	13	9.6 (5.2 - 15.8)
Sex		
Male	100	73.5 (65.3 - 80.7)
Female	36	26.5 (19.3 - 34.7)
HIV coinfection		
No	119	87.5 (80.7 - 92.6)
Yes	17	12.5 (7.5 - 19.3)
Bone marrow direct examination or culture for the presence of *Leishmania* sp		
Positive	86	77.5 (68.6 - 84.9)
Negative	25	22.5 (15.1 - 31.4)
Chance of death above 10%*		
No	102	75.0 (85.2 - 96.2)
Yes	34	25.0 (22.2 - 40.1)
Death		
No	129	95.9 (89.7 - 97.9)
Yes	7	5.2 (2.1 - 10.3)

CI: confidence interval for binomial proportions (exact Clopper–Pearson method).
*As estimated by the prognostic software Kala-Cal (available at www.sbmt.org/kalacal/).

Only three patients died before the 30th day and had no CRP measured at this time. Four died afterward but the precise day of death was not known. All those who survived and returned to follow-up (early survivors) improved substantially with the anti-*Leishmania* drug. Weight increased by about 1.6Kg on average. While almost all patients had splenomegaly at the diagnosis, only 40/108 (37%) had palpable spleen at day 30. None had any claim or observed hemorrhage or bacterial infection on the 30th day, while hemoglobin improved noticeably and WBC and platelets returned to reference values (Table 2).

Table 2. Clinical characteristics before and after the anti-*Leishmania* treatment.

Clinical characteristic	Before treatment	After treatment
Weight in kg, mean (95% CI)	34.7 (30.1 - 39.3)	36.3 (31.5 - 41.1)
Percent with splenomegaly (95% CI)	97.3 (92.4 - 99.4)	37.0 (27.9 - 46.9)
Percent with hemorrhagic events (95% CI)	23.7 (16.9 - 31.7)	None
Percent with bacterial infections (95% CI)	15.1 (9.6 - 22.2)	None
Hemoglobin in g/dL, mean (95% CI)	8.3 (8.0 - 8.7)	11.6 (11.3 - 11.9)
WBC in number of cells/mL of blood, mean (95% CI)	3,843.2 (3,413.2 - 4,273.3)	7,557.0 (6,903.9 - 8,210.2)
Neutrophils in number of cells/ml of blood, mean (95% CI)	1,687.2 (1,583.4 - 1,794.8)	3,378.0 (3,143.7 - 3,612.3)
Platelets in number of cells/ml of blood, mean (95% CI)	122,842.4 (109,071.4 - 136,613.5)	285,317.6 (263,640.8 - 306,994.4)

CI: Confidence interval; WBC: White blood cells; g/dL: grams per deciliters; kg: kilograms; mL: milliliters.

There were no associations between CRP concentration with age, sex, weight loss, HIV-infection, spleen size, bleeding, bacterial infection, disease severity, hemoglobin, WBC, platelets, serum albumin, and alanine aminotransferase, neither before treatment, at hospital admission, nor after anti-protozoal treatment, 30 days later. It is worthy of mentioning, however, that CRP was higher among survivors (median 66.5mg/l vs. 38.7mg/l) and among those with positive parasitology tests (median 65.8mg/l vs. 48.6mg/l), albeit with p-values of 0.069 and 0.079, respectively (Data not shown).

CRP was very high before treatment but became much lower 30 days later. The median and mean before treatment were 63.4mg/L and 88.8mg/L, respectively. Thirty days later, the median and mean fell 13 and 10 times, respectively, to 5.0 mg/L and 8.9 mg/L. Sensitivity (proportion with values above the reference) was 97.8% (95% CI: 93.7-99.5) before, but only 14.1% were still above the reference value of 10.0 mg/L at the 30th post-treatment day, e.g., 85.9% who had VL and were treated 30 days earlier had a normal concentration of CRP in the sera (test's specificity) (Table 2).

By regression analysis, correlation of CRP before anti-protozoa treatment and 30 days later was significant ($p<0.001$, adjusted $R^2=0.11$) albeit with weak Spearman's correlation coefficient (rho=0.2, p-value=0.024) (Figure).

Table 3. Median, mean and proportions of normal and high values of C-reactive proteins before and 30 days after the anti-*Leishmania* treatment.

Measure of CRP*	CRP before treatment	Interval	CRP 30 days after treatment	Interval
Median (mg/dL)	63.4	Q_1, Q_3: 34.1 -141.5	5.0	Q_1, Q_3: 5.0 - 7.7
Mean (mg/dL)	88.5	95% CI: 75.7 - 101.3	8.9	6.9 - 10.8
Proportion with normal values (%)	2.2	95% CI: 0.5 - 06.3	85.9	78.7 - 91.0

CI: confidence interval for binomial proportions (exact Clopper–Pearson method); CRP: C-reactive protein; Q1, Q2: 1st and 3rd quartiles.

*Cut-off point = 10 mg/dl.

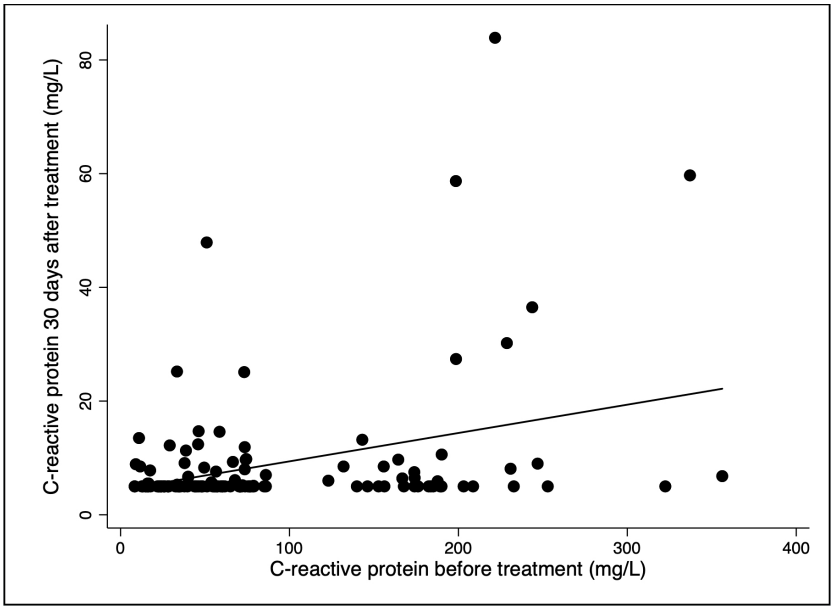

Figure. Correlation of C-reactive protein measured at the diagnosis of kala-azar and 30 days after the start of treatment.
Rho = 0.2; p-value = 0.024.

DISCUSSION

This study is the first to show the excellent performance of CRP as an adjunct test for the diagnosis of VL caused by *L. infantum*. It was performed in a representative sample, including patients following the expected distribution by sex and age, HIV infection, and severe disease. The research shows that CRP is helpful for patients with VL caused by *L. infantum* as it is for the disease caused by *L. donovani* in East Africa and South Asia (Wasunna et al., 1995; Singh et al., 2011). Accordingly, as CRP was high in almost all patients before treatment, it may help diagnose patients in whom parasites could not be detected.

Moreover, since patients with potentially lethal diseases like systemic lupus erythematosus (SLE) and leukemia may have fever, pancytopenia, and hepatosplenomegaly, similarly as in VL, any delay in diagnosis by assuming the diagnosis of VL based solely on clinical data or on serology can be catastrophic (Cunha, 2015). However, CRP usually is low in these diseases (Pepys & Hirschfield, 2003; Kilicarslan & Uysal, 2013). Thus, low levels of CRP in patients with the typical symptoms of VL indicates that this disease is less likely, and other suspicions should be raised. Women are at particular risk of misdiagnosis since they are less likely than men to get VL and are more likely to develop SLE (Rivas-Larrauri & Yamazaki-Nakashimada, 2016).

Among the 30 days survivors, there was a regular improvement of the clinical variables. However, almost a third of them remained with the palpable spleen, which was not associated with CRP levels. Similarly, signs, symptoms, and laboratory data at admission and the residual manifestations on the 30th day were also not associated with CRP (Pepys & Hirschfield, 2003; Black et al., 2004; Janciauskiene et al., 2011). VL's most typical clinical manifestations are fever, anorexia, wasting, paleness, edema, hepatosplenomegaly, loose stools, jaundice, bleeding phenomena such as bruising, and bacterial coinfections. Regularly, the laboratory changes are microcytic, hypochromic, anemia (iron deficient pattern), leukopenia with neutropenia, thrombocytopenia, hypoalbuminemia, polyclonal hypergammaglobulinemia, increased liver enzymes, and conjugated bilirubin, and prolonged coagulation tests (Kager & Rees, 1986; Costa et al., 2016; Burza et al., 2018). The injection of CRP to healthy volunteers resulted in no clinical manifestations, albeit generating a proinflammatory and procoagulant status , suggesting CRP itself is not a cause of clinical manifestations of VL (Bisoendial, 2005; Bisoendial et al., 2005).

However, other acute-phase reactants would contribute to disease. For instance, an increase of fibrinogen and other procoagulant proteins, on the one hand, and reduction of anticoagulants such as thrombomodulin, on the other hand, would promote the bleeding phenomena and changes in coagulation tests, as seen in VL (Davidson, 2013). Hepcidin would retain iron and contribute to iron-deficient anemia (Sangkhae & Nemeth, 2017), and hypoalbuminemia explains the edema present in the most severe disease case (Soeters et al., 2019).

Acute phase reaction is triggered mainly through the action of IL-6 on its IL-6R and gp-130 receptor complex on the surface of hepatocytes, leading to transduction signals, which result in activation of the acute-phase genes (Black et al., 2004; Janciauskiene et al., 2011; Hunter & Jones, 2015). Further, IL-6 broader actions mimic some abnormalities seen in VL. This interleukin activates tissue-factor expression, the main trigger of the disseminated intravascular coagulation (DIC) perceived in severe VL (Blount et al., 1980; Lomtadze et al., 2005; Costa et al., 2013; Boukhris et al., 2015). It promotes wasting and cachexia by inhibiting hunger and increasing muscular catabolism, and B-cell activation and proliferation results in hypergammaglobulinemia (Hunter & Jones, 2015), hence the well-known "albumin-globulin inversion" characteristically seen in VL (Thakur et al., 1981; Kumar et al., 2018). Therefore, the data shows that, indeed, CRP by itself does not seem to contribute to disease significantly but is instead a less critical, a marker of a broad innate response with many actors that promote the symptoms of VL. However, this conclusion does not diminish at all the potential role it may have on host defense against *L. infantum* infection (Black et al., 2004; Laurenti et al., 2004; Janciauskiene et al., 2011) since the lower concentration of CRP among the deceased patients in this study suggests such a role.

The weak association of post-treatment CRP concentration shows that CRP varies widely in VL, partially due to within-individual variation (Bower et al., 2012) and the burden of parasites (Ansari et al., 2007). The weak but noticeable positive association of CRP with the identification of parasites in the bone marrow would suggest that the more parasites are present in bone marrow, the more CRP is secreted (Wasunna et al., 1995b; Singh et al., 2011). The association of CRP with IL-6 in VL caused by *L. donovani* has already been demonstrated (Ansari et al., 2007; Pourcyrous et al., 1998), but the relationship of IL-6 with parasite burden is missing (Verma et al., 2010). Inter and within-individual variation (Verschuur et al., 2004; Bower et al., 2012), distinct study populations, and methodological asymmetries could justify the absence of a clear path of IL-6, acute-phase response, and *L. infantum* parasite burden, indicating a well-designed study with this purpose should be carried out.

The most interesting for VL diagnosis is the CRP sensitivity when measured at the diagnosis and the specificity when measured after cure. The high concentration of CRP before treatment and the high proportion of patients with abnormal values indicates that the test is quite helpful for diagnosing VL caused by *L. infantum* as patients with fever and splenomegaly and normal values of CRP seldom have VL. In addition, the study reveals that CRP is valuable also as a marker of cure, since the value fell dramatically after 30 days and most cured patients have abnormal values, confirm its value for following-up patients after treatment, as previously demonstrated (Wasunna et al., 1995; Singh et al., 2011), and should be used as a routine. However, the CRP value for identifying relapses in HIV-coinfected patients deserves further investigation since, albeit none relapsed, few HIV-infected patients were studied and the followed-up period was too short to detect relapses.

The principal study limitation is the short period of follow-up. A more extended period would certify the patients were cured; therefore, the test specificity would be better evaluated. The data suggest that CRP does not have a prominent role in symptoms of VL caused by *L. infantum* and seems not to be a marker of disease severity. On the other hand, the study decisively demonstrates the role of CRP as an additional, parallel test for the differential diagnosis and follow-up of VL caused by *L. infantum* and should be routinely required for the diagnosis and follow-up.

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CONFLICT OF INTEREST

The authors declare no conflict of interests

REFERENCES

1. Akuffo H, Costa C, van Griensven J, Burza S, Moreno J, Herrero M. New insights into leishmaniasis in the immunosuppressed. *PLoS Negl Trop Dis 12*: e0006375, 2018.
2. Alvar J, Vélez ID, Bern C, Herrero M, Desjeux P, Cano J, Jannin J, Boer MD Leishmaniasis Worldwide and Global Estimates of Its Incidence. *PLoS One 7*: e35671, 2012.
3. Ansari NA, Sharma P, Salotra P. Circulating nitric oxide and C-reactive protein levels in Indian kala azar patients: Correlation with clinical outcome. *Clin Immunol 122*: 343-348, 2007.
4. Bisoendial R. Letter to the Editor. *Circ Res 97*: e115-e116, 2005.
5. Bisoendial RJ, Kastelein JJP, Levels JHM, Zwaginga JJ, van den Bogaard B, Reitsma PH, Meijers JC, Hartman D, Levi M, Stroes ES. Activation of Inflammation and Coagulation After Infusion of C-Reactive Protein in Humans. *Circ Res 96:* 714-716, 2005.
6. Black S, Kushner I, Samols D. C-reactive Protein. *J Biol Chem 279*: 48487-48490, 2004.
7. Blount ER, Hartmann R, Nernoff J. Kala-Azar as a Cause of Disseminated Intravascular Coagulation. *Clin Pediatr (Phila) 19*: 139-140, 1980.
8. Boukhris I, Azzabi S, Chérif E, Kéchaou I, Mahjoub S, Kooli C, Aoun K, Khalfallah N. Hémophagocytose et coagulation intravasculaire disséminée au cours de la leishmaniose viscérale de l'adulte: trois nouveaux cas. *Pan Afr Med J 22*: 96, 2015.
9. Bower JK, Lazo M, Juraschek SP, Selvin E. Within-Person Variability in High-Sensitivity C-Reactive Protein. *Arch Intern Med 172*: 1519, 2012.
10. Burza S, Croft SL, Boelaert M. Leishmaniasis. *Lancet 392:* 951-970, 2018.
11. Costa CHN, Werneck GL, Costa DL, Holanda TA, Aguiar GB, Carvalho AS, Cavalcanti JC, Santos LS. Is severe visceral leishmaniasis a systemic inflammatory response syndrome? A case control study. *Rev Soc Bras Med Trop 43*: 386-392, 2010.

12. Costa DL, Rocha RL, Carvalho RMA, Lima-Neto AS, Harhay MO, Costa CHN, Barral-Neto M, Barral AP. Serum cytokines associated with severity and complications of kala-azar. *Pathog Glob Health 107*: 78-87, 2013.

13. Costa DL, Rocha RL, Chaves E de BF, Batista VG de V, Costa HL, Costa CHN. Predicting death from kala-azar: construction, development, and validation of a score set and accompanying software. *Rev Soc Bras Med Trop 49*: 728-740, 2016.

14. Cunha BA, Lortholary O, Cunha CB. Fever of Unknown Origin: A Clinical Approach. *Am J Med 128:* 1138.e1-1138.e15, 2015.

15. Davidson SJ. *Inflammation and Acute Phase Proteins in Haemostasis*. Acute Phase Proteins: Hannover Medical School, Germany. 2013. p.31-54.

16. Eckart A, Struja T, Kutz A, Baumgartner A, Baumgartner T, Zurfluh S, Neeser O, Huber A, Stanga Z, Mueller B, Schuetz P. Relationship of Nutritional Status, Inflammation, and Serum Albumin Levels During Acute Illness: A Prospective Study. *Am J Med 133*: 713-722.e7, 2020.

17. Hunter CA, Jones SA. IL-6 as a keystone cytokine in health and disease. *Nat Immunol 16:* 448-457, 2015.

18. Janciauskiene S, Welte T, Mahadev R. *Acute Phase Proteins: Structure and Function Relationship*. Acute Phase Proteins: Hannover Medical School, Germany. 2011. 382p.

19. Johansson PI, Sørensen AM, Perner A, Welling KL, Wanscher M, Larsen CF, Ostrowski SR. Disseminated intravascular coagulation or acute coagulopathy of trauma shock early after trauma? An observational study. *Crit Care 15*: R272, 2011.

20. Kager PA, Rees PH. Haematological investigations in visceral leishmaniasis. *Trop Geogr Med 38:* 371-379, 1986.

21. Kilicarslan A, Uysal A RE. Acute phase reactant. *Acta Medica 2*: 2-7, 2013.

22. Kumar V, Agarwal P, Marwah S, Nigam AS, Tiwari A. Spectrum of clinicohematological profile and its correlation with average parasite density in visceral leishmaniasis. *Cytojournal 15:* 19, 2018.

23. Laurenti MD, Örn A, Sinhorini IL, Corbett CEP. The role of complement in the early phase of *Leishmania (Leishmania) amazonensis* infection in BALB/c mice. *Brazilian J Med Biol Res 37*: 427-434, 2004.

24. Lomtadze M, Khochava M, Shalamberidze I, VI K, Vorob'eva E. [Study of intravascular coagulation activation markers in patients with visceral leishmaniasis]. *Georgian Med News 125:* 47-50, 2005.

25. Madu AJ, Ughasoro MD. Anaemia of Chronic Disease: An In-Depth Review. *Med Princ Pract 26:* 1-9, 2017.

26. Markanday A. Acute Phase Reactants in Infections: Evidence-Based Review and a Guide for Clinicians. *Open Forum Infect Dis 2*: 1-7, 2015.

27. Nylén S, Sacks D. Interleukin-10 and the pathogenesis of human visceral leishmaniasis. *Trends Immunol 28*: 378-384, 2007.

28. Pepys MB, Hirschfield GM. C-reactive protein: a critical update. *J Clin Invest 111*: 1805-1812, 2003.

29. Pourcyrous M, Korones S, Crouse D, Bada H s. Interleukin-6 (IL-6), C-reactive protein (CRP), and Cardiorespiratory Responses to Primary Routine Immunization Series in Premature Infants with Bronchopulmonary Dysplasia (BPD) 1467. *Pediatr Res 43*: 251-251, 1998.

30. Rivas-Larrauri F, Yamazaki-Nakashimada MA. Systemic Lupus Erythematosus: Is It One Disease? *Reumatol Clín 12*: 274-281, 2016.

31. Sangkhae V, Nemeth E. Regulation of the Iron Homeostatic Hormone Hepcidin. *Adv Nutr An Int Rev J 8*: 126-136, 2017.

32. Singh S, Sharma U, Mishra J. Post-kala-azar dermal leishmaniasis: recent developments. *Int J Dermatol 50:* 1099-1108, 2011.

33. Singh U. Prognostic value of serum C-reactive protein in kala-azar. *J Trop Pediatr 45*: 226-228, 1999.

34. Soeters PB, Wolfe RR, Shenkin A. Hypoalbuminemia: Pathogenesis and Clinical Significance. *J Parenter Enter Nutr 43*: 181-193, 2019.

35. Thakur CP, Kumar M, Pathak PK. Kala-azar hits again. *J Trop Med Hyg 84*: 271-276, 1981.

36. Verma S, Kumar R, Katara GK, Singh LC, Negi NS, Ramesh V, Salotra P. Quantification of Parasite Load in Clinical Samples of Leishmaniasis Patients: IL-10 Level Correlates with Parasite Load in Visceral Leishmaniasis. *PLoS One 5*: e10107, 2010.

37. Verschuur M, van der Beek MT, Tak HS, Visser LG, de Maat MP. Interindividual variation in the response by fibrinogen, C-reactive protein and interleukin-6 to yellow fever vaccination. *Blood Coagul Fibrinolysis 15*: 399-404, 2004.

38. Wasunna KM, Raynes JG, Were JB, Muigai R, Sherwood J, Gachihi G, Carpenter L, McAdam KP. Acute phase protein concentrations predict parasite clearance rate during therapy for visceral leishmaniasis. *Trans R Soc Trop Med Hyg 89*: 678-681, 1995.