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Case-Control Study

Interleukin-8 Predicts Fatal Kala-Azar: A Case-Control Study

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Abstract

Kala-azar is associated with lethality rates of up to 10%, even with timely treatment. Early identification of mortality biomarkers can significantly reduce this risk. This study, strengthened by a relatively high number of kala-azar-related deaths, aimed to identify serum cytokines as predictive biomarkers of fatal kala-azar. We compared 48 deceased patients with kala-azar to 42 survivors. The concentrations of IL-1β, IL-6, IL-8, IL-10, IL-12, and tumor necrosis factor-α (TNF-α) were measured by flow cytometry. Cytokine levels were compared between groups using the Wilcoxon rank-sum test. Receiver-operating-characteristic (ROC) analysis, coupled with Youden's index, defined the optimal diagnostic threshold. Upon admission, IL-8 concentrations were substantially higher in deceased kala-azar patients (median 76.5 pg/mL [IQR 35.2–242.4 pg/mL]) than in survivors (median 26.4 pg/mL [IQR 15.1–47.7 pg/mL]; $p < 0.0001$). ROC analysis identified 49.3 pg/mL as the optimal cutoff. When rounded to the clinically convenient value of 50 pg/mL, IL-8 predicted a fatal outcome with an area under the curve of 0.75, sensitivity of 70.8%, and specificity of 76.2%. In contrast, IL-1β, IL-6, IL-10, IL-12, and TNF-α showed no significant prognostic utility. Our findings suggest that IL-8 levels equal to or greater than 50 pg/mL is a reliable predictor of fatal kala-azar.

Keywords: visceral leishmaniasis; prognosis; cytokines; inflammation; mortality

1. Introduction

Kala-azar, or visceral leishmaniasis, is potentially fatal despite adequate treatment, with an estimated mortality rate of 10% [1]. Brazil accounts for 91% of kala-azar in the Americas, approximately 1461 cases, where this parasitosis represents a significant public health problem, particularly given the increasing trend in cases of coinfection with kala-azar and human immunodeficiency virus (HIV) [2].

The parasitic disease is caused by *Leishmania infantum* in the Middle East, Central Asia, South America, the Mediterranean Basin, and *L. donovani* in South Asia and East Africa [3]. In the New World, transmission occurs primarily by the bite of female insects of the genus *Lutzomyia* [4]. The most common symptoms of kala-azar are fever, splenomegaly, hepatomegaly, weight loss, and cutaneous

mucocutaneous pallor [5–7]. Beyond these clinical manifestations, bacterial infections and bleeding are associated to fatal outcomes [8–10].

The exacerbated profile of pro- and anti-inflammatory cytokines in patients with kala-azar appears related to disease worsening, particularly in the levels of interferon-gamma (IFN- γ), interleukin-6 (IL-6), IL-8, and IL-10, even in a population without signs of severity [11]. In addition to the significant increase in IFN- γ and IL-8 levels in patients with bleeding, the presence of IL-6 and IFN- γ associated with increased coagulation [12] and, more alarmingly, elevated levels of IL-6 in patients who progressed to death [13] suggest the importance of evaluating cytokines as biomarkers of severity and fatal outcomes in individuals with kala-azar.

Based on these observations, our case-control study was further designed to investigate the predictive power of these immune markers. This case-control study was enhanced by the high proportion of deceased patients compared with surviving controls, thus increasing its analytical power. We compared the pretreatment levels of IL-1 β , IL-6, IL-8, IL-10, IL-12, and TNF- α between kala-azar patients who survived and those who died, with the primary aim of investigating serum cytokines as biomarkers of mortality in patients with kala-azar.

2. Materials and Methods

2.1. Study Design and Setting

We conducted a case-control study in Piauí, Brazil, an area endemic for epidemic outbreaks [14]. The research centre was the Nathan Portella Institute of Tropical Diseases (IDTNP), the reference hospital for treating kala-azar in Teresina, Piauí. Participants were drawn from a cohort that reported 1,009 kala-azar cases and 80 deaths over five years. Deceased individuals were classified as cases, and surviving individuals were classified as controls.

2.2. Inclusion Criteria and Follow-Up

The inclusion criteria were consecutively admitted, newly diagnosed, and untreated symptomatic kala-azar patients proven by molecular, serological, or parasitological diagnostic techniques. There were no restrictions on sex or age, and no exclusion criteria. The attending physician and one of the researchers followed the participants prospectively from admission to the date of discharge or fatal outcome.

2.3. Serum Cytokines

Serum samples collected prior to treatment were stored at -20°C until cytokine analysis was conducted. The levels of IL-1 β , IL-6, IL-8, IL-10, TNF- α , and the interleukin 12 p70 heterodimer (IL-12) were measured by flow cytometry using the Human Inflammatory Cytokines CBA Kit (BD Biosciences, San Jose, CA) on a BD FACS Canto™ instrument (Becton Dickinson, USA) following the manufacturer's instructions, and the levels of clinical and laboratory markers of kala-azar activity were analyzed.

2.4. Clinical and Laboratory Variables

Direct medical interviews or clinical records were obtained using demographic, clinical, and laboratory variables. Haematologic analysis of peripheral blood, kidney and liver function tests, comorbidities, and complications were also evaluated as part of the routine investigation of patients with suspected kala-azar.

2.5. Statistical Analysis

The analysis was conducted using Stata Now/BE 15.1 software. Dichotomous variables were assessed with Fisher's exact or Chi-square tests. We determined the medians and interquartile ranges of cytokine levels in both deceased patients with kala-azar and survivors. Wilcoxon Rank-Sum tests were then employed to compare these median cytokine levels between non-survivors and survivors.

Additionally, we used the Wilcoxon rank-sum test to compare cytokine levels between patients with and without HIV coinfection, evaluating the impact of HIV on cytokine distribution across these groups.

To evaluate the potential of IL-1 β , IL-6, IL-8, IL-10, IL-12, and TNF- α as predictive biomarkers for identifying fatal cases of kala-azar, we conducted Receiver Operating Characteristic (ROC) curve analysis. We first assessed the cytokine levels in their continuous form against a dichotomous outcome (fatal versus non-fatal kala-azar) to determine their discriminatory power. We then established optimal cutoff points using the Youden index.

Only the cytokines that yielded an area under the curve (AUC) greater than 0.70 or less than 0.30, along with sensitivity and specificity equal to or greater than 70%, were selected for further analysis.

Cytokines that met the criteria related to AUC, sensitivity, and specificity were transformed into dichotomous variables based on their Youden-derived cutoff points. In this categorization, a value of 1 (one) was assigned to individuals with cytokine concentrations equal to or greater than the established cutoff point. In contrast, the value 0 (zero) was assigned to those with concentrations below this limit. Additionally, we employed the DeLong test to compare the areas under the curve (AUCs).

2.6. Ethical Approval

The study was approved by the Human Research Ethics Committee of the Federal University of Piauí (CAAE 0116.0.045.203-05) and conducted in accordance with the ethical standards outlined in the Declaration of Helsinki. All patients provided written informed consent.

3. Results

3.1. Study Population

Patients hospitalized at the IDTNP during the study period were considered for inclusion. One thousand nine patients met the case definition, and 80 of them died.

A total of 90 individuals with kala-azar were included in the study, comprising 48 deceased patients (53.3%) and 42 survivors (46.7%). The majority were male (73.3%) and adults (53.3%), with a mean age of 29.5 years (range: 5.7 months to 78 years). Among the nineteen participants living with HIV or AIDS, 16 (84.2%) were in the deceased group ($p = 0.002$). Individuals aged 60 years or older, as well as those presenting with hemorrhagic or infectious complications, were significantly more frequent among the deceased ($p < 0.05$) (**Table 1**).

Table 1. Demographic and clinical characteristics of kala-azar patients by outcome (survivors vs. deceased).

Variables	Survivors (n=42) n (%)	Deceased (n=48) n (%)	Total (n=90) n (%)	p-value
Sex				0.924 ^a
Male	31 (73.8)	35 (72.9)	66 (73.3)	
Female	11 (26.2)	13 (27.1)	24 (26.7)	
Age groups				
Child (age < 18 years)	23 (54.8)	9 (18.7)	32 (35.6)	< 0.0001 ^a
Adult (18-59 years)	18 (42.8)	30 (62.5)	48 (53.3)	0.06 ^a
Elderly (≥ 60 years)	1 (2.4)	9 (18.8)	10 (11.1)	0.014 ^a
Comorbidities				

HIV coinfection	3 (7.1)	16 (33.3)	19 (21.1)	0.002^b
Complications during hospitalization ^c				
Bacterial infection	12 (28.6)	27 (56.2)	39 (43.3)	0.008^a
Hemorrhage	1 (2.4)	22 (45.8)	23 (25.6)	< 0.0001^b

Abbreviations: HIV, human immunodeficiency virus; n, absolute number; %, relative frequency. ^a, Chi-squared test; ^b, Fisher’s Exact test. ^c Patients may have presented with more than one complication during hospitalization. Bold values indicate statistical significance ($p < 0.05$).

3.2. Clinical and Laboratory Findings by Outcome

The median and mean durations of fever until hospital admission were 30 and 60 days, respectively. The most frequent clinical manifestations were fever (90.0%), mucocutaneous pallor (89.0%), fatigue (84.4%), splenomegaly (81.1%), weight loss (77.8%), and hepatomegaly (56.2%). The mean length of the spleen was six cm, with a maximum length of 20 cm, and the mean length of the liver was three cm, with a maximum length of 14 cm. These signs and symptoms were similar between deceased patients and survivors ($p > 0.05$).

However, some laboratory data were statistically different between survivors and deceased patients. The last group exhibited significantly lower mean hemoglobin levels (7.4 g/dL vs. 8.1 g/dL; $p = 0.032$), reduced mean albumin levels (2.9 g/dL vs. 3.3 g/dL; $p = 0.043$), and elevated mean creatinine levels (1.8 mg/dL vs. 0.9 mg/dL; $p = 0.008$) compared to survivors. Conversely, no statistically significant disparities were noted between the deceased and survivor cohorts regarding mean leukocyte count (3,183.5/mm³), mean neutrophil count (1,426.6/mm³), mean platelet count (100,800/mm³), mean AST concentration (82.2 U/L), or mean ALT concentration (53.2 U/L).

3.3. Serum Cytokine Levels in Survivors and Non-Survivors with Kala-Azar

Table 2 presents the mean and median (interquartile range, IQR) cytokine concentrations in patients with kala-azar, stratified by outcome (survivors vs. deceased). At the hospital admission, IL-8 levels were statistically significantly higher in deceased patients ($p < 0.0001$), while IL-1 β , IL-12, and TNF- α levels were significantly lower ($p < 0.002$). The concentrations of IL-6 and IL-10 did not differ significantly between the groups, although IL-6 was near two- times higher among those who lately died (Table 2).

Table 2. Serum cytokine levels (pg/mL) in individuals with kala-azar, stratified by outcome.

Cytokine	Survivors (n=42)		Deceased (n=48)		Total (n=90)		p-value*
	Mean	Median (IQR)	Mean	Median (IQR)	Mean	Median (IQR)	
IL-1 β	22.7	2.5 (1.0–6.6)	3.6	0.0 (0.0–4.0)	12.5	1.7 (0.0 –6.0)	0.001
IL-6	161.3	26.7 (11.2–54.7)	334.0	49.9 (13.1–125.1)	253.4	30.1 (11.4–77.1)	0.100
IL-8	176.5	26.4 (15.1–47.7)	267.0	76.5 (35.2–242.4)	224.8	46.6 (21.9–120.5)	< 0.0001
IL-10	21.4	15.9 (8.0–30.6)	27.4	13.1 (4.8–29.5)	24.6	13.8 (5.6–30.6)	0.571
IL-12	1.8	0.9 (0.0–2.0)	1.5	0.0 (0.0–0.0)	1.6	0.0 (0.0–1.1)	< 0.0001
TNF- α	6.1	1.7 (0.3–6.5)	2.5	0.0 (0.0–2.4)	4.2	0.8 (0.0–3.9)	< 0.001

Abbreviations: IL, interleukin; IL-1 β , interleukin 1 beta; IQR, interquartile range; pg/mL, picograms per milliliter; TNF- α , tumor necrosis factor-alpha. Notes: * *p*-values were calculated using the Wilcoxon Rank-Sum test to compare cytokine levels between groups. Bold values indicate statistical significance (*p*<0.05). Values of 0.0 indicate concentrations below the limit of detection.

3.4. Diagnostic Performance of Serum Cytokines in Predicting Fatal Kala-Azar

Table 3 presents the diagnostic potential of IL-1 β , IL-6, IL-8, IL-10, IL-12, and TNF- α in predicting fatal kala-azar. Among the cytokines evaluated, IL-8 emerged as the most promising biomarker for predicting mortality in individuals with kala-azar. It showed good discriminative power, with an AUC of 0.75 and a 95% confidence interval (95% CI) of 0.65 to 0.86. At its optimal cutoff of 49.3 pg/mL, IL-8 achieved a sensitivity of 70.8% and a specificity of 76.2%. Conversely, IL-6 exhibited low discriminatory power, with an AUC of 0.60 (95% CI: 0.48–0.72). Even more notably, IL-1 β (AUC: 0.31, 95% CI: 0.20–0.42), IL-10 (AUC: 0.47, 95% CI: 0.34–0.59), IL-12 (AUC: 0.30, 95% CI: 0.17–0.37), and TNF- α (AUC: 0.29, 95% CI: 0.18–0.40) all presented AUC values below 0.5.

A reliable cutoff point could not be determined for IL-1 β , IL-12, and TNF- α due to a high frequency of zero values, which limited the performance of logistic regression analysis.

Table 3. Diagnostic performance of serum cytokines (IL-1 β , IL-6, IL-8, IL-10, IL-12, and TNF- α) in predicting death in patients with kala-azar (n=90).

Cytokine	Optimal Cutoff (pg/mL)	Sensitivity (%)	Specificity (%)	AUC (95 % CI)
IL-1 β	0.6	56.3	88.1	0.31 (0.20 – 0.42)
IL-6	49.5	52.1	73.8	0.60 (0.48 – 0.72)
IL-8	49.3	70.8	76.2	0.75 (0.65 – 0.86)
IL-10	45.3	16.7	92.9	0.47 (0.34 – 0.59)
IL-12	0.3	83.3	69.0	0.30 (0.17 – 0.37)
TNF- α	0.0	62.5	85.7	0.29 (0.18 – 0.40)

Abbreviations: AUC: Area under the receiver operating characteristic curve; CI: Confidence interval; IL, interleukin; IL-1 β , interleukin-1 beta; TNF- α , tumor necrosis factor-alpha. Notes: The sample size (n = 90) represents the total number of patients with valid cytokine measurements included in the analysis for each respective cytokine. Optimal cutoff points were determined using the Youden Index.

3.5. Serum Cytokine Profiles by HIV Coinfection Status

As shown in Table 4, we evaluated cytokine levels (means, medians, and interquartile ranges) in kala-azar patients stratified by HIV coinfection status. This analysis was prompted by the observation that 33.3% of patients who died were HIV coinfectd. Our findings indicate that IL-6, IL-8, and IL-10 concentrations were similar in HIV-coinfectd and non-HIV-coinfectd kala-azar patients (*p* > 0.05). In contrast, IL-1 β , IL-12, and TNF- α levels were significantly lower in HIV-coinfectd patients (*p* < 0.05).

Table 4. Serum cytokine levels (pg/mL) in individuals with kala-azar, stratified by HIV coinfection status.

Cytokine	Kala-azar without HIV (n=72)		Kala-azar with HIV (n=19)		Total (n=90)		<i>p</i> - value*
	Mean	Median (IQR)	Mean	Median (IQR)	Mean	Median (IQR)	
IL-1 β	15.6	2.3 (0.7–7.0)	0.9	0.0 (0.0–0.7)	12.5	1.7 (0.0–6.0)	0.0002

IL-6	292.5	29.8 (11.7–77.1)	107.2	34.7 (10.5–129.4)	253.4	30.1 (11.4–77.1)	0.9404
IL-8	247.6	45.5 (19.2–124.1)	139.5	53.4 (25.8–104.5)	224.8	46.6 (21.9–120.5)	0.9606
IL-10	27.0	15.0 (8.1–30.6)	15.5	5.6 (1.5–30.8)	24.6	13.8 (5.6–30.6)	0.0551
IL-12	2.0	0.0 (0.0–1.5)	0.3	0.0 (0.0–0.0)	1.6	0.0 (0.0–1.1)	0.0123
TNF-α	5.2	1.7 (0.0–6.9)	0.2	0.0 (0.0–0.0)	4.2	0.8 (0.0–3.9)	< 0.0001

Abbreviations: HIV, human immunodeficiency virus; IL, interleukin; IL-1β, interleukin 1 beta; IQR, interquartile range; pg/mL, picograms per milliliter; TNF-α, tumor necrosis factor-alpha. Notes: * *p*-values were calculated using the Wilcoxon Rank-Sum test to compare cytokine levels between groups. Bold values indicate statistical significance (*p*<0.05). Values of 0.0 indicate concentrations below the limit of detection.

3.6. Diagnostic Performance of Dichotomized IL-8 in Predicting Fatal Kala-Azar

IL-8 was the only cytokine that met all predefined criteria to be considered a biomarker for fatal kala-azar, demonstrating an AUC greater than 0.70, with both sensitivity and specificity exceeding 70%, as shown in Table 3. In the subsequent analysis, IL-8 was dichotomized using a cut-off of 50 pg/mL, a clinically convenient threshold that closely approximates the Youden-derived optimum of 49.3 pg/mL. With this binary classification, IL-8 maintained robust discriminatory performance, producing an AUC of 0.71 (95% CI, 0.62–0.81) and demonstrating no loss of information relative to the continuous-variable analysis (*p* = 0.1857) (Figure 1).

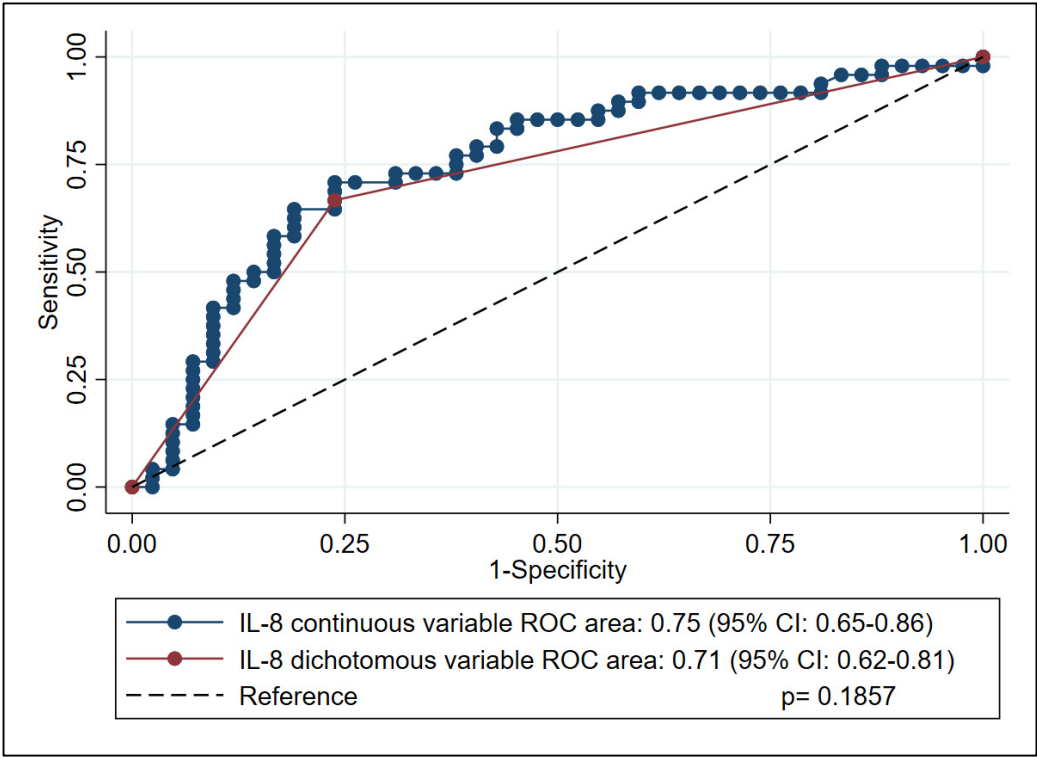


Figure 1. Receiver operating characteristic (ROC) curve analysis of interleukin-8 (IL-8) performance in predicting mortality in kala-azar patients, comparing continuous and dichotomized forms.

This figure illustrates the predictive performance of IL-8 (in both its continuous and dichotomized forms) for mortality in patients with kala-azar. The comparison of their respective areas under the curve (AUCs) was performed using DeLong’s test.

3.7. Influence of HIV Coinfection on IL-8 Discriminatory Power

To assess whether HIV coinfection affects the performance of IL-8 as a biomarker for fatal outcomes in kala-azar, we conducted separate AUC analyses for HIV-coinfected and non-coinfected patients. No significant differences were observed between the groups, as shown in Table 5.

Table 5. Diagnostic performance of interleukin-8 (IL-8) for predicting mortality in patients with kala-azar, stratified by HIV coinfection status (Total n=90).

Parameter	n	AUC (95 % CI)	p-value (DeLong Test)
IL-8 in patients without HIV	71	0.79 (0.67–0.89)	–
IL-8 in patients with HIV	19	0.77 (0.51–1.00)	0.9216

Abbreviations: AUC, Area under the Receiver Operating Characteristic curve; CI, Confidence Interval; IL, interleukin. Notes: *p*-values were calculated using the DeLong test to compare the AUC of IL-8 across HIV coinfection strata. The sample size (n) for each subgroup represents the number of patients with valid IL-8 measurements included in the ROC analysis.

4. Discussion

In our study, the proportion of deceased patients with kala-azar (cases) was 1.1:1 relative to survivors (controls). This balanced ratio of deaths to survivors aimed to optimize the identification of cytokines as biomarkers predictive of an unfavourable prognosis. Furthermore, we randomized cases from the same healthcare facility and hospitalized them concurrently with the controls. Based on the selection process, these cases seem to be representative of the overall study population.

We employed ROC curve analysis to thoroughly assess the potential of IL-1 β , IL-6, IL-8, IL-10, IL-12, and TNF- α as predictive biomarkers for identifying fatal cases of kala-azar. Our findings indicate that measuring IL-8 at the initial clinical encounter can effectively predict fatal outcomes in kala-azar patients, demonstrating good sensitivity and specificity. Notably, IL-8 maintained consistent predictive performance in both HIV-coinfected and non-coinfected individuals. With a Youden-derived cutoff point of approximately 50 pg/mL, IL-8 showed meaningful discriminatory power in identifying individuals at increased risk of death.

IL-8 is a pro-inflammatory chemokine secreted chiefly by activated monocytes, macrophages, and endothelial cells. It functions as the principal chemoattractant for neutrophils and a potent regulator of their activation [15–17]. In severe kala-azar, characterized by an exaggerated systemic inflammatory response, circulating IL-8 concentrations are markedly elevated. Because the cytokine can be quantified early in the course of illness through a simple peripheral venepuncture, IL-8 offers a minimally invasive biomarker for identifying patients at heightened risk of adverse outcomes and guiding timely clinical intervention.

Prior studies have already indicated the importance of IL-8 in kala-azar. However, the association between IL-8 and mortality in this population had not been previously reported. It was observed that symptomatic individuals exhibited higher IL-8 levels compared to asymptomatic ones [18] and that this chemokine served as an early marker of treatment response in patients without severe signs [11]. Additionally, IL-8 has been independently associated with hemorrhage, a key marker of disease severity [12].

This novel finding aligns with the broader established role of IL-8 as a valuable prognostic biomarker across various inflammatory and infectious conditions [19–23]. For example, high IL-8 levels have been correlated with increased multiple organ failure, sepsis, and mortality in pediatric patients with severe burns [20]. Furthermore, IL-8 has been proven to be a sensitive and specific biomarker for neonatal infection, and its association with severity has been established [21]. Among adults with sepsis, plasma IL-8 levels measured within the first 24 hours of diagnosis can predict

disease severity and 28-day mortality [23]. Conversely, in children younger than ten years with septic shock, serum IL-8 levels below 220 pg/mL predict 28-day survival with 95% accuracy [24].

Our findings showed that patients who progressed to death had significantly lower levels of IL-1 β , IL-12, and TNF- α , similar to those identified in individuals coinfecting with HIV. Notably, 33% of the fatal cases occurred in HIV-positive patients, suggesting that the reduced cytokine levels may, at least in part, be due to the immunomodulatory effects associated with HIV coinfection. However, these cytokines did not exhibit acceptable discriminatory performance in ROC curve analysis.

In a previous publication by our group, when we examined these same cytokines as potential early indicators of therapeutic response in patients without severe clinical signs of the disease, we found that IL-1 β , IL-12, and TNF- α did not reliably indicate ongoing disease activity in this subgroup [11].

Elevated levels of IL-1 β , IL-12, and TNF- α may have a protective effect in individuals with kala-azar; however, in the present study, these cytokines did not demonstrate adequate diagnostic utility.

In summary, our findings indicate that a serum IL-8 concentration of 50 pg/mL or greater, measured at the initial clinical encounter, can effectively distinguish between fatal and non-fatal cases of kala-azar with high sensitivity and specificity, thereby aiding clinical decisions at the point of care. Therefore, early quantification of IL-8 emerges as a promising tool for risk stratification. By facilitating the prompt identification of critically ill patients, this strategy enables clinicians to intensify surveillance and institute early, targeted interventions and measures that could lower the case-fatality rate of kala-azar, which stubbornly remains near 10 % even when appropriate therapy is initiated without delay.

5. Conclusions

Our data demonstrate that serum IL-8 levels equal to or above 50 pg/mL at the initial clinical encounter are a reliable predictor of fatal kala-azar.

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Informed Consent Statement: All patients provided written informed consent.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Conflicts of Interest: The authors declare that they have no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

AUC	Area under the curve
CI	Confidence interval
CNPq	Conselho Nacional de Desenvolvimento Científico e Tecnológico
HIV	Human immunodeficiency virus
IFN- γ	Interferon-gamma
IL	Interleukin
IL-1 β	Interleukin 1 beta
ROC	Receiver operating characteristic curve
TNF- α	Tumor necrosis factor-alpha

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