Late diagnosis: a factor associated with death from visceral leishmaniasis in elderly patients

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Introduction: Visceral leishmaniasis (VL) is among the seven global endemic diseases assigned a high priority by the World Health Organization. In Latin America, most cases occur in Brazil. Despite the availability of intensive treatment resources and protocols for specific treatment, lethality rates for VL have increased in several regions in the country over the past 10 years, particularly in patients under one and over 50 years of age. As the growth of the elderly population accelerates in Brazil, VL poses a greater challenge to public health. Given the scarcity of studies addressing the disease in this age group, the purpose of this study was to identify factors associated with VL lethality among the elderly.

Methods/Key findings: This analytical, cross-sectional epidemiological study comprised 80 elderly patients who sought treatment at the teaching hospital of the Universidade Federal de Mato Grosso do Sul, Campo Grande, MS, Brazil, in the period 2000–2013. Clinical, laboratory and treatment variables were investigated from records of elderly patients with VL diagnosis confirmed by at least one laboratory test positive (culture for parasite or direct parasitological examination; reactive immunofluorescence; immunochromatographic test with recombinant antigens) or patients without laboratory confirmation who lived in endemic areas and responded favorably to therapeutic trial, as defined by the Brazilian Ministry of Health. Of the 80 patients included, 78 tested positive to at least one exam; in two cases, diagnosis was based on clinical and epidemiological criteria. The lethality rate was 20%. Multivariate analysis revealed an association between death and time elapsed from symptom onset.

Keywords: Visceral leishmaniasis, Epidemiology, Neglected diseases, Communicable diseases, Parasitic diseases, Aged, Aging, Mortality

Introduction

Visceral leishmaniasis (VL) is a growing public health concern in Brazil, with high lethality rates, wide geographical distribution, considerable regional differences and a tendency to expand into non-endemic areas.¹ Despite the global reach of the disease, over 90% of cases occur in Brazil, India, Nepal, Bangladesh and Sudan. Once prevalent in rural and periurban areas, VL has recently been reported from urban centres.² Currently, autochthonous cases are being reported from most Brazilian states.³

Despite the availability of specific resources and protocols for intensive treatment of VL, lethality rates have risen in several regions of the country in

the past 10 years, particularly in patients under 1 and over 50 years of age.⁴

The demographical changes that have been taking place in Brazil over the past century have increased life expectancy to an advanced age,⁵ rendering the population as a whole more vulnerable to the severe form of VL. Immunosenescence, atypical presentations, comorbidities and polypharmacy are among the factors to be taken into account when evaluating individuals over 60.^{6,7}

Studies on the demographic profile of fatal cases have shown a higher risk for elderly individuals, although key factors contributing to a higher lethality in this age group have not, to our knowledge, been investigated.

As the Brazilian elderly population grows at a fast pace,⁸ VL poses a greater public health challenge in the states where it is endemic. Given the scarcity

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of studies on this disease in this demographic group, the purpose of this study was to identify factors associated with VL lethality among the elderly in Brazil.

Method

This analytical, cross-sectional epidemiological study comprised elderly patients who sought treatment for VL at the teaching hospital of the Universidade Federal de Mato Grosso do Sul (UFMS), Campo Grande, MS, Brazil, in the period of 2000–2013.

All medical records of elderly patients diagnosed under ICD code B55.0 in the study period, retrieved from the hospital's Medical Records Division, were investigated. The study included all patients aged 60 years who met at least one of the following criteria: positive culture for parasite or direct parasitological examination; reactive immunofluorescence reaction with titre of 1:80 or higher, provided that other diagnoses were ruled out; or positive immunochromatographic tests (rapid tests) using recombinant antigens.⁴

Patients without laboratory confirmation who lived in endemic areas and exhibited favourable response to therapeutic trial were also considered as having VL.⁴

Clinical, laboratory and therapeutic variables were investigated from the medical records.

The chi-squared test, chi-squared test for trends, and Fisher's exact test were used to identify possible associations between variables. Prevalence ratios were calculated adopting a 95% confidence interval. For variables with significance $\leq 20\%$, Cox regression (with time equal to one unit) was employed to estimate adjusted prevalence ratios. BioEstat software (version 5.3, Sociedade Mamirauá, Belém, PA, Brazil) and Epi Info software (version 7, Centers for Disease Control and Prevention, Atlanta, GA, USA) were used for statistical analyses.

The study was approved by the UFMS Ethics Committee for Research on Humans (permit 277 192, issued May 2013).

Results

Among the 80 patients investigated, diagnosis was confirmed by direct detection of parasites in bone marrow aspirate in 25 (31.25%), serological tests in 24 (30%), and both bone marrow aspirate and serology in 29 (36.25%). In two patients, diagnosis was based on clinical and epidemiological criteria. Bone marrow aspirates were collected from 68 patients (85%), proving positive in 54 (67.5%).

A lethality rate of 20% was detected among the 80 patients investigated. For all 16 patients who died, diagnosis had been confirmed by laboratory methods. Four of these had not been subjected to bone marrow aspirate examination, but diagnosis

had been confirmed by serology – namely, indirect immunofluorescence (1:80 in two cases and 1:160 in one) or rapid test (one case).

Relapse occurred in four of the 80 patients (5%), without association with death ($P=1.000$).

Fifty-seven (71.2%) had some type of associated non-infectious chronic disease, predominantly hypertension (56.25%), diabetes (23.75%), renal failure (10%), heart failure (10%) and coronary failure (3.75%). Sixty-seven (83.7%) were under continued drug therapy. No associations were observed between comorbidity ($P=0.566$) or continued drug therapy ($P=1.000$) and death. Two patients (2.5%) had AIDS, which was not associated with death ($P=0.566$).

Death incidence rose with longer time elapsed from symptom onset to diagnosis. For intervening periods of 61–120 days, the death prevalence was 26.7%, increasing to 100% for longer periods (Table 1). No deaths occurred among patients diagnosed within 60 days of symptom onset.

Fever was reported in 84.7% of medical records (61/72), weight loss in 81.4% (57/70), hepatomegaly in 58.3% (42/72), and splenomegaly in 51.4% (37/72). No cases of lymphadenomegaly were reported. No association was found between these signs and symptoms and the occurrence of death (Table 1).

No statistical associations were observed between death and haematological levels, biochemical levels, secondary bacterial infection, bleeding, type or number of antileishmanial drugs (Table 1).

Multivariate analysis (Table 2) revealed an association between death and time elapsed from symptom onset to diagnosis.

Discussion

The main purpose of the Brazilian Program for Vigilance and Control of VL is to promote timely diagnosis and treatment so as to reduce the numbers of cases and deaths. Despite these efforts, lethality increased by 67.6% from 1994 to 2009.⁴ Mean lethality rate in the 2010–2013 period was 6.8%.⁹ In the present sample, lethality was 20%.

Identification of associated clinical and laboratory factors can facilitate early diagnosis, crucial in patients at higher risk of death from the disease. Botelho and Natal (2009) found this risk to be higher in individuals aged 40 years and older, but although VL incidence was high among children, lethality in this younger group was lower.¹⁰ Oliveira *et al.* (2010) found mortality rates of 3.1/100 in children younger than 1 year and 20/100 in adults over 60 years.¹¹

Although the disease was prevalent in males (a feature corroborated by other studies),^{11,12} gender was not associated with death from VL,

Table 1 Distribution of elderly patients with visceral leishmaniasis, by fatal outcomes and study variables. Campo Grande, MS, Brazil, 2014 (*n*=80)

Variable	Death				Prevalence ratio (95% CI)	P
	Yes		No			
	n	%	n	%		
Gender						¹ 0.736
Female	4	23.5	13	76.5	1	
Male	12	19.0	51	81.0	1.24 (0.46-3.35)	
Age range (years)						² 0.299
60–69	9	21.4	33	78.6	1	
70–79	7	25.0	21	75.0	0.86 (0.36–2.03)	
80 And older	–	–	10	100.0	–	
Time elapsed between symptom onset and diagnosis (days)						² < 0.001
> 120	8	100.0	–	–	1	
61–120	4	26.7	11	73.3	3.75 (1.62–8.68)	
≤ 60	–	–	53	100.0	–	
No data*	4	100.0	–	–	–	
Fever						¹ 0.174
Yes	7	11.5	54	88.5	1	
No	3	27.3	8	72.7	0.42 (0.13-1.38)	
No data*	6	75.0	2	25.0	–	
Weight loss > 10%						¹ 0.441
Yes	11	19.3	46	80.7	1	
No	1	7.7	12	92.3	2.51 (0.35-17.75)	
No data*	4	40.0	6	60.0	–	
Hepatomegaly						² 0.199
Yes	5	11.9	37	88.1	1	
No	7	23.3	23	76.7	0.51 (0.18–1.45)	
No data*	4	50.0	4	50.0	–	
Splenomegaly						² 0.916
Yes	6	16.2	31	83.8	1	
No	6	17.1	29	82.9	0.95 (0.34–2.66)	
No data*	4	50.0	4	50.0	–	
Haemoglobin						¹ 0.260
≤ 7 g/dl	2	40.0	3	60.0	1	
> 7 g/dl	14	18.7	61	81.3	2.14 (0.66–6.92)	
Leucocytes						¹ 1.000
≤ 1000/ml	1	20.0	4	80.0	1	
> 1000/ml	15	20.0	60	80.0	1.00 (0.16–6.11)	
Platelets						¹ 0.333
≤ 50 000/ml	2	10.5	17	89.5	1	
> 50 000/ml	14	22.9	47	77.1	0.46 (0.11–1.84)	
Albumin						² 0.449
≤ 2.5 mg/ml	8	26.7	22	73.3	1	
> 2.5 mg/ml	7	18.9	30	81.1	1.41 (0.58–3.44)	
No data*	1	7.7	12	92.3	–	
Creatinine						¹ 0.202
≥ 2.4 mg/dl	4	40.0	6	60.0	1	
< 2.4 mg/dl	12	17.9	55	82.1	2.23 (0.89–5.58)	
No data*	–	–	3	100.0	–	
Bacterial infection						² 0.364
Yes	5	15.2	28	84.8	1	
No	11	23.4	36	76.6	0.65 (0.25–1.69)	
Bleeding						¹ 1.000
Yes	–	–	2	100.0	–	
No	12	17.1	58	82.9	–	
No data*	4	50.0	4	50.0	–	
Diagnosis						¹ 0.592
Parasitologic	11	23.4	43	79.6		
Serology	5	20.8	19	79.2		
Clinical-epidemiological	–	–	2	100.0		
Use of antileishmanial drugs						² 0.016
1	15	27.3	40	72.7	1	
> 1	1	4.0	24	96.0	6.82 (0.95–48.81)	
Initial drug						² 0.307
Liposomal amphotericin	10	25.6	29	74.4	1	
Glucantime	3	23.1	10	76.9	1.11 (0.36–3.43)	
Amphotericin B	3	10.7	25	89.3	2.39 (0.72–7.91)	

* Medical records devoid of this information were excluded from statistical tests.

¹ Fisher's exact test.² Chi-squared test for trend.

Table 2 Cox regression for death in elderly patients diagnosed with visceral leishmaniasis. Campo Grande, MS, Brazil, 2014 (*n*=80)

Variable	<i>P</i>	Prevalence ratio	95% CI
Time elapsed between symptom onset and diagnosis	<0.001	3.06	1.61–5.82
Fever	0.063	0.39	0.14–1.05
Number of concomitant antileishmanial drugs	0.362	2.68	0.32–20.18
Creatinine	0.903	1.08	0.33–3.49

suggesting that males have a higher predisposition to acquire the disease, but not to overcome it.

In other studies, the risk of death was found to increase with age in the 45- to 60-year range,^{11,13–16} but the underlying causes for this pattern were not investigated. One possible reason was misdiagnosis, since VL predominantly affects children.

Ageing is a complex process that involves cellular and molecular changes, affecting tissue integrity both quantitatively and qualitatively. The immunological system is no exception, as shown by the development of immunosenescence. Cellular immunity is a major defence mechanism against *Leishmania*,¹⁷ and immunological function undergoes changes with ageing, including decreased T cell production,^{7,18} changes in cytokine production (with higher levels of Th2 cytokines, such as IL-10 and TGF- β) and lower production of Th1 cytokines (IFN- γ and IL-12), contributing to protozoan survival.¹⁷ In some cases, elderly patients are undergoing immunosuppressive drug therapy for underlying diseases such as rheumatoid arthritis¹⁹ or lymphoma,²⁰ with an additional risk for VL development.

In the present study, none of the 10 patients aged 80 years and over died. Recently, cure following treatment was reported for an 86-year-old woman diagnosed with VL.²¹ Ageing has multiple causes, and genes participating in many important biological pathways can affect ageing rates in animal models.²² Individuals who reach the age of 80 years and over are likely to have a favourable genetic background that predisposes them to lower risk for disease or at least to a more favourable performance in counteracting inner homeostasis imbalances, for instance during infection.

Relapse was neither the most frequent cause of hospitalisation nor the most associated with death. More frequent relapses are expected among the elderly, considering their poorer immune responses. In some patients, however, the disease may have relapsed after discharge, and treatment been sought at a different hospital. Relapse can therefore be more effectively evaluated by prospective cohort studies.

Late diagnosis and the fact that VL affects individuals with comorbidities are factors identified as contributing to lethality.^{11,12,16,23,24} In the present study, comorbidities were not associated with death, a finding that can be explained by the frequent medical

monitoring required for these patients, as well as by clinical stability of the underlying disease.

In HIV-infected patients, VL relapses are more frequent and lethality rates are higher.²⁵ In the present study, the number of patients with confirmed HIV diagnosis was small, preventing further statistical analysis. However, because AIDS is an emerging disease in individuals over 50 years of age, HIV infection may increase mortality rates in VL patients.²⁶

In the present study, late diagnosis may also have resulted from difficulties in collecting information directly from patients at the time of infection – among elderly individuals, poorly articulated complaints are frequent and tend to be overlooked – hence the importance of taking into account the specificities of this age group.⁶ Also, given the higher prevalence of chronic diseases among these patients, most were in use of drug therapy, often with accompanying risks and detrimental outcomes, particularly in the case of multidrug therapy. The likelihood and severity of drug interaction can be higher in elderly patients with organic and functional disorders, which can compromise drug pharmacokinetics. In effect, adverse reactions caused by these factors are three to four times more frequent in elderly than in young individuals.⁶ Multidrug therapy not only renders drug interaction more likely; it can also cause cumulative toxicity, consequently increasing morbidity and mortality rates.²⁴ Despite these risks, multidrug therapy did not influence the mortality rate.

Early implementation of effective measures can decrease VL lethality rates. Knowledge of the laboratory and clinical profile of cases and its association with death from the disease can improve VL management and reduce lethality.^{11,13,16}

Difficulties in identifying suspected cases, as well as incomplete information in medical records, indicate shortcomings in public health services, interfering with early detection.^{10,27} In the present study, however, incompleteness of medical records did not preclude obtaining relevant information on the profile of VL in elderly patients.

Association between late diagnosis and mortality is probably related to clinical specificities of particular age groups. Among Tunisian children with VL, delays of over 56 days between symptom onset and first clinical examination were among the seven

major factors associated with poor prognosis.²⁸ A Brazilian study conducted in Mossoró County, in the northeastern state of Rio Grande do Norte, identified that late diagnosis is a key factor for high mortality.²⁹

Fever, splenomegaly, hepatomegaly, weight loss and pallor are the most frequent symptoms of VL. Cough and diarrhoea have also been described.^{23,30–34} Among patients who died of the disease, Oliveira *et al.* (2010)¹¹ found a lower occurrence of fever and splenomegaly than did the previously cited studies or an investigation of patients with VL–HIV co-infection.³⁵

Conditions such as lymphadenomegaly, common in India and Sudan, were not found in the present study or elsewhere in Brazil^{15,36–38} – an important feature for differential diagnosis of myeloproliferative diseases.

Splenomegaly, for instance – a classic sign of VL^{30–33} and one of the clinic-epidemiological criteria for diagnosis⁴ – was observed in <51% of the present sample, indicating that the criteria usually applied to patients over 60 years of age should be reviewed.

Most patients had fever, although this symptom does not consistently manifest in elderly individuals under conditions that would typically elicit fever in younger patients.³⁹ Absence of fever, however – which might suggest poor immune response and poorer prognosis – did not translate to higher mortality rates. Fatigue, a common, unspecific symptom in this age group, can be mistaken for a symptom of other diseases, or even as a manifestation of the ageing process.³⁹

Weight loss, which can accompany metabolic, neoplastic and psychological diseases, occurs as a side effect of medication, or even result from social neglect, requires further investigation.⁴⁰ Visceral leishmaniasis should be consistently considered as a differential diagnosis in elderly individuals living in endemic areas who present with weight loss, given that symptoms can be unspecific in this age group. Reducing mortality rates requires early diagnosis, which in turn depends on early suspicion followed by prompt intervention.⁴¹

Anaemia, neutropaenia and thrombocytopaenia are frequently seen in VL cases,^{30,32–34,42} reflecting the occurrence of bone marrow suppression, aemolysis and splenic sequestration,⁴³ conditions that predispose to infection and haemorrhage, increasing the risk of death.

A study conducted in São Paulo found that lethality was associated with haemoglobin levels <5 g/dl, but not with leucocyte or platelet counts.⁴⁴ Costa *et al.* (2010) found that leucocyte levels $\geq 7000/\text{mm}^3$ were associated with death from VL.⁴⁵ Oliveira *et al.* (2010) found that low albumin levels were not

associated with higher mortality, in contrast with increased creatinine.¹¹ Comparing individuals who had creatinine levels above and below 1.3 mg/dl, Daher *et al.* found no differences in clinical presentations, despite the fact that mean age was higher in patients with elevated creatinine levels.³⁰

In the present study, lethality was not associated with lower blood cell levels, lower albumin or higher creatinine. More detailed studies are needed, particularly because the populations investigated in the studies cited,^{11,30,32–34,42,44} were largely heterogeneous or comprised only children.

Infection and haemorrhage are classic complications of VL.⁴⁶ Bacterial infection is among the factors possibly associated with increased lethality.^{4,16} Costa *et al.* (2010) suggested that immunosuppression can coexist with inflammation, and a balance between these two processes determines patient response: either immune paralysis or severe inflammation. They also view haemorrhage as part of the expression of systemic inflammatory response.⁴⁵ Considering the age group investigated in the present study, the absence of association with increased mortality may be explained by early administration of antimicrobials.

Five patients, diagnosed exclusively by serology, died. In endemic areas, however, serology (rapid test and indirect fluorescent antibody test) is positive for a considerable percentage of asymptomatic individuals.⁴⁷ This feature precludes ruling out the possibility that patients diagnosed exclusively by positive serology died from another undetected underlying disease, despite the efforts made, during hospitalisation, to achieve differential diagnosis and rule out other conditions.

Concomitant use of two or more antileishmanial drugs did not influence mortality rate. Early detection of drug non-responsiveness, side effects, or drug toxicity, with consequent changes in medication, may have contributed to this finding. No association was found between mortality and initial drug, even for glucantime, currently contraindicated for patients older than 50 years.⁵ Most patients were initially treated with liposomal amphotericin B, the drug recommended by the Brazilian Ministry of Health.⁵

Conclusion

Late diagnosis of VL in elderly patients was associated with higher lethality rates, underscoring the importance of early differential diagnosis in patients in this age group who live in or have visited areas endemic for LV. Splenomegaly was largely absent, suggesting that the definition of suspected cases for individuals older than 60 years should be reviewed. No association was found between death and the other variables investigated.

Disclaimer Statements

Contributors

Marta Driemeier: data collection and preparation of the article. Polianna Alvarenga de Oliveira: data collection. Angelita Fernandes Druzian: data collection. Laís Ferreira Lopes Brum: data collection. Elenir Rose Jardim Cury Pontes: statistical calculations. Maria Elizabeth Cavalheiros Dorval: review of the article. Anamaria Mello Miranda Paniago: review of the article.

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Conflicts of interest

The authors have no conflicts-of-interest.

Ethics approval

The study was approved by the UFMS Ethics Committee for Research on Humans (permit 277 192, issued May 2013).

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