Immunologic Biomarkers, Morbidity and Mortality Among HIV Patients Hospitalized in a Tertiary Care Hospital in the Brazilian Amazon

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Abstract

Background: While antiretroviral therapy (ART) has significantly improved survival rates of people living with HIV, some regions in Brazil still show a linear trend of growth in the opportunistic infections that cause HIV-associated mortality. We aimed to describe HIV-associated morbidity and mortality among hospitalized medical patients in a tertiary care hospital in Manaus, in the Brazilian Amazon, by investigating clinical data and immunologic biomarkers in order to assess predictive factors of mortality in this patient group.

Methods: We prospectively measured concentrations of cytokines Th1/Th2/Th17 and soluble CD14 (sCD14) and reviewed the laboratory parameters and opportunistic infections in outcomes of either death or discharge of eighty-three HIV/AIDS patients who were admitted in 2017-2018 to the Fundação de Medicina Tropical Doutor Heitor Vieira Dourado (FMT-HVD) in Manaus.

Results: The mortality in the sample studied was 20.5%. Tuberculosis (TB) showed a relative risk (RR) =1.86 (confidence interval (CI) 1.14 to 2.81: and p = 0.026), and weight loss was the symptom (RR=1.81; CI: 1.21 to 2.53 and p = 0.007) most highly associated with the death outcome in HIV/AIDS inpatients. Univariable analyses showed that the eosinophil count, platelet distribution width (PDW), and alanine aminotransferase were the only laboratory parameters that differed among patients who died. In relation to cytokines and sCD14 levels, no differences were found between those who died or were discharged. A multivariable logistic regression model was used to predict mortality and showed that individuals with no digestive syndrome (especially the absence of oropharyngeal candidiasis), nor TB are 63% to 76% less likely to die, respectively. In addition, increases in PDW values also decreased the probability of death. Curiously, patients who were discharged showed a trend towards a concomitant increase in PDW and mean platelet volume (MPV) in relation to those who died.

Conclusions: Opportunistic infections continue to be major events in morbidity and mortality of HIV/AIDS patients, and the relationship between increased PDW and the likelihood of survival suggests the need for future studies on innate immune response of platelets in HIV/AIDS inpatients.

Background

Brazil has been successfully combating the HIV/AIDS epidemic by providing state-of-the-art antiretroviral therapy (ART) and a unified, universal and free public health system widely disseminated to the population[1,2]. The mortality rate due to HIV/AIDS in Brazil has fallen significantly in the last twenty years, especially in the southeastern, southern and mid-western regions. This has been principally influenced by a government policy of linking HIV/AIDS treatment and medication dispensing to compulsory notification within the public health system (Sistema Único de Saúde/SUS)[1,3]. This revised system has been assisting decision-making at the individual and programmatic levels of different antiretroviral treatment regimens for all people living with HIV4.

Since the beginning of the epidemic in 1982, more than three hundred thousand deaths with HIV/AIDS being attributed as the cause have been reported. The number of new cases of HIV/AIDS has been gradually decreasing in recent years in Brazil, influenced by the southeast, southern and central western regions, while the northern and northeastern regions still show a linear trend of growth in the detection of new cases[3]. As of 2018, more than 900,000 AIDS cases had been reported in Brazil and approximately 600,000 are on ART[1]. After a significant reversal in mortality due to the adoption of ART in 1996, deaths related to the HIV/AIDS epidemic have been showing a downward trend in Brazil as a whole, however this trend is still subtle in the northern and northeastern regions[1,3,5].

Located in the north of Brazil, the State of Amazonas has peculiar characteristics, such as geographic isolation and a lack of access to full health services, mainly in the interior of the State. Manaus is the capital and the largest city of the state, and the decentralized health units are located in the neighborhoods and semi-urban areas. These are generally more accessible and promote the highly active antiretroviral therapy (HAART)[2]. The Fundação de Medicina Tropical Doutor Heitor Vieira Dourado (FMT-HVD), located in the middle of the city, is a tertiary care hospital which performs HAART services and admits moderate to severely ill people living with HIV. This represents a contingent greater than 85% of all patients receiving ART, as well as concentrating data on incidence levels of AIDS, mortality rates and late diagnosis[2,5]. Studies on the HIV/AIDS epidemic in the region have indicated over time that the trend in the number of cases continues to increase principally in men, that tuberculosis (TB) is still the leading cause of death, and that respiratory failure is the main cause of hospital admission for these patients[5–8].

The hallmark of HIV infection is the progressive depletion of CD4 T cells caused by an active replication of the virus in the lymph nodes and blood stream, which, over time, lead to a chronic HIV infection[9–11]. In addition, HIV cripples the immune system and allows reactivation of dormant pathogens or increases susceptibility to exogenous pathogens. These co-infections further fuel the immune activation that characterizes a strong predictor of disease progression in HIV infection[12,13]. Although morbidity and mortality caused by opportunistic diseases have decreased due to HAART, coinfections continue to be important factors for the pathogenesis of chronic HIV-infected patients. In our prospective study, in which HIV/AIDS inpatients were classified by either death or hospital discharge, we assessed which comorbidities, coinfections and concentrations of serological biomarkers were predictors of the risk of death.

Methods
Study type

This prospective study involved HIV/AIDS inpatients admitted to the Fundação de Medicina Tropical Doutor Heitor Vieira Dourado (FMT-HVD), the tertiary referral hospital for infectious diseases in Manaus. This is a prospective study, in which sampling was done for convenience. The target population consisted of HIV/AIDS patients of either sex who were admitted to the FMT-HVD in years 2017 and 2018. Eighty-

three participants aged between 12–70 were enrolled in this study, the sampling was by convenience and we enrolled those patients that were admitted in FMT-HVD within than 72 hours. After signature of the informed consent form, the blood collection was performed. All had their CD4 count and viral load monitored. Plasma for the tests was stored at -80 °C.

Clinical data

During first contact with the patients, socio-demographic data were collected, such as name, age, gender and use of ART. Through access to the electronic medical records (iDoctor®) of the patients, the following clinical data were collected: general health status, comorbidities, co-infections, treatment, clinical manifestations (weight loss, diarrhea, vomiting) and death.

Laboratory data were obtained at the hospital itself, which currently uses software to store medical records and electronic prescriptions. This instrument also provided laboratory data such as: blood count, serology (Cytomegalovirus - CMV, Toxoplasmosis, Epstein Barr - EBV, Herpes virus, hepatitis B virus - HBV, hepatitis C virus - HCV), immunological markers (viral load and T cell count CD4 + T and CD8 + T) and biochemical markers (bilirubin, creatinine, lactic dehydrogenase, GT range, albumin, alkaline phosphatase, GPT and OGT).

General characteristics of patient comorbidities

Comorbidities were obtained from the electronic medical database at the FMT-HVD, and the outcomes of interest were survival (hospital discharge) and death. Both were verified via either a death certificate or discharge authorization registered in the electronic medical record.

Participants considered as having tuberculosis were those with positive smear microscopy for Mycobacterium tuberculosis. Comorbidities were defined as signs and/or symptoms of respiratory, neurological and digestive origin, of infectious and non-infectious origin, with or without chronicity. Respiratory syndromes were classified when patients reported dyspnea (shortness of breath or difficulty in breathing), atypical chest performance with abnormal noises, long-term or productive cough, vesicular breathing sounds, abnormal respiratory murmurs, pleural effusion, gasping respiration and wheezing.

Pulmonary co-infections were tuberculosis, pneumocytosis and pulmonary histoplasmosis. Neurological syndromes were classified as disorientation, seizure, paralysis, movement deficit, mental confusion, tumor, hemiplegia, depression and mental disorder, psychotic depression, organic mental disorder and dementia. Neurological co-infections were neurocriptococcosis, neurotoxoplasmosis, neurotuberculosis, and meningoencephalitis. Circulatory syndromes were hypertension, endocarditis, pericardial tuberculosis, heart failure, megaloblastic anemia, edemigenic syndrome and hematological syndrome. Digestive syndromes were classified into erosive esophagitis, ulcerative esophagitis, digestive hemorrhage, gastritis and epigastria, abdominal pain, odynophagia, oroscopy, intestinal lymphadenomegaly, nephrotoxicity, gastritis, gastrointestinal syndrome, diarrheal syndrome and diarrhea. The majority of digestive syndromes were oral candidiasis, moniliasis with whitish lesions, esophageal candidiasis, intestinal tuberculosis, amoebic colitis and anal condyloma. Other comorbidities, such as

chronic lymphocytic leukemia, Hodgkin's disease, aplastic anemia, and neurological disorders like multiple sclerosis and myasthenia gravis, were monitored.

Blood sample collection

On the same day of the patient's enrollment, after the interview and signing of the consent form, 5 mL of blood was collected by vacuum venipuncture. The samples, collected in dry tubes, were centrifuged at 3,500RPM for 5 minutes at 25 ºC to obtain the serum and aliquoted (1 mL) for analysis of inflammatory markers. The whole blood collected in an anticoagulant tube was homogenized, aliquoted (1 mL) and stored for future analysis. Both were stored at -80 ºC until use.

Serum cytokine markers

The measurement of serum cytokines was performed using the Flow Cytometry technique CBA (Cytometric Bead Array) with the human cocktail Th1/Th2/Th17 Cytokine for IL-2, IL-4, IL-6, IL-10, TNF-α, IFN-γ and IL17 and inflammatory cytokines IL-8, IL-1β, and IL-12 (Biosciences, USA). Serum concentrations of soluble CD14 were determined by Enzyme-Linked Immunosorbent Assay, using anti-human CD14 Antibody (R&D Systems) and anti-human CD14 Biotinylated Antibody; (R&D Systems). Recombinant human CD14 protein (R&D Systems) was used as a standard.

Statistical analysis

Data were tabulated in an Excel database created by the researchers and analyzed using the GraphPad Prism program, version 7. For data analysis, patients were classified in two groups: Death (those who died during hospitalization) and Survival (those who were discharged from hospital). Descriptive analysis was performed with mean and standard deviation for numerical variables, with normal and median distribution for the others. For comparison between categorical variables, the Chi-Square test was used to determine relative risk. Serum soluble CD14 and cytokine concentrations were compared between Death and Survival groups using the Mann Whitney Test. Logistic regression predictions were performed, the level of statistical significance defined in both cases was $p < 0.05$.

Results

The prospective study allowed us to quantify the specific comorbidities of HIV/AIDS patients in which respiratory syndromes had the highest incidence (62.1%), followed by neurological syndromes (37.9%), gastrointestinal syndromes (21.0%) and cardiovascular disorders (5.2%). Among respiratory syndromes, tuberculosis had the highest incidence (56.30%), followed by crackles (22.40%), atypical chest (7.00%), atypical chest + severe cough (5.30%), respiratory failure (5.30%), and pneumocystosis (3.70%). Among neurological syndromes, the most predominant were neurotoxoplasmosis (44.4%), neurocryptococcosis 8.3%, and brain tuberculosis 2.8%. Patients showed neurological symptoms such as confusion, paralysis, poor coordination (30.6%), tremors and asthenia 11.1%, tumours 11.1%, seizures 5.6%, and meningoencephalitis 2.8%. Digestive syndromes were candidiasis 55.0%, odynophagia 5.0%, intestinal

lymphadenomegaly 5.0%, esophageal candidiasis 5.0%, esophagitis + epigastria 5.0%. Twenty-five percent of patients reported abdominal pain 25.0%.

Laboratory data were obtained from electronic medical records, followed by blood collection. As previously mentioned, almost all of the patients were classified as having AIDS due CD4 T counts being 350 cells/mL. The mortality in the sample studied was 20.5%. Univariable analyses with of HIV-RNA > 1000 copies and CD4 T < 350 cells/mL showed no relative risk for death (Table 1). Comorbidities were assessed to identify predictive factors of mortality. Among the clinical data, weight loss showed a relative risk (RR) of 1.81 with a confidence interval (CI) of between 1.18 and 2.97 ($p = 0.013$). The active coinfections were diagnosed by microbiological or serological tests. Tuberculosis was the only risk associated with death, with a RR of 3.44 (CI 2.33–5.08; p = 0.001). The IgG serological tests used for previous infections did not differ between HIV/AIDS patients who died or were discharged from hospital.

Table 1
Assessment of comorbidities in the identification of predictive factors for mortality.

Clinical data	Death	Discharge	Relative Risk	95% CI	p	
	N = 17	N = 66				
Age (mean, std.)	37.6 (9.9)	36.2 (9.4)	-	-	0.590	
Gender (male)	11 (64.7)	54 (81.8)	0.79	0.42 to 1.05	0.184	
HAART	12 (70.6)	55 (83.3)	0.84	0.55 to 1.09	0.300	
CD4 T < 350 cells/mL	14 (82.4)	58 (87.9)	0.93	0.66 to 1.12	0.688	
HIV-RNA > 1000 copies	14 (82.4)	47 (71.2)	1.15	0.80 to 1.46	0.539	
Weight loss	14 (82.4)	30 (45.5)	1.81	1.21 to 2.53	0.007	**
Very Good Condition	7 (41.2)	35 (53.0)	0.77	0.39 to 1.30	0.425	
Lucid and space-oriented	8 (47.1)	40 (60.6)	0.77	0.42 to 1.22	0.410	
Anemia	15 (88.2)	43 (65.2)	1.35	0.97 to 1.71	0.079	
Respiratory syndrome	14 (82.4)	42 (63.6)	1.29	0.89 to 1.68	0.244	
Neurological syndrome	8 (47.1)	29 (43.9)	1.07	0.56 to 1.76	0.999	
Cardiovascular disorder	2 (11.8)	2 (3.0)	3.88	0.70 to 20.49	0.184	
Digestive syndromes	8 (47.1)	16 (24.2)	1.94	0.96 to 3.58	0.077	
Diarrhea	4 (23.5)	24 (36.4)	0.64	0.25 to 1.42	0.397	
Vomiting	8 (47.1)	25 (37.9)	1.24	0.64 to 2.10	0.581	
Active coinfections						
Tuberculosis	12 (70.6)	25 (37.9)	1.86	1.14 to 2.81	0.026	*
Parasites in stools	3 (17.6)	13 (19.7)	0.89	0.28 to 2.44	0.999	
Toxoplasmosis IgM	1 (5.9)	7 (10.6)	0.55	0.09 to 3.00	0.999	
Cytomegalovirus IgM	1 (5.9)	2 (3.0)	1.94	0.25 to 13.79	0.502	
Epstein Bar virus IgM	17 (100)	66 (100)	1.00	0.81 to 1.05	0.999	
Herpes virus IgM	0 (0)	3 (4.5)	0.00	0.0 to 4.51	0.999	
Previous infections						
HCV IgG	1 (5.9)	2 (3.0)	1.94	0.25 to 13.79	0.502	
Cytomegalovirus IgG	13 (76.5)	53 (80.3)	0.95	0.64 to 1.20	0.741	

Clinical data	Death	Discharge	Relative Risk	95% CI	p
	N = 17	N = 66			
Toxoplasmosis IgG	13 (76.5)	52 (78.8)	0.97	0.65 to 1.22	0.999
Epstein Bar virus IgG	12 (70.6)	49 (74.2)	0.95	0.61 to 1.25	0.764
Herpes virus IgG	12 (70.6)	44 (66.7)	1.05	0.68 to 1.42	0.999

Table 1 shows the relative risk of anemia in HIV/AIDS patients and indicated a trend to death (p = 0.079), which it was also revealed by lower hemoglobin concentration, as seen in Table 2, although they were not statically different. In contrast, total leukocytes, neutrophils, and monocytes did not differ between groups and remained in normal ranges in most of HIV/AIDS patients (Table 2). In addition, eosinophil counts were reduced in both groups in relation to normal range, but counts in those who died were even lower (p = 0.03). Table 1 also shows a trend towards digestive syndromes in HIV/AIDS patients who died. In the assessed biochemical tests, HIV/AIDS patients preserved their liver and renal functions.

Univariable analyses of laboratory variables were also carried out to identify predictive factors of mortality (Table 2). The percentage of lymphocytes of those who died showed a tendency to be lower. The CD4 T counts were very reduced for AIDS patients in both groups, however they did not differ statistically. In relation to CD8 T cells, the death group presented normal values, while patients that were discharged tended towards a slight increase, but not sufficient to be statistically different. The ratio of CD4:CD8 cells below 0.20 due reduction of CD4-T provided a notion of how weak the immunity of the HIV/AIDS patients was (Table 2).

Despite anemia being common between them, renal function was able to maintain bilirubin, albumin, and creatinine levels in normal ranges (Table 2). Hepatic tests did not indicate liver disorders, although gamma-glutamyltransferase levels were higher, alkaline phosphatase levels were slightly elevated in relation to normal ranges. The same was observed with aspartate aminotransferase and alanine aminotransferase levels that showed slight elevations.

Table 2
Univariable analyses with laboratory variables to identify predictive factors of mortality.

Laboratory tests	Death	Discharge	p	
	N = 17	N = 66		
Hematological data (NVA)				
HIV-RNA copies/mL ##	126578 (3247;273012)	25258.5 (391.5;145976.8)	0.590	
Lymphocytes (percent) # (25.0–40.0%)	20.5 (10.3)	27.4 (13.5)	0.055	
CD4-T cells/μL ## (600 to 1;500/mm3)	44 (7;232)	79 (30;198.2)	0.140	
CD8-T cells/μL ## (200 to 800/mm3)	402 (198;776)	544.5 (359.2;1143.8)	0.312	
CD4/CD8 ratio ## (1.0 and 4.0)	0.1 (0;0.4)	0.1 (0.1;0.3)	0.123	
Hemoglobin (13.0–18.0 g/dL)	9.3 (2.7)	10.8 (2.4)	0.165	
Leukocytes ## (4;500 – 11;000/mL)	4740 (3510;8600)	4215 (3275;5757.5)	0.527	
Neutrophils ## (1;800-7;700/mL)	2660 (1679;3234)	3118 (2109;4907.5)	0.113	
Monocytes ## (80 – 1;100/mL)	249 (161;379)	329 (187.2;488.2)	0.370	
Eosinophils ## (40–550/mL)	2 (2;4)	4 (2.6;7)	0.030	*
Platelets # (150;000-4000;000 × 10^3/mL)	299352.9 (207630.4)	273947.0 (132454.6)	0.536	
MPV ## (8.8 fL a 12.5 fL)	8.2 (7.8;8.8)	8.1 (7.4;8.7)	0.446	
PDW ## (9.3 fL a 16.0 fL)	15.8 (13;18.7)	13.5 (11.2;16.5)	0.037	*
Biochemistry parameters (NVA)				
Bilirubin ## (< 1.0 mg/dL)	0.4 (0.2;0.9)	0.4 (0.3;0.7)	0.999	
Creatinine ## (0.6–1.35 mg/dL)	1 (0.7;1.3)	0.8 (0.6;0.9)	0.069	
DHL ## (120 e 246 U/uL)	435 (332;534)	367.5 (300.2;474.2)	0.159	
Gamma-glutamyltransferase ## $	88 (42;203)	103.5 (57.8;290)	0.876	
Albumin ## (3.5-5.0 g/dL)	3.4 (2.8;4)	3.8 (3.3;4.5)	0.129	

NVA: Normal values in adults. # mean and std. ##; median (25 and 75 interquartilel) $ References values of Gamma-glutamyltransferase (men: 10 to 50 U/uL and women: 7 to 32 U/uL).

Laboratory tests	Death	Discharge	p	
	N = 17	N = 66		
Alkaline phosphatase[##] (65.0-330.0 U/uL)	282 (200;459)	281 (201;386)	0.835	
Aspartate aminotransferase [##] (2-38.0U/uL)	44 (26;82)	34 (25;58)	0.280	
Alanine aminotransferase [##](2-44.0U/uL)	31 (22;41)	43 (31;77)	0.038	*

NVA: Normal values in adults. # mean and std. ##; median (25 and 75 interquartilel) $ References values of Gamma-glutamyltransferase (men: 10 to 50 U/uL and women: 7 to 32 U/uL).

The soluble CD14, the chemokine IL-8 and Th1/Th2/Th17 cytokines were assessed between the two groups. Though no differences were observed, it is worth mentioning the stratification in levels of inflammatory biomarkers sCD14, IL-8, IL-6, and IFNγ (Fig. 1A-D) in relation to Th1/Th2/Th17 cytokines (Fig. 1E-L). We observed predominance of IL-17A and IL-4 over IL-12 and IL-2 in both groups (Fig. 1E-H). Lastly, we observed a trend towards a predominance of the anti-inflammatory IL-10 over TNFα and IL-1β in HIV/AIDS patients who died (Fig. 1I-L).

A multivariable logistic regression model assessed all biomarkers for likelihood of death in HIV/AIDS patients (Table 3). Those without digestive syndrome were 63% less likely to die (crude OR = 0.37), while 76% were likely to survive when all biomarkers were assessed together (adjusted OR = 0.24). As mentioned previously, more than half of the digestive syndromes were due oropharyngeal-esophageal candidiasis. Odds ratios for tuberculosis were more pronounced, individually, those without TB were 76% less likely to die (crude OR = 0.24), while 86% were likely to survive when all biomarkers were assessed together (adjusted OR = 0.14). Among continuum biomarkers, only PDW predicted death in HIV/AIDS patients, both individually (crude OR = 0.84) and with all biomarkers (adjusted OR = 0.85, Table 3).

Table 3
Logistic regression for predictive factors of mortality in HIV/AIDS patients.

	crude OR (95%CI)	adj. OR (95%CI)	P (Wald's test)	P (LR-test)
Digestive syndromes	0.37 (0.12;1.11)	0.24 (0.06;0.94)	0.041	0.035
Tuberculosis	0.24 (0.08;0.78)	0.14 (0.04;0.57)	0.006	0.003
PDW	0.84 (0.72;0.97)	0.85 (0.72;1)	0.046	0.026

OR: odds ratio. CI: confidence interval.

To understand these data, Fig. 2A allows us to explain how as the PDW values increase, the probability of death decreases. Furthermore, the direct relation between PDW and MPV was common in all HIV/AIDS patients. However, patients who were discharged showed a trend towards a concomitant increase of PDW and MPV in relation to those who died (Fig. 2B-C).

Discussion

Despite mortality rate due to HIV/AIDS falling significantly in Brazil, the northern and northeastern regions of the country have seen an increase and, between these two regions, the trend has been increasing at a greater rate in the state of Amazonas[3,5]. Our study found respiratory syndromes, followed by neurological and digestive syndromes, to be the main causes of hospitalization. In most of these cases, opportunistic pathogens were observed, especially TB, neurotoxoplasmosis and oropharyngeal-esophageal candidiasis. These data demonstrate that, although the country is progressing towards reaching the UNAIDS 90-90-90 treatment target, the northern and northeastern regions are no in line with the general trend. Here in the Brazilian Amazon, opportunistic infections continue to be the main causes of death of people living with HIV, which is a similar situation for those coming from cities in other regions with low economic power[14-19].

Regarding the risk of death, our multivariable logistic regression model indicated two main causes of death, HIV/TB co-infection and digestive syndrome. Tuberculosis was highly prevalent in HIV/AIDS patients admitted at the FMT-HVD, as has been occurring for the past ten years[5]. The prevalence of HIV/AIDS co-infection can be much higher, since TB is generally paucibacillary in HIV-infected individuals, which makes diagnosis difficult[7,20]. As the HIV infection weakens the host's immune response to TB, HIV/TB co-infection results in a more dramatic progression[7,16,17,20-22]. In relation to digestive syndrome, it is worth highlighting that more than half of digestive syndromes were due to oropharyngeal-esophageal candidiasis. The occurrence of oral candidiasis has been recognized as an indicator of immune suppression. It is known that some patients did not wish to receive HAART due to skepticism, social factors, or because, even while taking medications, they experienced virological and therapeutic failure[23]. Here, CD4 counts lower than 200cells/ul were observed in almost all HIV-infected patients. In this context, the occurrence of oropharyngeal-esophageal candidiasis can help physicians to diagnose the clinical progression of HIV infection, as observed in other countries [24,25].

HIV/AIDS is characterized by a state of chronic hyperactivation. In this sense, several serological markers are being investigated in the search for a better predictor of the risk of death[12,26-29]. In the present study, no difference was observed in sCD14, IL-8 and Th1/Th2/Th17 cytokines between patients who died or were discharged. Biomarker concentrations are observable outcomes of complex biological processes that are only partially understood, and this may be the reason for a lack of consensus regarding immunological markers in prediction of mortality in HIV/AIDS patients[30-34]. Curiously, we observed much higher levels of inflammatory biomarkers sCD14, IL-8, and IL-6 than for other cytokines, which is consistent with the inflammatory status of the HIV/AIDS patients[35]. In relation to the main Th1/Th2/Th17 cytokines, the very low concentrations could be explained by immunosuppression of CD4 T cells. Thus, elevated levels of three markers are consistent with inflammatory loadings of soluble receptors, chemokines, and proinflammatory cytokines in HIV/AIDS patients[27].

According to multivariable analysis, PDW was the only parameter which indicated a likelihood of death in HIV/AIDS patients. This became clearer through the trend towards a concomitant increase of PDW and MPV in patients who were discharged. MPV and PDW are easily measured platelet indexes, which

increase during platelet activation. However, PDW seems to be a more specific activation marker due to the increase in the number and size of pseudopodia, under activation, and possibly affects platelet distribution width[36]. Platelets are small, anucleate cells that have hemostatic and inflammatory proprieties and are considered to be not so innocent bystanders. Whereas platelets appear to support the host response to extracellular infections, in intracellular infections they contribute to immune evasion[37]. The same authors reported that reduced platelet counts in patients with active TB are associated with mortality[37]. Here, some patients showed thrombocytopenia, but it was not a predictor mortality. Conversely, our multivariate analysis showed that, as the PDW values increased, the probability of death decreased. Thus, our data suggest the role of platelets as host defense effectors in agreement with these studies[38–42].

The limitation of this study is related to the study design, which did not allow patient follow-up, with only one blood sample being taken after recruiting patients for the investigation of serological markers. Another limitation was that the information regarding deaths of patients was obtained solely via electronic medical records; neither did we have access to patients who evolved negatively and were admitted to the intensive care unit.

Among the immunological markers, we could not perceive any differences, as the blood collection was obtained only once and within 72 hours of admission to the hospital, though perhaps we could not distinguish them because all patients were very weak and ill. That is why it is important to perform tests on admission and a follow-up at least once right after the worsening of the state of patient, as well as another before discharge. The data collected corresponded to those closest to the day of blood collection used for the markers. No data was obtained for TB-immune reconstitution inflammatory syndrome.

Conclusions

In conclusion, the opportunistic infections continue to be the major contributory events in mortality of HIV/AIDS patients. The risk of mortality found in HIV-tuberculosis co-infected patients underlines how difficult its management still is. The relation of increased PDW with likelihood of survival evidenced the role of second most common circulating blood cell at the front line of antimicrobial host defense in HIV/AIDS patients under severe immunosuppression. Therefore, future studies are needed on innate immune response of platelets in HIV/AIDS inpatients.

Abbreviations

AIDS – Acquired Immunodeficiency Syndrome

ART - Antiretroviral Therapy

CBA - Cytometric Bead Array

CI - Confidence Interval

CMV - Cytomegalovirus

EBV - Epstein Barr

FMT-HVD – Fundação de Medicina Tropical Dr Heitor Vieira Dourado

GPT - Glutamic-pyruvic Transaminase

HAART - Highly Active Antiretroviral Therapy

HBV - Hepatitis B Virus

HCV - Hepatitis C Virus

HIV – Human Immunodeficiency Virus

IFN - Interferon

IL - Interleukin

MPV - Mean Platelet Volume

NVA: Normal Values in Adults

OGT - Oxalacetic Glutamic Transaminase

OR – Odds Ratio

PDW - Platelet Distribution Width

RR – Relative Risk

TB – Tuberculosis

Th - T helper

TNF - Tumor Necrosis Factor

Declarations

Ethics approval and consent to participate

All protocols and consent forms were approved by the Fundação de Medicina Tropical Dr. Heitor Vieira Dourado Ethics Review Board (CAAE: 57330116.6.0000.0005).

Consent for publication

All consent forms were obtained in writing and signed by the research participants, being stored for future verification.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

WM, GM, DS, YC, DF, PO were responsible for the data collection from medical records. FP, TA, BB and VS performed the statistical analysis. AB, MS, MF, RG and AC participated in study design. WM, TA, CF and PN wrote the first draft of the manuscript. WM, TA, CF, LF, ML and PN elaborated the final version of manuscript. All authors read and approved the final manuscript.

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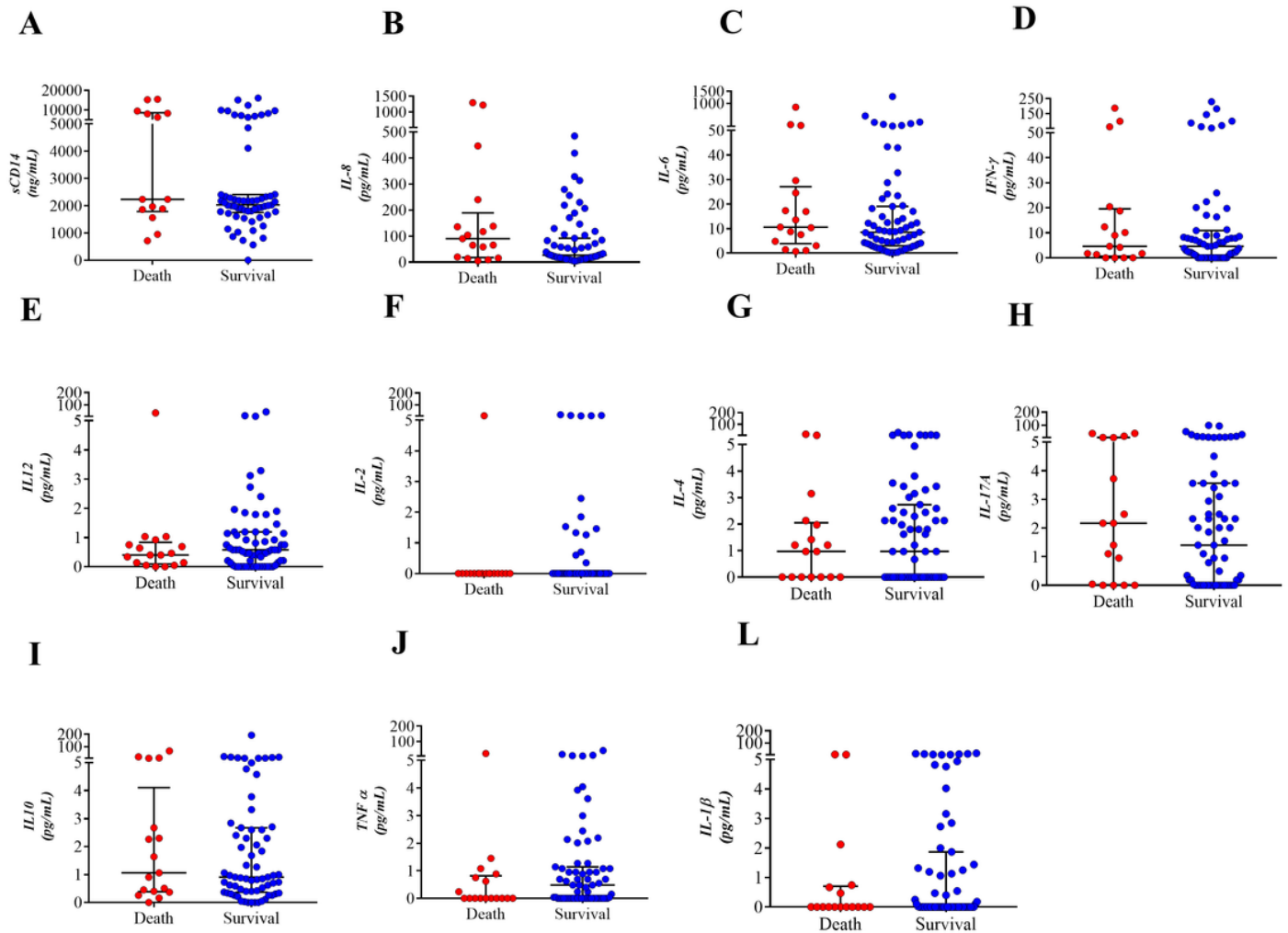
Figures

Figure 1

Serological markers as predictors of mortality. The soluble CD14, the chemokine IL-8 and Th1/Th2/Th17 cytokines were compared between the patients who died and those who survived. A) sCD14; B) IL-8; C) IL-6; D) IFNγ; E) IL-12; F) IL-2; G) IL-4; H) IL-17A; I) IL-10; J) TNFα and L) IL-1β. Concentrations were compared between Death and Survival groups using the Mann Whitney Test.
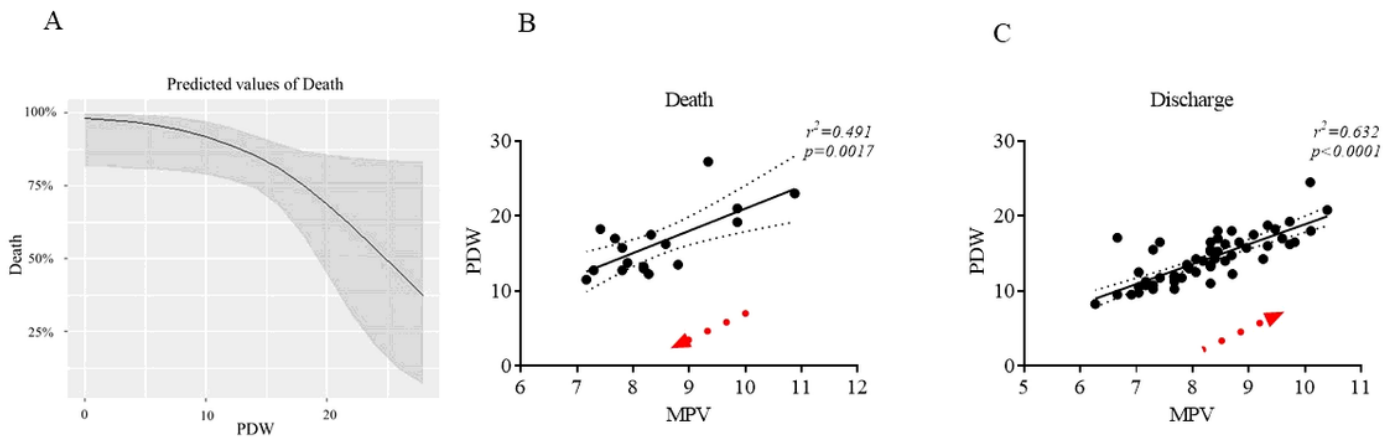
Figure 2

Assessment of predicted PDW values in death of HIV/AIDS patients. A) Logistic function between PDW values and percentage of death in HIV/AIDS patients. As the PDW values increase the probability of death decreases. B) Linear regression between platelet distribution width (PDW) and mean platelet volume (MPV) among those who evolved to death; C) Linear regression between PDW and MPV among those who were discharged.