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Association between cytokine and increased risk of death in ART- naïve and ART-non-adherence patients hospitalized with advanced HIV disease

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Abstract

Background Despite progress in healthcare for people living with HIV/AIDS (PLWHA), many still present with advanced HIV, thus increasing their risk of death. Late initiation of treatment and poor adherence to antiretroviral therapy (ART) are key contributing factors. This study aimed to evaluate cytokines as mortality predictors among hospitalized PLWHA. It assessed the risk of death between ART-naïve and ART-non-adherent PLWHA with advanced HIV and quantified immunological markers in post-mortem samples to determine the influence of irregular ART use.

Methods A longitudinal observational study was conducted at the Fundação de Medicina Tropical Doutor Heitor Vieira Dourado (FMT-HVD) in Manaus, Brazil, with 111 participants recruited between 2018 and 2019. Clinical and laboratory data were obtained from electronic medical records. Plasma samples were analyzed for 27 cytokines/chemokines using the Luminex® multiplex assay within 72 h of admission and 6 h after post-mortem.

Results ART-naïve PLWHA had a higher risk of death. Most of the 27 immunological markers analyzed in the post-mortem were elevated in those who died compared to those who were discharged. Increased levels of IFNγ, CCL2, and CCL3 were associated with death. Elevated immunological markers in ART-naïve PLWHA correlated with CD4 cell counts. Notably, IL-17 increased in ART-naïve PLWHA, while IL-2 increased in ART-non-adherent PLWHA, indicating a dichotomy. T helper-2 responses were marked by IL-9 in ART-naïve and IL-5 in ART-non-adherent PLWHA.

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Conclusions ART-naïve PLWHA hospitalized with advanced HIV have a higher risk of death. Some immunological markers are possible predictors of death upon hospital admission due to HIV/AIDS, and their levels were found to be increased in post-mortem blood samples. Our findings suggest a polarized response among ART-naïve and ART-non-adherent PLWHA.

Keywords HIV/AIDS, Opportunistic infections, Late presentation, Therapeutic failure, Immunological markers, Mortality biomarkers

Background

With the availability of antiretroviral therapy (ART), acquired immunodeficiency syndrome (AIDS) cases are no longer expected to be prevalent. Effective and uninterrupted ART reduces viral load, improves immune function, and significantly decreases mortality while enhancing the quality of life for people living with HIV/AIDS (PLWHA) [1, 2]. Brazil is one of the countries that has responded positively in the fight against HIV/AIDS, offering state-of-the-art antiretroviral therapy through a unified public health system that is universal and free for the entire population [1–4]. Despite this, we are entering the fifth decade, and the HIV/AIDS epidemic in Brazil is far from being stabilized. Although 89% of PLWHA are diagnosed, only 82% are on ART, and while 95% of those on treatment have achieved viral load < 1,000 copies/mL, these numbers remain far from ideal [5]. Misinformation, non-adherence to ART, poverty and social stigmas seem to be barriers to ending the AIDS epidemic in Brazil and late diagnosis and late presentation to healthcare is a major of them [1, 6].

Late presentation occurs when PLWHA seek care at an advanced stage of the disease, typically with a CD4 + count below 350 cells/mm^3 or an AIDS-defining condition [6–12]. Individuals at this stage face significantly higher risks of opportunistic infections and mortality, which have been the leading cause of hospitalization among PLWHA for over a decade, particularly in the region of the present study [13–16]. ART-naïve individuals are more likely to present late due to delayed diagnosis, leading to worse disease progression [6–8]. Insufficient adherence to ART is also associated with disease progression [17–21]. As the immune system deteriorates, the disease advances to a stage defined by a CD4 + count of less than 200 cells/mm^3 or by the World Health Organization (WHO) criteria for stages 3 and 4, which are linked to specific opportunistic infections [7, 9, 22–24].

The burden of advanced disease continues to pose a significant risk of death among ART-naïve patients and ART-non-adherent patients who have interrupted their treatment in Brazil [6, 25]. Recently, we identified a specific chemokine as a potential predictor of mortality in hospitalized HIV patients with advanced disease [26, 27]. A key question remains whether irregular ART use affects circulating cytokine levels and influences mortality differently between ART-naïve and ART-non-adherent PLWHA in late-stage disease.

Cytokines and other soluble immunological markers, such as chemokines and growth factors, have prognostic significance as potential predictors of mortality in untreated PLWHA [28]. Regardless of ART status—whether naïve or non-adherent—disease progression weakens immune regulation, leading to impaired viral control and worsened outcomes. Irregular ART adherence disrupts cytokine production, increasing inflammation and diminishing antiviral responses [29]. In ART-naïve individuals, HIV shifts the immune response toward a Th2-dominated profile, with heightened pro-inflammatory activity and reduced antiviral defenses [18, 30].

Although several studies provide insights into the broader effects of ART on immune function and cytokine profiles [18, 31–36], no studies have specifically compared cytokine levels among PLWHA who have died, either regardless of ART status. In this context, the study evaluated the risk of death using a 27-plex cytokine and chemokine panel and given the complexity of cytokine responses in disease progression, it also assessed post-mortem cytokine profiles in ART-naïve and ART-non-adherent PLWHA who died while hospitalized.

Methods

Study population and study design

This cross-sectional and longitudinal observational study evaluated immunological and clinical biomarkers associated with death or discharge of patients with HIV/AIDS, of either sex, who were admitted to the Fundação de Medicina Tropical Doutor Heitor Vieira Dourado (FMT-HVD) between 2018 and 2019. A total of 111 participants aged between 18 and 70 were enrolled in this study within 72 h of admission. After signing the informed consent form, blood was collected from each patient to measure serum cytokines and chemokines. Of the 111 patients included, 77 were discharged and 34 died. All patients were followed up until discharge or death.

Participant selection of ART-naïve and ART-non-adherent PLWHA

Participants in this study were classified into two groups: ART-naïve PLWHA and ART-non-adherent PLWHA. This classification was based on structured interviews

conducted with each participant. The information obtained from these interviews was further validated by cross-referencing the electronic medical database at the FMT-HVD, medication dispensing records, and data from the Laboratory Examination Control System (called SISCEL, *Sistema de Controle de Exames Laboratoriais*) and Brazilian Logistics System for the Distribution and Control of Medication for PLWHA (called SICLOM, *sistema de controle logístico de medicamento*). This comprehensive approach ensured accurate categorization of participants, allowing for reliable analysis of their treatment adherence and health status.

General characteristics of patient comorbidities

Information regarding coinfections and comorbidities was obtained from the electronic medical database at the FMT-HVD. The outcomes of interest were survival (hospital discharge) and death, verified via either a death certificate or a discharge authorization registered in the electronic medical record. Comorbidities or disorders were defined as signs and/or symptoms of respiratory, neurological, cardiovascular or digestive origin, of both infectious and non-infectious causes, with or without chronicity.

Respiratory syndromes encompass a variety of pathogenic conditions that affect the respiratory tract, including infectious and non-infectious signs and symptoms such as dyspnea (shortness of breath or difficulty breathing), abnormal lung auscultation, long-term and/or productive cough, and pleural effusion. For ART-experienced PLWHA, positive cultures for *Mycobacterium tuberculosis* or positive GeneXpert® MTB/RIF results were collected from their electronic medical records for previous hospitalizations. For tuberculosis diagnosis in ART-naïve PLWHA, those who had died or were discharged were diagnosed based on positive culture, GeneXpert® MTB/RIF results, or suspected TB in chest X-rays, which was characterized by lesions such as infiltrates in the upper lobe, cavitations, nodules, or hilar lymphadenopathy. Neurological syndromes include those of infectious and non-infectious etiology, with signs and symptoms such as alterations in consciousness, sensory loss and/or movement disorders (poor coordination, tremors, asthenia), paralysis and seizures. Gastrointestinal candidiasis was diagnosed by examining lesions in the mouth associated with odynophagia, dysphagia, esophagitis, gastritis, vomiting and diarrhea. Other diagnoses, such as herpes simplex types 1 and 2, Epstein-Barr virus, varicella zoster virus, cytomegalovirus, John Cunningham virus, BK virus, *Toxoplasma gondii*, *Pneumocystis jirovecii*, and HTLV-1/2, were identified according to the protocols described by [37]. *Cryptococcus* sp. was detected using the cryptococcal antigen (CrAg), Nankin ink staining, and culture. *Histoplasmosis* sp. was detected using Nankin ink staining, and culture.

Clinical management of hospitalized patients with advanced AIDS

The clinical management involves a comprehensive approach [38]. Antiretroviral therapy (ART) initiation is typically delayed until the infection is stabilized to avoid immune reconstitution inflammatory syndrome (IRIS). Treatment for opportunistic infections includes management of fungal infections such as pneumocystosis (*Pneumocystis jirovecii*) with sulfamethoxazole/trimethoprim (SMX/TMP), which can be combined with corticosteroids in severe cases. Amphotericin B, followed by fluconazole, is used for cryptococcal or neurocryptococcal meningitis, or histoplasmosis, while rifampicin and isoniazid are administered for tuberculosis, with adjustments to account for drug interactions with ART. Sulfadiazine with pyrimethamine and folinic acid is the first-line treatment for cerebral toxoplasmosis, and ganciclovir or valganciclovir are recommended for cytomegalovirus (CMV) infections. In cases of severe pneumocystosis, corticosteroids are added to reduce pulmonary inflammation. Routine laboratory evaluations include monitoring CD4 counts and viral loads to assess immune status and ART effectiveness, alongside cultures and imaging to aid in diagnosing and managing opportunistic infections. Complications such as IRIS, if occurring after ART initiation, are managed with anti-inflammatory agents and corticosteroids in severe cases. Metabolic and nutritional disorders are addressed through electrolyte monitoring, nutritional support, and correction of malnutrition or anemia, which are critical components of care.

Causes of death reported on death certificates

All the individuals had advanced AIDS with opportunistic respiratory infections (Supplementary data). According to their death certificates, advanced AIDS was associated with several opportunistic infections leading to septic shock with or without acute respiratory failure. Based on medical histories obtained from the electronic medical records, these patients were admitted to the hospital with worsening conditions (as evidenced by the average length of hospital stay), which led to respiratory failure requiring ventilatory support. In summary, the disease had already been manifesting itself, then worsened, and there was little time to provide sufficient support to diagnose the etiological agent and prescribe antimicrobial therapy before initiating antiretroviral medication. In other words, the exacerbation indicated by the short hospitalization time suggests that they suffered from pulmonary dysfunction caused by an acute exacerbation of a chronic disease. Additionally, patients

were also suffering from drug-induced hepatitis caused by empirical polypharmacy.

Sociodemographic and laboratory data of cases and control participants

During the first contact with the patients, sociodemographic data such as name, age, gender and use of ART were collected. The following data were collected through the patient's electronic medical records: Clinical data: general health status, comorbidities, coinfections, treatment, clinical manifestations (weight loss, diarrhea, vomiting), discharge, and death; Laboratory data: blood count, immunological markers (viral load and CD4 + T cell count), and biochemical markers (glucose, calcium, total protein, albumin, globulin, urea, creatinine, bilirubin, gamma GT, alkaline phosphatase, AST and ALT, phosphorus, sodium, potassium and C-reactive protein).

Collection of blood samples

On patient enrollment, after the interview and signing of the consent form, 5 mL of blood was collected by venipuncture in vacuum tubes with EDTA anticoagulant. Collection occurred upon admission to the ward, and patients were monitored until discharge or death. In the event of patient death, their family members/caregivers were approached regarding the possibility of conducting a post-mortem examination for the purposes of epidemiological and patient care monitoring and education, and as a complement to in vivi data. In these cases, 4 mL of blood was collected via peripheral venipuncture using vacuum EDTA tubes. After collecting all the samples (taken on admission or during post-mortem), each sample was centrifuged at 500 g for 5 min at 25 °C to obtain the plasma. One milliliter was aliquoted immediately for marker measurement and then stored at − 80 °C, as described by [26], for future analysis.

Dosage of immunological markers

The BioPlex Pro™ Human Cytokine 27plex assay kit (cat. no. M500KCAF0Y) is composed of a magnetic bead and microsphere panel that quantifies FGF, Eotaxin (CCL11), G-CSF, GM-CSF, IFN-γ, IL-1β, IL-1ra, IL-2, IL-4, IL- 5, IL-6, IL-7, IL-8 (CXCL8) , IL-9, IL-10 (CXCL10), IL-12(p70), IL-13, IL-15, IL-17, IP-10, MCP-1 (CCL2), MIP − 1α (CCL3), MIP-1β (CCL4), PDGF-BB, RANTES (CCL5), TNF-α and VEGF in volumes as low as 25 µL. In summary, the Luminex XMAP technique has a similar principle to the enzyme-linked immunosorbent assay (ELISA) [39]. Magnetic microspheres covalently bind to capture antibodies that target specific cytokines/chemokines. Plasma samples are prepared in a 96-well plate and, after washing, detection antibodies are added to form a sandwich complex. The final detection uses streptavidin-phycoerythrin conjugate as a fluorescence indicator. Cytokine/Growth factors/Chemokine levels are measured in pg/mL using the Luminex XMAP analytical instrument according to the manufacturer's protocol (Bio-Rad).

Statistical analysis

Data were tabulated in an Excel database created by the researchers and analyzed using the GraphPad Prism program, version 7, for univariate analysis, and the STATA program for multivariate analysis. The chi-square (χ^2) test was applied to categorical variables, including causes of hospitalization, relative risk of death among ART-naïve, ART-non-adherenceand undetectable viral load patients, and for comparison of numerical data (hematological, biochemical, and immunological markers). When comparisons were made between two groups, the non-parametric Mann-Whitney U-test was used. The Spearman's correlation was employed to compare CD4 counts, viral load and immunological markers of PLWHA who died (grouped into ART-naïve and non-adherent to ART). Two paired samples of the same patients were assessed using the Wilcoxon signed-rank test to compare cytokine/Growth factors/Chemokine levels in samples taken on admission and during post-mortem. Multivariate analyses were conducted to identify parameters that predicted death by comparing admission data between groups of patients that were discharged and those that died. We evaluated relative risk to determine the association of immunological markers that changed throughout hospitalization, as determined in the post-mortem, between PLWHA grouped into ART-naïve and ART-non-adherent.

Results

Of the 111 hospitalized PLWHA, 34 died (26 males and 8 females). The median age and interquartile range were 34 years (25; 44). Seventy-seven PLWHA were discharged (54 males and 23 females). The median age and interquartile range were 35 years (29; 39.5). The most frequent cause of hospitalization was respiratory syndromes (76.47%), followed by the presence of more than two opportunistic infections: tuberculosis (35.29%), gastrointestinal candidiasis (35.29%), and neurological syndrome (32.35%) (Table 1). Univariate analyses showed that a respiratory syndrome increased the relative risk (RR) of death to 2.05 when compared to PLWHA who were discharged, while neurotoxoplasmosis did not (Table 1). The HIV-RNA > 1,000 copies differed significantly between groups ($p = 0.001$), with a higher concentration in patients who died. CD4 T cell counts were lower in those who died, 27.5×10^3 cells/mL and the interquartile range (IQR) of 17-140.5×10^3 cells versus 82.0×10^3 cells/mL and IQR of 36.50-204.05×10^3 cells ($p = 0.0178$). Both

Table 1 Assessment of causes of hospitalization in HIV/AIDS inpatients

Cause of hospitalization	Death *N* = 33	Discharge *N* = 77	RR	95% CI	*P*
CD4 counts (x cells/mm3) in admission	27.5 (17-140.5)	82.0 (36.50–204.0)			0.0178*
CD4 < 350	32	70	1.412	0.5347 to 5.085	0.5681
CD4 > 350	2	7			
HIV-RNA copies/mL	894.838 (401.167–1.388.510)	126.368 (73.664-179.071)			0.001*
Hemoglobin (13.0–18.0 g/dL)	9.266 (8.365–10.166)	10.741 (10.243–11.239)			0.003*
Anemia	32	61	3.097	1.001 to 11.35	0.0497*
No	2	16			
Respiratory syndrome	26	42	2.055	1.071 to 4.16	0.035*
No	8	35			
Neurological syndrome	11	39	0.5835	0.3134 to 1.051	0.098
No	23	38			
Other	10	17	1.296	0.693 to 2.26	0.473
No	24	60			
Tuberculosis	12	22	1.235	0.6818 to 2.139	0.508
No	22	55			
Histoplasmosis	2	7	0.7083	0.1967 to 1.87	0.719
No	32	70			
Cryptococcosis	1	2	1.091	0.1982 to 2.846	0.999
No	33	75			
Neurotoxoplasmosis	3	20	0.3703	0.125 to 0.9628	0.044*
No	31	57			
Bacterial Pneumonia	2	6	0.8047	0.2245 to 2.049	0.999
No	32	71			
Pneumocistosis	3	2	2.052	0.7613 to 3.522	0.166
No	31	75			
CMV	1	0	3.333	0.6772 to 17.74	0.306
No	33	77			
Herpes zoster	0	2	0	0 to 2.217	0.999
No	34	75			
Disseminated infection by mycobacteria	0	1	0	0 to 2.729	0.999
No	34	76			
Gastrointestinal Candidiasis	12	18	1.473	0.8182 to 2.519	0.246
No	22	59			
No coinfection	11	15	1.564	0.8592 to 2.666	0.152
No	23	62			
More than 2 opportunistic infections	24	66	0.56	0.3327 to 1.028	0.070
No	10	11			

N = simple number; RR = risk ratio; IC = confidence interval; * significant p-value

groups showed evidence of advanced disease, as nearly all PLWHA had CD4 counts below 200 cells/10^3 mm^3.

We compared laboratory parameters between those who died and those who were discharged (Supplementary Table 1). Although anemia was observed in both groups, the patients who died had an RR of 3.0 and lower hemoglobin levels. Among the hematological and biochemical parameters, no significant alterations were observed (Supplementary Tables 1 and 2). The values of calcium, total protein and albumin were significantly different between the groups. In general, liver function was preserved without significant alterations in liver markers. Alkaline phosphatase and transaminase levels were elevated in both groups. Renal function was able to maintain creatinine levels within normal limits. The other laboratory parameters did not show significant differences between admission and death (Supplementary Table 1).

To assess whether soluble immune response factors (cytokines, growth factors and chemokines) were associated with the outcome of death (Fig. 1), we measured 27 immunological markers. Of the total markers evaluated, 23 were observed to be increased in patients who died when compared to those who were discharged. Thus, several of these immunological markers are promising predictors of mortality.

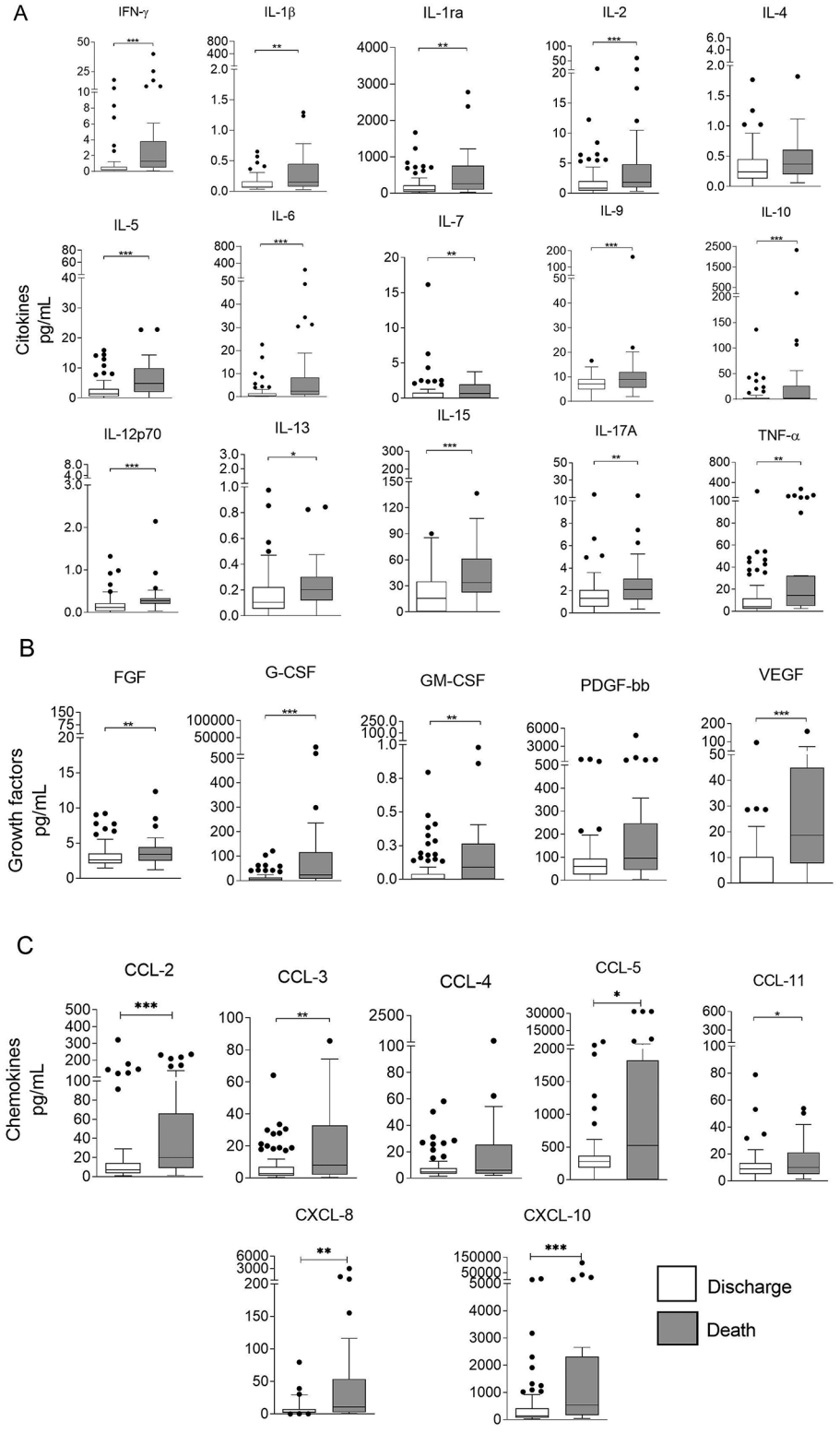

Fig. 1 Cytokines, chemokines, and growth factors as predictors of mortality. Measurement of immunological mediators (Bio-Plex Pro human cytokine 27-plex kit) among HIV/AIDS patients who were discharged or died. Concentrations were compared using the Mann-Whitney test. **A**) Cytokines are shown in alphabetical sequence. **B**) Growth factors are shown in alphabetical sequence. **C**) Chemokines are shown in alphabetical sequence

Table 2 Relative risk of laboratory parameters and mediator markers in the mortality of hospitalized PLWHA

Predictors	Outcome		
	Risk Relative	95%CI	*P*
(Intercept)	0.0001	0.0000–0.0001	0.004
Hematocrit	0.8879	0.8064–0.9777	0.016
CCHM g/dL	2.1777	1.3302–3.5651	0.002
Leukocytes/mm^3	1.0002	1.0000–1.0003	0.013
Sodium mmol/L	1.0393	0.9549–1.1311	0.373
IFN-γ	1.9110	1.0018–3.6455	0.049
CCL-2	0.9608	0.9246–0.9984	0.041
CCL-3	1.0656	1.0073–1.1273	0.027
CCL-5	1.0007	0.9999–1.0016	0.098

Multivariate analysis was conducted to evaluate relative risk

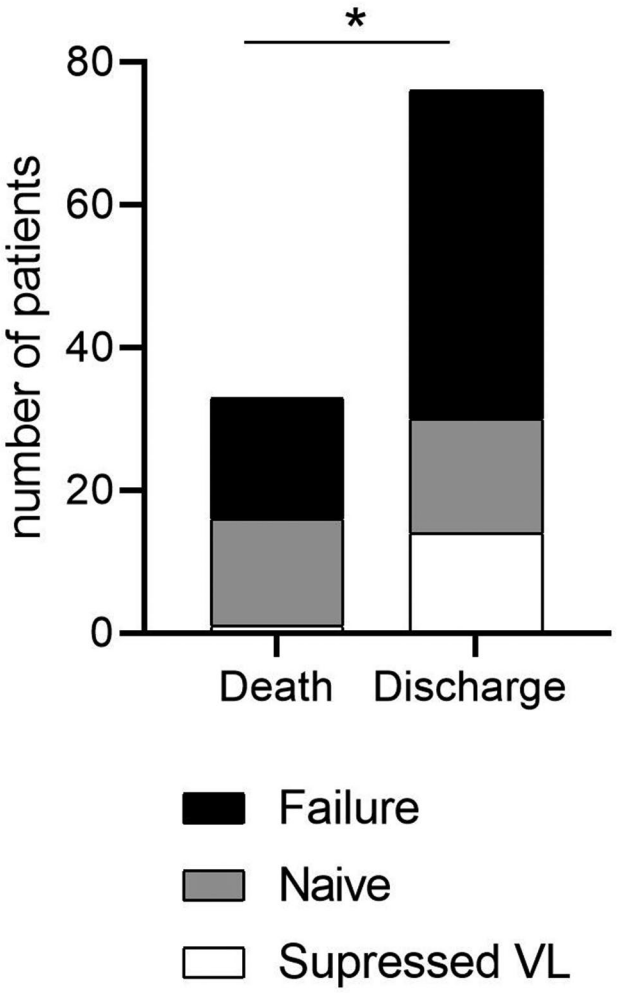

Fig. 2 Relative risk of death in hospitalized HIV patients according to ART status. In the period of 2018 and 2019, our study enrolled by convenience 111 hospitalized PWLHA of which 77 were discharged and 34 died. Relative risk of death was calculated using χ^2 among 31 ART-naïve people, 63 ART-non-adherent people, and 17 PWLHA with suppressed viremia. Among those who died, 15 were ART-naïve, 17 ART-non-adherent and one had suppressed viremia

We evaluated the association of laboratory variables and immunological markers with the risk of death (Table 2). Using a relative risk model, hematological, biochemical factors, and immunological assessment markers were identified as independent predictive factors for death. The relative risk indicated a higher risk of death in patients with reduced levels of hematocrit, MCHC, leukocytes, and high levels of INF-γ, CCL-2 and CCL3.

Hospitalized PLWHA were stratified considering the use of antiretroviral therapy, virological failure, treatment-naïve status and suppressed viral load (Fig. 2). Based on this stratification, the death and discharge groups were compared using the χ^2 test. The RR indicated an increased risk of death in patients with virological failure and treatment-naïve status compared to those who were discharged (RR: 2.62; 95% CI: 1.23 to 5.58; $p = 0.013$).

Regarding ART status among those who died, the hospitalization time for ART-naïve PLWHA was shorter than for ART-non-adherent PLWHA ($p = 0.031$). The median number of hospitalization days for ART-naïve PLWHA who died was 9 days, with an IQR of 8.5 to 22.5 days. The minimum hospitalization time was 3 days, and the maximum was 48 days. The median number of hospitalization days for ART-non-adherent PLWHA who died was 8 days, with an IQR of 4 to 11 days. The minimum hospitalization time was 2 days, and the maximum was 65 days. Viral load and CD4 counts did not differ between ART-naïve and ART-non-adherent PLWHA. The median viral load for ART-naïve PLWHA was 181,092 copies of HIV RNA/mL, with an IQR of 46,442 to 494,805 copies/mL, while the median viral load for ART-non-adherent PLWHA was 287,753 copies of HIV RNA/mL, with an IQR of 40,807 to 760,571 copies/mL ($p = 0.922$). The median CD4 T-cell count for ART-naïve PLWHA was 29.5×10^3 cells/mL (IQR 19.2 to 136.8×10^3 cells/mL), while for ART-non-adherent PLWHA it was 27.5×10^3 cells/mL (IQR 13.7 to 223×10^3 cells/mL) ($p = 0.707$).

Since ART-naïve PLWHA have no prior experience with antiretroviral drugs, we specifically assessed the correlations of these immunological markers with CD4 + T cell counts in both ART-naïve and ART-non-adherent PLWHA, focusing on those who either died or were discharged (Fig. 3). In the ART-naïve PLWHA who died, CD4 + T cell counts were inversely correlated with several immunological markers, IL-1β (r=-0.69, $p = 0.0212$); IL-2 (r=-0.74, $p = 0.012$); IL-4 (r=-0.80, $p = 0.0049$); IL-5 (-0.84, $p = 0.003$); IL-6 (r=-0.62, $p = 0.027$); IL-9 (r=-0.77, $p = 0.008$); IL-15 (r=-0.81, $p = 0.003$); IL-17 (r=-0.86, $p = 0.002$); FGF (r=-0.79, $p = 0.007$); G-CSF (r=-0.65, $p = 0.029$); CCL2 (r=-0.84, $p = 0.002$); CCL3 (r=-0.70, $p = 0.018$) e CXCL8 (r=-0.84, $p = 0.003$), and positively correlated with CCL4 ($r = 0.78$, $p = 0.032$) and VEGF ($r = 0.85$, $p = 0.0009$). However, no significant correlations

Variables	ART-Naive PWLHA				ART-Non adherent PWLHA			
	Discharged		Death		Discharged		Death	
	r	*P-value*	*r*	*P-value*	*r*	*P-value*	*r*	*P-value*
IFNγ	0,03721	0,8835	-0,6059	0,0732	-0,184	0,2435	-0,1533	0,5544
IL-1β	-0,1724	0,4939	-0,6925	**0,0212***	-0,1902	0,2275	-0,2747	0,2838
IL-1ra	-0,1074	0,6715	-0,4545	0,1474	-0,09387	0,5543	-0,05028	0,8482
IL-2	-0,4369	0,0699	-0,7455	**0,0129***	-0,1895	0,2294	-0,1877	0,4674
IL-4	0,2377	0,3422	-0,8091	**0,0049***	-0,4047	**0,0078***	-0,1609	0,5337
IL-5	-0,1115	0,6596	-0,8455	**0,0033***	-0,2335	0,1367	-0,1717	0,5071
IL-6	0,2589	0,2995	-0,6273	**0,0278***	-0,04367	0,7836	0,008584	0,9756
IL-7	-0,09301	0,7136	-0,09081	0,7914	0,1072	0,4991	0,04386	0,8665
IL-9	-0,1725	0,4936	-0,7727	**0,0087***	-0,08829	0,5782	-0,1386	0,5935
IL-10	-0,07625	0,7636	-0,5364	0,0839	-0,2073	0,1877	0,08216	0,753
IL-12p70	-0,2773	0,2653	-0,4977	0,1692	0,2006	0,2027	-0,3595	0,1559
IL-13	0,04021	0,8741	0,03636	0,9739	-0,1361	0,3901	-0,4346	0,0822
IL-15	-0,246	0,3252	-0,8182	**0,0038***	-0,0249	0,8756	-0,1313	0,6128
IL-17	0,1436	0,5697	-0,8636	**0,0025***	-0,2719	0,0816	-0,07485	0,7741
TNFα	-0,4471	0,0629	0,4909	0,1616	-0,2929	0,0597	-0,13	0,6167
FGF	0,1911	0,4475	-0,7909	**0,007***	-0,1216	0,4431	0,04047	0,8779
G-CSF	-0,3279	0,184	-0,6545	**0,0299***	0,04244	0,7896	-0,09571	0,7129
GM-CSF	-0,1648	0,5135	-0,8374	**0,0021***	-0,2483	0,1128	0,1534	0,5523
PDGFbb	0,04956	0,8452	-0,1545	0,4851	0,3426	**0,0264***	-0,06009	0,8186
VEGF	-0,01057	0,9668	0,8532	**0,0009***	0,06699	0,6734	-0,3294	0,1951
CCL2	0,2447	0,3277	-0,8455	**0,0025***	-0,1977	0,2095	-0,3225	0,2057
CCL3	-0,2552	0,3068	-0,7091	**0,0185***	-0,1344	0,3961	-0,1239	0,6338
CCL4	-0,1931	0,4427	0,7818	**0,0323***	0,05431	0,7326	-0,1655	0,5228
CCL5	-0,2426	0,332	0,1959	0,7111	-0,0586	0,7124	-0,3613	0,1537
CCL11	0,3511	0,1532	-0,5727	0,0667	-0,3052	**0,0494***	-0,1471	0,5704
CXCL8	-0,1373	0,5869	-0,8455	**0,0033***	-0,203	0,1973	-0,01839	0,9454
CXCL10	0,03511	0,89	-0,4727	0,1275	-0,1722	0,2754	-0,05763	0,8259

Fig. 3 Correlations between immunological markers and CD4 T cell counts according to ART status. Each immunological marker was correlated with the CD4 T cell count on admission among PLWHA who died or were discharged, classified into ART-naïve, ART-non-adherent or PLWHA with suppressed viremia. The correlation between CD4 T cell counts for each marker was calculated using Spearman's correlation coefficient (r = Rho value). Positive correlations are in blue gradient scale and negative correlations are in red gradient scale. Only the p-values with statistically significant differences are depicted with an asterisk: *$p < 0.05$; **$p < 0.005$

were observed in those who were discharged. Conversely, in ART-non-adherent PLWHA, IL-4 and CCL11 levels were inversely correlated with CD4 + T cell counts, and PDGFbb levels were positively correlated in those who were discharged, with no significant correlations in those who died. The extensive correlations observed in ART-naïve PLWHA who died indicate that the exacerbation of these markers is associated with lower CD4 + T cell counts.

Overall, levels of most of the 27 immunological markers were increased in the post-mortem samples compared to the admission samples (Fig. 4A-C). Exceptions were the growth factors PDGFbb, and VEGF (Fig. 4B), and the chemokines CCL3, CCL5 and CXCL10 (Fig. 4C), which did not increase compared to levels on admission. When comparing ART-naïve PLWHA and ART-non-adherent PLWHA, Table 3 shows an increase in both groups post-mortem for IFN-γ, IL-1β, IL-1RA, IL-4, IL-6, IL-10, IL-12p70, IL-13, IL-15, FGF, G-CSF, CCL2, CCL11 and CXCL8. Of these immunological markers, only CCL3 ($p = 0.0114$) and CXCL8 ($p = 0.040$) measured post-mortem differed between ART-naïve PLWHA and ART-non-adherent PLWHA. Nonetheless, the levels of IL-17 ($p = 0.012$), IL-7 ($p = 0.008$) and IL-9 ($p = 0.008$) were increased in the post-mortem samples of ART-naïve PLWHA but not in those who reported non-adherence to ART. Figure 5A-F illustrate these differences post-mortem compared to admission. Conversely, IL-2 ($p = 0.003$) and IL-5 ($p = 0.0001$) were increased in the post-mortem samples of those who reported non-adherence to ART but not in ART-naïve PLWHA (Table 3; Fig. 5G-J). Figure 5L-M show that the increase in IFN-γ in the post-mortem samples occurred in both groups; however, the increase observed in ART-non-adherent PLWHA was very abrupt.

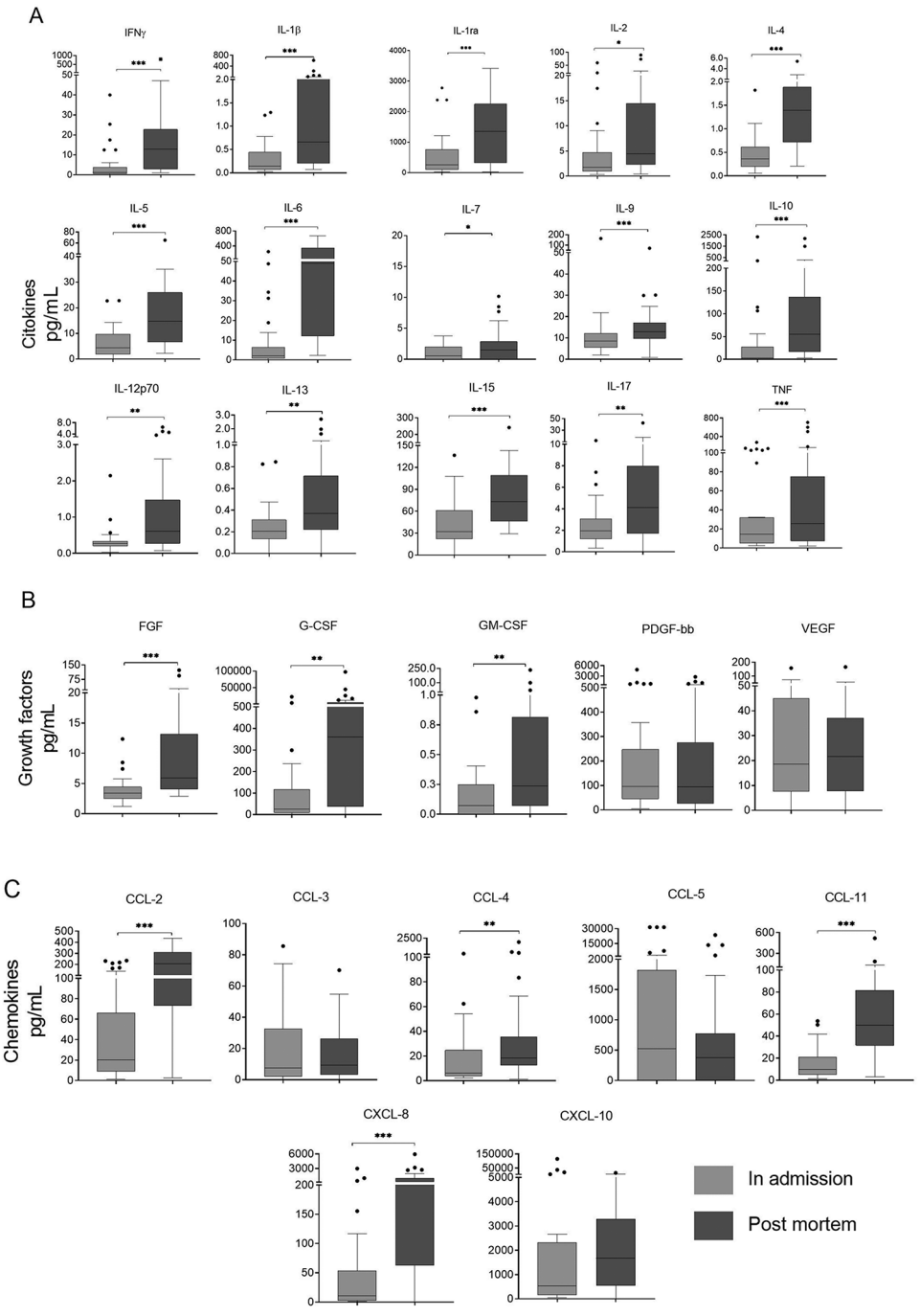

Fig. 4 Temporal comparison of the cytokines, chemokines, and growth factors between samples taken on admission and during post-mortem. Measurement of immunological mediators (Bio-Plex Pro human cytokine 27-plex kit) among HIV/AIDS patients who were discharged or died. Concentrations were compared using the Wilcoxon test. **A**) Cytokines are shown in alphabetical sequence. **B**) Growth factors are shown in alphabetical sequence. **C**) Chemokines are shown in alphabetical sequence

Discussion

As of 2023, the state of Amazonas reported 31% of PLWHA start ART with CD4 counts below 200 cells/mm^3 [5], a stage that has been the leading cause of hospitalization among this population for over a decade, as these individuals face significantly higher risks of opportunistic infections and mortality [13–16]. This study is the first to report a higher risk of death in hospitalized ART-naïve PLWHA, and it should be highlighted that sociodemographic determinants, such as disinformation, poverty and social stigmas, contribute to non-adherence to ART [6]. Additionally, our study indicates that the burden of

Table 3 Correlation of inflammatory mediators with CD4 cell count in HIV/AIDS patients hospitalized treatment naive with virological failure (failure) on admission and at postmortem

	ART-naïve PLWHA			ART non-adherence PLWHA			Comparison between groups	
	N = 15			*N* = 17			Data in admission	Data in postmortem
	In admission	Postmortem	*p*	In admission	Postmortem	*p*	*p*	*p*
IFNγ	3.015 (0.88: 12.51)	13.89 (4.34: 20.64)	**0.012**	0.355 (0.865: 1.488)	11.55 (2.67: 22.8)	**0.001**	0.059	0.576
IL-1β	0.125 (0.07: 0.66)	1.335 (0.36: 9.575)	**0.010**	0.0725 (0.145: 0.2425)	0.3 (0.13: 2.695)	**0.009**	0.918	0.249
IL-1ra	224.5 (104.2: 1188)	1544 (852.3: 2625)	**0.003**	75.21 (254.8: 490.8)	1014 (278.2: 1657)	**0.003**	0.312	0.140
IL-2	1.615 (0.99: 9.05)	4.695 (2.495: 15.76)	0.107	0.8525 (1.92: 4.028)	3.675 (2.243: 13)	**0.003**	0.647	0.433
IL-4	0.305 (0.2: 0.825)	1.665 (0.615: 1.885)	**0.005**	0.165 (0.375: 0.535)	1.21 (0.7125: 1.998)	**0.0001**	0.992	0.984
IL-5	6.065 (2.75: 14.01)	14.76 (6.775: 29.37)	0.054	1.348 (3.245: 7.28)	14.09 (6.145: 25.95)	**0.0001**	0.053	0.975
IL-6	1.23 (0.845: 7.215)	126 (10.99: 351.2)	**0.008**	0.375 (3.23: 8.558)	46.15 (11.38: 305.3)	**0.001**	0.978	0.765
IL-7	0 (0: 1.565)	2.655(0.83: 3.6)	**0.008**	0.285 (1.07: 2.115)	1.435 (0: 2.158)	0.999	0.057	0.128
IL-9	8.42 (5.1: 11.81)	14.46 (8.85: 18.31)	**0.008**	7.513 (8.455: 12.56)	12.92 (10.51: 15.65)	0.174	0.411	0.550
IL-10	4.76 (1.335: 41.03)	21.03 (15.42: 91.4)	**0.049**	0.575 (1.595: 25.15)	62.04 (14.15: 186.2)	**0.010**	0.176	0.526
IL-12p70	0.27 (0.185: 0.43)	1.185 (0.305: 2.125)	**0.012**	0.2 (0.265: 0.3175)	0.535 (0.255: 1.208)	**0.011**	0.634	0.472
IL-13	0.2 (0.105: 0.4)	0.59 (0.22: 0.93)	**0.039**	0.1375 (0.215: 0.2775)	0.37 (0.2: 0.6)	**0.009**	0.674	0.727
IL-15	38.68 (22.23: 63.1)	73.34 (45.03: 110.9)	**0.015**	18.09 (29.59: 49.19)	63.41 (47.59: 106.8)	**0.0002**	0.294	0.881
IL-17	1.775 (1.21: 5.255)	4.77 (2: 11.84)	**0.021**	1.16 (2.355: 2.893)	3.395 (1.49: 5.715)	0.330	0.888	0.261
TNFα	10.8 (3.425: 28.14)	25.03 (5.325: 82.24)	0.330	7.078 (14.42: 60.5)	26.97 (8.27: 62.48)	0.611	0.411	0.433
FGF	3.405 (2.57: 5.76)	8.72 (4.095: 14.7)	**0.005**	2.365 (3.41: 4.443)	5.55 (4.095: 11.35)	**0.001**	0.801	0.621
G-CSF	44.07 (10.82: 165.2)	469.6 (63.88: 11519)	**0.043**	6.565 (18.43: 63.12)	64.27 (8.33: 1485)	**0.015**	0.261	0.113
GM-CSF	0.07 (0.0: 0.34)	0.235 (0.075: 0.755)	0.148	0.06 (0.0: 0.25)	0.22 (0.035: 1.02)	0.052	0.693	0.977
PDGFbb	54.49 (17.37: 221.8)	130.5 (17.22: 312.5)	0.595	69.62 (115.8: 302.5)	65.6 (27.14: 146.6)	0.224	0.105	0.550
VEGF	14.28 (6.66: 44.98)	21.61 (9.27: 35.64)	0.719	5.17 (18.64: 38.45)	15.01 (3.33: 38.38)	0.979	0.940	0.829
CCL2	45.75 (12.28: 170.4)	193.6 (87.52: 271.3)	**0.025**	3.975 (14.22: 23.31)	234 (80.13: 317.1)	**0.0001**	**0.048**	0.832
CCL3	8.645 (3.86: 59.27)	26.12 (7.755: 31.75)	0.719	1.183 (3.36: 13.5)	7.12 (1.508: 16.04)	0.611	0.142	**0.011**
CCL4	10.13 (3.535: 23.19)	11.69 (7.455: 24.65)	0.990	5.118 (15.07: 27.38)	13.33 (5.52: 65.51)	0.377	0.526	0.628
CCL5	744.3 (3.59: 2144)	240.9 (0: 541.8)	0.426	437.9 (0: 3484)	449 (2.23: 885.4)	0.743	0.799	0.405
CCL11	7.895 (4.895: 22.8)	42.74 (12.97: 76.41)	**0.002**	5.575 (11.37: 19.77)	62.3 (35.99: 88.89)	**0.0001**	0.852	0.277
CXCL8	16.08 (3.295: 94.18)	654.7 (151.8: 1894)	**0.012**	2.155 (7.145: 19.24)	178.8 (46.57: 442.5)	**0.0001**	0.153	**0.048**
CXCL10	649.7 (383.3: 2546)	1412 (531.4: 2604)	0.719	115.8 (321.2: 1593)	2058 (451.7: 3305)	0.120	0.113	0.635

#: Median(IQ25:IQ75) *: p-value < 0.05; ** $p < 0.005$

hospitalization remains high among those with advanced HIV, regardless of whether the PLWHA had experience with ART, which is consistent with the findings of this systematic study [14, 15]. The higher mortality in ART-naïve PLWHA presented as late presenters underscores the need for particular attention from the government authorities that are responsible for addressing the social vulnerabilities of PLWHA in the state of Amazonas, as the persistence of advanced HIV is leading to increased AIDS-related mortality in this ART era, especially due to a new wave [40], despite some positive results [1–3, 41].

The massive depletion of CD4 + T cells and HIV viremia lead to extensive immune activation and progression to advanced HIV, which is associated with an increased risk of all-cause mortality [42]. Although the average CD4 count of those who died was lower, nearly all of these patients were in advanced AIDS with severe CD4 depletion. However, the average viremia in those who died was significantly higher compared to those who were discharged. Therefore, the very high levels of several cytokines, chemokines, and growth factors observed in PLWHA who died after hospital admission, as seen in the univariate analysis, are more strongly associated with viral replication than with low CD4 counts. Krastinova and colleagues [43] found an association between viral load and disease progression, showing that higher HIV viral load was significantly correlated with elevated levels of markers, including CCL2, compared to those with lower viral loads. Thus, our findings align with the understanding that immune activation, characterized by excessive cytokine and chemokine production, is associated with rapid disease progression and increased mortality risk [26, 43–48].

During HIV-1 infection, various cytokines and chemokines are activated, influencing viral replication either positively or negatively [49]. While several serological

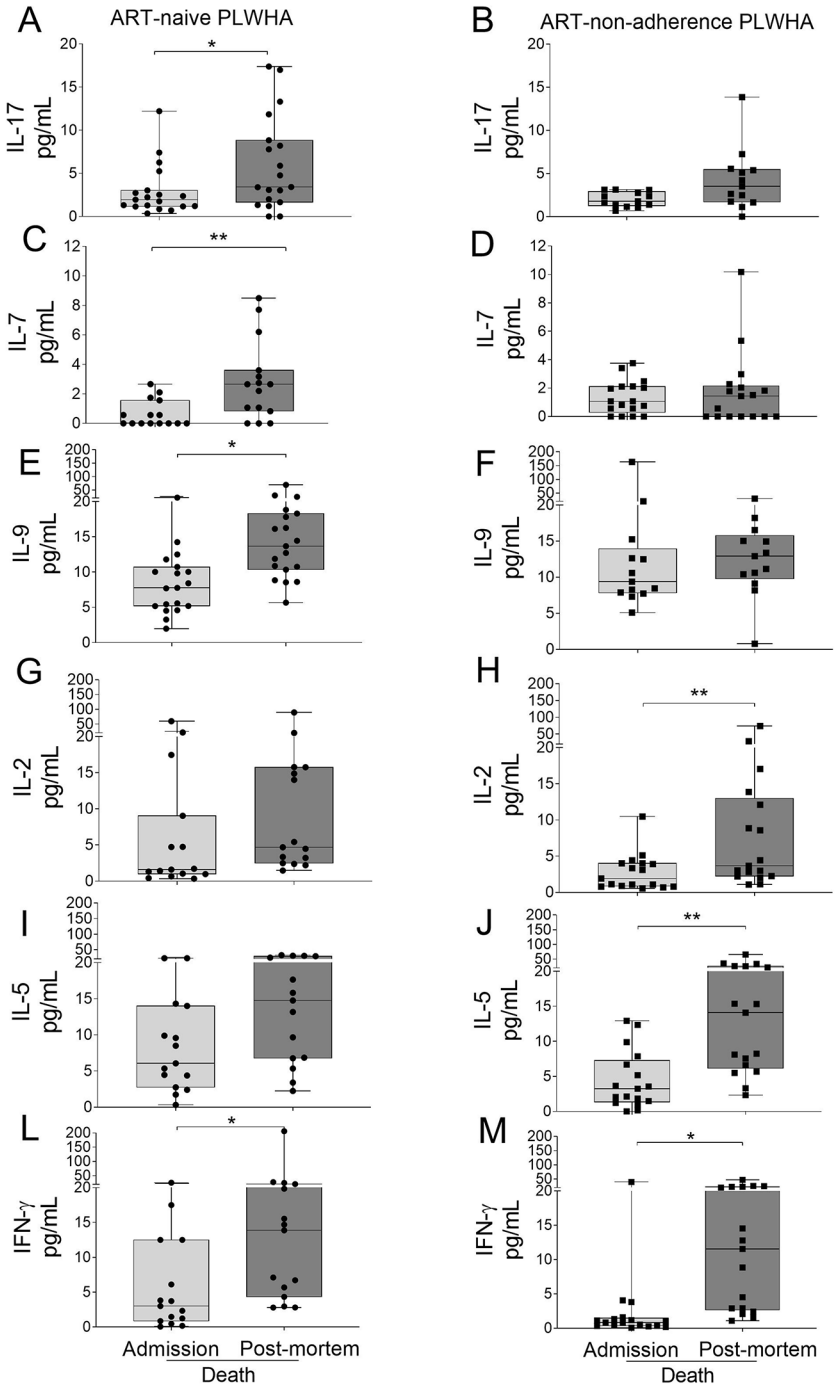

Fig. 5 Differences between levels of IL-17, IL-7, IL-9, IL-2, IL-5 and IFNγ in samples taken post-mortem in relation to the samples taken on admission to hospital among ART-naïve and ART-non-adherence PWLHA. Concentrations were compared using the Wilcoxon test

markers were elevated in those who succumbed to the disease, INF-γ, CCL2, and CCL3 were the only factors identified as significant predictors of mortality in our multivariate analysis, building upon earlier findings [26]. A study highlights the role of gamma interferon (IFN-γ) in PLWHA that experienced treatment failure with a significant increase in IFN-γ levels suggesting a link to a pro-inflammatory immune state associated with high viral loads [32]. In relation to chemokines, we observed divergent associations between the relative risks of CCL2 and CCL3 with outcomes (Table 2). While CCL2 acted as a protective factor, CCL3 emerged as a risk factor. Meanwhile, CCL5 approached statistical significance. Additionally, we observed divergent associations

between CD4 counts and the chemokines CCL2, CCL3, and CCL4 in treatment-naive PLHIV who died. CCL2 and CCL3 showed an inverse correlation with CD4, while CCL4 had a direct correlation. This result aligns with the known roles of chemokines and their receptors in HIV infection, where CCR2b and CCR3, ligands for CCL2 and CCL3, may have inhibitory effects on viral entry, though possibly less potent than other pathways [49, 50]. Chemokines such as CCL5, CCL3, and CCL4, ligands for CCR5, are known to block the entry of early-stage HIV strains, while SDF-1α, the ligand for CXCR4, inhibits later-stage strains [50]. Campbell et al. demonstrated that CCL2 levels correlate with HIV viral load, indicating that CCL2 may enhance CD4 + T cell susceptibility to infection by upregulating CXCR4, facilitating X4-tropic HIV strain entry [51]. Other studies suggest that CCL2 may indirectly block viral entry by promoting receptor dimerization, impacting CCR5 and CXCR4 without downregulating these receptors [52, 53]. These multifaceted roles of chemokines provide insight into the divergent risk associations observed in our analysis, prompting further questions about how the host's chemokine system influences viral replication and tropism. This is particularly relevant given the high prevalence of subtype B in the region, as documented in previous studies [54, 55].

In addition to analyzing mortality outcomes, our study reveals important differences in cytokine profiles between ART-naïve PLWHA and those who have undergone antiretroviral therapy. Some studies have reported that IL-4, TNF-α and IFN-γ are inversely associated with CD4 T cell counts [17, 18]. In our study, several markers were inversely associated with CD4 T cell counts in ART-naïve PLWHA. Benjamin Amoani et al. [17] discuss the modulation of cytokines by ART, noting that as the viral load decreases, these cytokine levels normalize while anti-inflammatory cytokines decrease. This aligns with our data, which shows that the inverse correlation between CD4 counts and certain cytokines in ART-naïve PLWHA may disappear with ART-experience, even with non-adherence. Despite this, more studies are necessary to underscore the impact of ART on cytokine modulation and immune response.

A unique aspect of this study is the comparison of 27 immunological markers in post-mortem samples. With the exception of PDGFbb, VEGF, CCL3, CCL5 and CXCL10, all other markers were exacerbated post-mortem compared to levels on admission (Fig. 3). According to Maes et al. [39], the 27-plex can be separated into an immune profile, such as the M1 macrophage (IL-1β, sIL-1RA, IL-6, TNF-α, CXCL8, CCL3), T helper-1 (IL-2, IFN-γ, IL-12), T helper-2 (IL-4, IL-5, IL-9, IL-13) and T helper-17 (IL-6, IL-17). Additionally, it includes systems such as the broad immune-inflammatory response system (IRS), which is composed of IL-1β, IL-6, TNF-α, CXCL8, CCL3, IL-2, IFN-γ, IL-12, IL-17, IL-15, G-CSF, GM-CSF, CXCL10, CCL5 and CCL2, and the compensatory immunoregulatory system (CIRS), which is composed of IL-4, IL-10 and sIL-1RA. All the immune profiles were significantly exacerbated in post-mortem samples. Additionally, post-mortem levels of these markers, especially IL-6, were significantly elevated, closely resembling the cytokine storm observed in critically ill SARS-CoV-2 patient [56–59]. Therefore, our findings may help identify generic immunological markers to predict mortality in advanced HIV.

As highlighted earlier, the risk of death is higher among ART-naïve PLWHA. Very few studies have compared cytokine levels between ART-naive and ART-nonadherent PLWHA. Musa and colleagues evaluated a few cytokines in ART-naive and ART non-adherent PLWHA from the outpatient clinic of the comprehensive HIV care unit at the referral hospital. Although pro-inflammatory cytokine levels tended to be higher in ART-naive PLWHA, the difference was not statistically significant, and both groups exhibited immune dysregulation with significantly reduced levels of IFN-γ [18]. Two studies highlight cytokine dysregulation in ART-naive PLWHA, focusing on a limited set of cytokines [29, 30]. Both show elevated levels of both pro-inflammatory and anti-inflammatory cytokines, reduced IFN-γ levels, and IL-17 A levels remaining relatively stable, indicating an immune response skewed towards Th2 cytokines. Our study is the first to evaluate this in hospitalized patients, including post-mortem analysis. Given the treatment of hospitalized patients with advanced AIDS, standard hospital protocol may minimize individual variations in opportunistic infections and treatment by employing antimicrobial prophylaxis, delayed ART until stabilization, metabolic management, and corticosteroid support when necessary. By comparing levels at admission and autopsy, the increase in postmortem IL-17 levels in ART-naïve PLWHA, which is not seen in ART-non-adherent PLWHA, contrasts with the rise in IL-2 levels in the latter group, not observed in ART-naïve PLWHA, suggesting a dichotomy (Table 3; Fig. 5). In addition, T helper-2 was represented by distinct cytokines, namely IL-9 in ART-naïve PLWHA and IL-5 in ART-non-adherent PLWHA, in agreement with [29, 30]. Curiously, the increases of postmortem IFN-γ levels in relation to admission in both groups. Although these data were observed in patients who died, who had advanced AIDS and high viral loads, our findings suggest that standard hospital protocols may help mitigate the imbalance caused by incomplete immune recovery and persistent immune activation, which can hinder effective viral control. Further studies are needed to clarify the observed dichotomy between ART-naïve PLWHA and those with ART experience, and to evaluate whether derepression of cytokine imbalance can be achieved through immune recovery.

The study presents several limitations that could impact the interpretation of its findings. One of the primary

limitations is the relatively small sample size of 111 patients, which may limit the generalizability of the findings to a broader population of individuals living with HIV/AIDS. Second, the patients in this study had advanced AIDS and were highly susceptible to opportunistic infections, such as tuberculosis and candidiasis, which may have acted as confounding factors by influencing cytokine levels. However, despite the heterogeneity of co-infections, none, including tuberculosis, showed a significant association with the risk of death. This lack of association makes it challenging to directly attribute the immune markers to mortality or hospital discharge. Furthermore, both intracellular and extracellular pathogens elicit distinct immune responses, and the presence of multiple co-infections likely contributes to a complex cytokine environment. As a result, the disproportionate elevations in cytokines, chemokines, and growth factors observed in patients who died may reflect not only the cumulative effects of these infections but also a broader immune dysregulation typical of terminal stages, rather than pathogen-specific responses. Importantly, the individuals who died had significantly higher HIV viral loads compared to those discharged, suggesting that the viral burden itself could be the primary driver of the observed immune dysregulation. Third, the study lacks a detailed explanation of factors leading to virological failure, which could offer more context regarding its relationship with mortality. Fourth, non-infectious comorbidities, while relevant in other stages of HIV, were not prioritized in our analysis because their influence is likely overshadowed by the more immediate impact of opportunistic infections and the underlying high viral load typical of advanced AIDS. At this stage, the immune system is heavily compromised, and the primary drivers of immune activation and cytokine elevation are related to the host's response to these infections and viral replication rather than non-infectious comorbidities. Fifth, socioeconomic and environmental factors have not been included although they may have impacted ART adherence and patient health. While these variables are crucial for interpreting mortality outcomes in resource-limited settings like the state of Amazonas, they are difficult to quantify and would shift the focus of the study.

This highlights the possibility of leveraging these chemokines not only as biomarkers for disease progression but also as therapeutic agents, potentially improving outcomes in patients with advanced HIV/AIDS who are non-adherent or untreated with antiretroviral therapy. Further investigation into the modulation of chemokine pathways could open new avenues for clinical management and targeted therapies in these high-risk groups.

Conclusions

The main causes of advanced HIV are late treatment initiation and low adherence to ART, leading to early respiratory syndromes and opportunistic infections. Soluble immunological markers have prognostic significance and can predict mortality among hospitalized PLWHA. This study identified three markers (IFNγ, CCL2, and CCL3) that are potential predictors of mortality due to their high levels in ART-naïve patients who died. Further studies are needed to confirm these findings, which could improve clinical outcomes and prevent death in PLWHA.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12879-024-10260-z.

Supplementary Material 1

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Author contributions

WMG, GM, BJB, MFS, TVRA, AA da SB, AG da C, OAM-F, MVGL, LC de LF, ZMS, HN dos SI, ATC and YOC were responsible for the data collection from medical records. TX, MMM, RSP, JMS were responsible for the data collection from medical records, death certificate. WMG, YOC, and AG da C performed immunoassays. ZMS, MVGL, ASB and PAN performed the statistical analyses, MVGL, TVRAA, MFS, LC de LF and PAN participated in study design. TVRA, MFS, LC de LF and PAN wrote the first draft of the manuscript. YOC, WMG, ZMS, ASB and PAN elaborated the final version of the manuscript. All authors read and approved the final manuscript.

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Data availability

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

Declarations

Ethical approval and consent to participate

Informed consent was obtained from all of the participants in the study. All the protocols and consent forms were approved by the Fundação de Medicina Tropical Dr. Heitor Vieira Dourado Ethics Review Board (CAAE: 57330116.6.0000.0005) in accordance with Resolution No. 466/12 of the Brazilian National Health Committee and in compliance with the Declaration of Helsinki.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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