Dissecting drivers of immune activation in chronic HIV-1 infection

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Summary

Background Immune activation is a significant contributor to HIV pathogenesis and disease progression. In virally-suppressed individuals on ART, low-level immune activation has been linked to several non-infectious comorbid diseases. However, studies have not been systematically performed in sub-Saharan Africa and thus the impact of demographics, ART and regional endemic co-infections on immune activation is not known. We therefore comprehensively evaluated in a large multinational African cohort markers for immune activation and its distribution in various settings.

Methods 2747 specimens from 2240 people living with HIV (PLWH) and 477 without HIV from the observational African Cohort Study (AFRICOS) were analyzed for 13 immune parameters. Samples were collected along with medical history, sociodemographic and comorbidity data at 12 HIV clinics across 5 programs in Uganda, Kenya, Tanzania and Nigeria. Data were analyzed with univariate and multivariate methods such as random forests and principal component analysis.

Findings Immune activation was markedly different between PLWH with detectable viral loads, and individuals without HIV across sites. Among viremic PLWH, we found that all immune parameters were significantly correlated with viral load except for IFN-α. The overall inflammatory profile was distinct between men and women living with HIV, in individuals off ART and with HIV viremia. We observed stronger differences in the immune activation profile with increasing viremia. Using machine learning methods, we found that geographic differences contributed to unique inflammatory profiles. We also found that among PLWH, age and the presence of infectious and/or noninfectious comorbidities showed distinct inflammatory patterns, and biomarkers may be used to predict the presence of some comorbidities.

Interpretation Our findings show that chronic immune activation in HIV-1 infection is influenced by HIV viral load, sex, age, region and ART use. These predictors, as well as associations among some biomarkers and coinfections, influence biomarkers associated with noncommunicable diseases.

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Keywords: Antiretroviral therapy; HIV; Immune activation; Inflammation

Research in context

Evidence before this study

We searched PubMed with no language restrictions through November 2021, using the search terms "HIV-1," "immune activation," OR "inflammatory biomarkers." While we did not limit the scope of our search to a particular country or geographic region, we were particularly interested in studies based in Sub-Saharan Africa and resource-limited settings. Prior studies from resource-rich settings have shown that specific inflammatory biomarkers, including sCD14, sCD163, and TNF-α are strong predictors of morbidity and mortality in chronic HIV-1 infection; however, inflammatory profiles of PLWH in sub-Saharan Africa are not as well described. In the Pan African Study to Evaluate Resistance Monitoring (PASER), a cohort study enrolling participants in Kenya, Uganda, Nigeria, South Africa, and Zambia, investigators recently used Luminex panels to evaluate samples from 398 adults living with HIV and 90 adults living without HIV for 8 inflammatory biomarkers, including sCD14, sCD163, CRP, CXCL10, IL-6, CCL2, and CXCL9. The PASER investigators found that for PLWH, IL-6, CCL2, and CXCL9 concentrations were normalized by ART, while CXCL10, CRP, sCD163, and sCD14 concentrations remained elevated when compared to HIV-uninfected controls. Immune profiles differed with the presence of co-infections, like tuberculosis and hepatitis B and C.

Added value of this study

AFRICOS is the largest HIV cohort study that characterizes immune activation and measures soluble inflammatory markers in PLWH from different countries and distinct regions in Sub-Saharan Africa. The large sample size (2270 PLWH and 477 without) and the breadth of soluble markers evaluated (13 different cytokines and chemokines, including IL- 6, CXCL10, IL-10, CCL2, IL-1β, IFN-γ, MIP-1β, CD163, CD25, CXCL9, IFN-α, TNF-α and TNF RII) are major strengths of the study. Furthermore, our analysis includes samples from participants enrolled in HIV care and treatment programs between 2013 and 2018, spanning multiple ART paradigms and WHO HIV guideline eras. We found that nearly all immune parameters were significantly associated with HIV-1 viral load, and that CXCL10 most strongly correlated with increasing viremia. We learnt that there are significant differences in immune activation profiles between men and women living with HIV, driven by differences in CXCL10, CCL2, CXCL9 and CD163 expression. Association between age of the participants and plasma biomarker levels was most evident for CCL2, TNF-RII, IL-6, MIP-1β, and CD25, which is consistent with results from other studies in various settings. However, we also observed differences in biomarker expression by country. Compared to other countries, the average CCL2 concentrations were lower in Uganda, and MIP-1β concentrations were higher in South Rift Valley, Kenya. Our study also showed that specific infectious and non-infectious comorbidities contributed to distinct inflammatory profiles that warrant further characterization.

Implications of all the available evidence

Taken together, data from our study shows that HIV-1 inflammatory profiles vary considerably with HIV viremia, by age, sex, and region, and with the presence of infectious and/or nonifectious comorbidities. Further research in is needed to characterize these seemingly distinct immune profiles, to elucidate immunologic pathways and their clinical implications, and explore opportunities to improve long-term outcomes for PLWH in Sub Saharan Africa.

Introduction

Combination antiretroviral therapy (cART) has transformed HIV infection from a uniformly fatal disease to a chronic condition, with life expectancy among people living with HIV (PLWH) approaching that of the general population. However, PLWH still have higher risk for the development of diseases typically associated with aging, including cardiovascular disease, diabetes, cancer and frailty.[1-3] While confounding lifestyle factors (e.g., smoking, alcohol and drug consumption) may explain some of this increased risk, it has been hypothesized

that low level persistent immune activation and inflammation is significantly contributing to this process.[4] Indeed, levels of markers of innate and adaptive immune activation remain abnormal in individuals that are on ART with fully suppressed viremia.[5,6] These levels have been shown to be strong predictors of comorbidity development and even mortality.[7–10] While the root drivers of this inflammatory state are incompletely characterized, it is possible that low level viral replication in the HIV reservoir, coinfections, and microbial translocation may contribute to this inflammatory state.[11,12] However, less well studied are other factors such as gender, local environmental factors, and nutrition that may contribute to low level immune activation, bias other scientific findings, and impact mortality and morbidity of PLWH.

Studies comparing the natural course of HIV infection between women and men have demonstrated significant sex differences in the manifestations of HIV disease.[13] While women living with HIV present with lower viral load early in HIV infection, women with the same HIV viral load as men have a 1.6-fold higher risk of developing AIDS.[13–15] This has been linked to a stronger immune activation and IFN-α response in women compared to men.[16] Furthermore, it has been suggested that serum vitamin D levels are associated with lower immune activation. However, understanding the factors for persistent low level inflammation in ART treated and untreated PLWH will be critical to elucidate underlying pathomechanisms of HIV disease and associated comorbidities.

To evaluate factors associated with low level immune activation with consideration for the likely multifactorial etiology of low level inflammation promoting a progressive proinflammatory phenotype, we evaluated immune-activation in the African Cohort Study (AFRICOS), a large prospective cohort encompassing a diversity of geographical regions, co-infections, educational, and socioeconomic parameters.

Methods

Study population and design

All participants were enrolled in the African Cohort Study (AFRICOS), an open-ended prospective cohort study implemented by the US Military HIV Research Program (MHRP) at 12 HIV clinics supported by the President's Emergency plan for AIDS relief (PEPFAR).[17] AFRICOS is a longitudinal study aiming to assess the impact of clinical practices, biological factors, and socio-behavioral issues on HIV infection and disease progression in Africa. Preliminary analyses conducted with clinical data from this cohort have been previously published.[17–18]

The study population comprised males and females aged 18 years old and older, living with or at risk for HIV and receiving medical care within five geographically distinct African regions, including the South Rift Valley (SRV) and Kisumu West in Kenya, the city of Mbeya in the southern highlands of Tanzania, Kayunga District in Uganda, and the cities of Abuja and Lagos in Nigeria. Inclusion criteria among PLWH were known HIV infection, signed informed consent, intent of long-term residency in the area, receiving HIV-specific care, and ability to provide contact information. Exclusion criteria included any significant condition that, in the opinion of the investigators, would interfere with study conduct. Similar inclusion and exclusion criteria were applied to those without HIV, with the addition of a requirement to consent to HIV testing and pre- and post-test counseling.

Enrollment procedures included a medical history and physical as well as an interviewer-administered questionnaire. Participants provided blood and/or sputum as indicated for clinical tests relating to general health and screening for infections or other comorbidities. Results for clinical tests were shared with the participant and their routine clinical providers for management and follow-up. For HIV-infected participants, CD4 T-lymphocyte count and HIV RNA viral load were also measured. HIV RNA was quantified using several different platforms over the duration of the study: Roche Cobas Ampliprep/Cobas TaqMan HIV Type 1 (HIV-1) Test, v2.0 (linear range 20–10,0000,000 or 48–10,000,000 copies/mL); Roche High Pure/COBAS TaqMan HIV-1 Test, v2.0 (linear range 34–10,000,000 copies/mL); COBAS® AmpliPrep/COBAS® TaqMan® 48 HIV-1 Test (linear range 48–10,000,000 copies/mL); or Abbott Real Time HIV-1 Viral Load assay (linear range 40–10,000,000 copies/mL). CD4 T-cell count was assessed using BD FACSCount, BD FACSCalibur, BD FACSCanto II, or BD FACSPresto.

Ethics

This study was approved by the institutional review boards of the Walter Reed Army Institute of Research (#1897), Makerere University School of Public Health (#173), University Duisburg-Essen (16-7098-BO), Uganda National Committee of Science and Technology (HS-1175), Kenya Medical Research Institute Science and Ethics Review Unit (SSC# 2396, 2371), Tenwek Institutional Ethics Review Committee (SSC# 2371), Tanzania National Institute of Medical Research (NIMR/HQ/R.8a/Vol.IX/1060), Mbeya Medical and Research Ethics Committee (NIMR/HQ/R.8a/Vol.IX/1060), and the Nigeria Ministry of Defense Health Research and Ethics Committee (#3726112019). All participants provided written informed consent prior to enrollment.

Data from this study cannot be made publicly available owing to restrictions in the participants' informed consent documents, as public availability may compromise participant confidentiality. However, data are

available to interested researchers on request with the agreement of the US MHRP, AFRICOS investigators and collaborating partners (requests should be directed to the corresponding author).

Definition of communicable diseases (CDs) and noncommunicable diseases (NCDs)

All subjects were screened for the following communicable diseases (CDs): syphilis (*treponema pallidum*), hepatitis B and hepatitis C. Cases of syphilis were identified by a reactive non-treponemal serologic test, such as venereal disease research laboratory (VDRL) or rapid plasma reagin (RPR) test; the result was confirmed by a reactive treponemal serologic test such as *T. pallidum* particle agglutination (TP-PA), enzyme immunoassay (EIA) or chemiluminescence immunoassay (CIA). HC Co-infections with hepatitis B were identified by screening and confirmatory serology positive for hepatitis B surface antigen (HBsAg). Confirmed cases of hepatitis C were diagnosed with a positive anti-HCV antibody assay, with confirmation of positive testing by repeat EIA or HCV viral load.

HIV-infected participants were requested to produce a sputum sample for Xpert® MTB/RIF testing (Cepheid, Sunnyvale, CA) to identify active pulmonary tuberculosis.

Elevated blood pressure, hypercholesterolemia, hyperglycemia and renal insufficiency were measured as part of vital sign and clinical chemistry measurements and used to define NCDs. Elevated blood pressure was defined as a single systolic blood pressure measurement >139 mmHg, a single diastolic blood pressure measurement >89 mmHg, or receipt of antihypertensive medications. Hypercholesterolemia was defined as a total fasting cholesterol >199 mg/dL or receipt of lipid-lowering medications. Hyperglycemia was defined as a fasting glucose >99 mg/dL, non-fasting glucose >199 mg/dL, or receipt of hypoglycemic medications. Renal insufficiency was defined as an estimated glomerular filtration rate (GFR) <60 mL/min/1.73 m^2, calculated using the Modification of Diet in Renal Disease (MDRD) equation based on serum creatinine.[19]

Specimen collection

Peripheral blood samples were collected from each study participant at enrollment. Plasma was separated from cells by centrifugation of the whole blood aliquoted and stored at −80 °C or in liquid nitrogen. Sputum specimens for detection of active tuberculosis were required from HIV-infected subjects regardless of symptoms. All samples were processed following Good Clinical Laboratory Practices (GCLP) and archived in the AFRICOS Repository (AFRICOSR).

Cytokine/chemokine profiling by Luminex

Biomarkers were measured using a Luminex Screening Assay according to the manufacturer's protocol. Briefly, microparticles, standards, and plasma were plated into 96-well plates and the immobilized antibodies bind the biological markers [IL- 6, CXCL10, IL-10, CCL2, IL-1β, IFN-γ, MIP-1β, CD163, CD25, CXCL9, TNF-α and TNF RII]. After washing away unbound microparticles and adding biotinylated antibody cocktail specific to the biological markers, the incubation with streptavidin-phycoerythrin conjugate (Streptavidin-PE) was done. A final wash to remove unbound Streptavidin-PE was performed, and the microparticles were resuspended and levels quantified in a Luminex analyzer.

Quantification of soluble IFN-α using ELISA

IFN-α measurement was performed using VeriKine™ Human IFN Alpha ELISA kit®, following the manufacturer's instructions. Briefly, the kit uses a human IFN-α-specific antibody conjugated to horseradish peroxidase (HRP), which shows no cross reactivity to human IFN-γ, human IFN-β or human IFN-ω.

Statistical analysis

The unpaired t test for continuous variables was used to compare demographic/clinical factors between the HIV-infected and HIV-uninfected groups and one-way ANOVA analysis was used to compare differences among the countries. To quantify possible immune parameter differences between gender, region and age, we applied a multilevel Bayesian regression model with the log10-scaled immune parameter as the explained variable and *female* (binary), *region* (5 levels) and *age* (continuous) as explanatory variables. *Viral load* (continuous), *education* (3 levels), *HIV status* (3 levels), *Hepatitis B* (binary), *Hepatitis C* (binary), *Tuberculosis* (binary) and *Syphilis* (binary) are also included to adjust for possible confounding effects. The estimates for *female* are additionally allowed to vary by HIV status. For the communicable- and non-communicable diseases, a Bayesian logistic regression model was used to quantify how each immune parameter is associated with each disease. Correlation between immune parameters and viral load and among immune parameters were measured using Spearman's ρ. Classification accuracy was measured using Cohen's κ. Cohen's κ is defined as $\kappa=(p_o-p_e)/(1-p_e)$, where p_o is the overall accuracy of the model and p_e the expected accuracy achieved by random guessing. Intuitively, Cohen's κ is therefore the improvement of accuracy over random guessing, normalized to the largest possible improvement. At $\kappa = 0$, the model has the same overall accuracy as can be achieved using random guessing; at $\kappa = 1$, the model classifies all samples correctly.

Role of the funding source

Funders did not contribute to study design, data collection, data analysis, or writing of the manuscript. Study

design, data collection, data analysis, and data interpretation were completed by collaborators at the US MHRP, University of Duisberg-Essen, and University of Bonn; writing and review of the manuscript was completed by the authors.

Results

HIV viral load is the main driver of immune activation

From January 2013 to 1 June 2018, we enrolled 3380 participants into AFRICOS, including 2820 PLWH and 560 without HIV. Of these, specimens and clinical data were available for analysis from 2747 participants from Kenya, Tanzania, Uganda and Nigeria: 2270 PLWH and 477 without. Table 1 and Supplemental Table 1 summarize baseline characteristics of all participants. Median age was 39.8 years [Standard Deviation (SD) ±10.4)] for participants with HIV and 37.1 years (SD± 10.4) for participants without HIV. Gender among study participants was well balanced with 59.1% women in PLWH and 54.1% in those without, respectively. The average time since HIV diagnosis was 3.8 years (SD± 3.6). Two thirds of the study participants (67%) were on antiretroviral therapy (ART) and 49.2% had suppressed viral load (<50 copies/ml).

We first investigated the general influence of HIV-1 viral load on levels of inflammation employing a panel of 13 different cytokines and chemokines that we previously identified to be differently regulated in acute and chronic HIV infection as well as in long-term ART treated individuals.[20] These included IL-6, CXCL10, IL-10, CCL2, IL-1β, IFN-γ, MIP-1β, CD163, CD25, CXCL9, IFN-α, TNF-α and TNF RII. Among the 2270 participants with HIV, 1154 had

Characteristics	All participants	HIV-uninfected	PLWH
N by Program Site (%)	*2.747*	477 (17.4%)	2270 (82.6%)
South Rift Valey, Kenya	1095 (39.9%)	175 (36.7%)	920 (40.5%)
Kayunga, Uganda	544 (19.8%)	101 (21.2%)	443 (19.5%)
Mbeya, Tanzania	424 (15.4%)	63 (13.2%)	361 (15.9%)
Kisumu West, Kenya	388 (14.1%)	88 (18.4%)	300 (13.2%)
Abuja & Lagos, Nigeria	296 (10.8%)	50 (10.5%)	246 (10.8%)
Median age, yr (±SD)	39.3 (±10.5)	37.1 (±10.4)	39,8 (±10.4)
Female sex, no. (%)	1603 (58.4%)	262 (54.9%)	1341 (59.1%)
Education, no. (%)			
None or Some Primary	937 (34.1%)	154 (32.3%)	783 (34.5%)
Primary or Some Secondary	1054 (38.4%)	192 (40.3%)	862 (38%)
Secondary +	754 (27.5%)	131 (27.5%)	623 (27.4%)
Missing/Unknown	2 (0.1%)	0	2 (0.1%)
BMI (kg/m 2), mean (±SD)	23.2 (±4.6)	24.5 (±10.4)	22.9 (±4.3)
Alcohol user, no. (%)	549 (20%)	121 (25.4%)	428 (18.9%)
Current Smoker, no. (%)	141 (5.2%)	32 (6.7%)	109 (4.8%)
Injection drug user, no. (%)	2 (0.1%)	0 (0.0%)	2 (0.1%)
Noncommunicable Diseases, no. (%)			
elevated blood pressure	396 (14.4%)	92 (19.3%)	304 (13.4%)
Hypercholesterolemia	448 (16.3%)	15 (3.1%)	433 (19.1%)
Renal insufficiency	260 (9.5%)	16 (3.4%)	244 (10.7%)
Hyperglycemia	455 (16.6%)	107 (22.4%)	348 (15.3%)
Communicable Diseases, no.(%)			
Hepatitis B	121 (4.4%)	22 (4.6%)	99 (4.4%)
Hepatitis C	69 (2.5%)	16 (3.4%)	53 (2.3%)
Syphilis	156 (5.6%)	24 (5%)	132 (5.8%)
Tuberculosis	63 (2.2%)	1 (0.2%)	62 (2.7%)
Average time of HIV infection, yr (±SD)	3.8 (±3.6)		3.8 (±3.6)
NADIR CD 4+ T cells (cells/mm 3), mean (±SD)	243.8 (±211.2)		243.8 (±211.2)
CD 4+ T cells count at enrollment (cells/mm 3), mean (SD)	420.4 (259)		420.4 (259)
On Antiretroviral Therapy (cART), no. (%)	1520 (67%)		1520 (67%)
Estimated years on cART, mean (±SD)	3.9 (±3.0)		3.9 (±3.0)
Undetectable viral load (HIV RNA ≤ 50 cp/ml), no. (%)*	1116 (49.2%)		1116 (49.2%)
Log10 plasma HIV RNA, mean (±SD)	5.3 (±4.8)		5.3 (±4.8)

***Table 1*: Demographic and clinical characteristics of AFRICOS participants.**
*P values were calculated using t test.
**Percentage calculated considering the total of participants on cART.

detectable viral loads ≥ 50 copies/ml. Within these individuals we found that all immune parameters were significantly correlated with viral load with the exception of IFN-α (Bayesian P-=0·15; Spearman's ρ=0·06). Most strongly correlating with HIV viral loads was CXCL10 (P-= 0.00025; ρ=0.56). While MIP-1beta (P-= <0.00025; ρ=0.17) was only weakly associated with viral loads (Figure 1A). Many of the inflammatory markers were interrelated, meaning that an increased concentration of one biomarker was also typically associated with an increase of the other biomarkers. TNF-α had the strongest associations, namely to CD25 (Spearman's ρ=0.68) and CXCL10 (ρ=0.61); furthermore IFN-γ was associated with IL-1β (ρ=0.63), TNF-RII with CD25 (ρ=0.62). Only INF-α showed contrary behavior and a negative correlation with CXCL9 (ρ=-0.09), MIP-1β (ρ=-0.08), INF-γ (ρ=-0.04) and TNF-α (ρ=-0.03). These data illustrate an interrelated inflammatory network that is upregulated during viremia in HIV infection (Supplementary Figure 1).

Influence of sex on immune parameters

We next assessed whether there are differences in immune activation profiles between sexes. Given the influence of viral loads on immune activation profiles, we categorized individuals into three groups: participants without HIV, PLWH with suppressed viral load, and viremic PLWH. While we did not find major differences in immune activation profiles between men and women among those without HIV (Figure 1B), we observed with increasing viremia stronger differences in the immune activation profile between men and women. Indeed, the overall inflammatory profile was distinct between men and women in individuals off ART and with HIV viremia (Figure 1B). Using regression analysis we found that CXCL10, CCL2, CXCL9 and CD163 contributed to significant differences in immune activation profiles between men and women. For example, CXCL10 was on average 20% higher in women compared to men, while CD25 plasma levels were nearly 10% lower in women compared to men (Figure 1B) suggesting important differences in the immunpathogenesis between sexes.

Distinct regional differences in inflammatory profiles

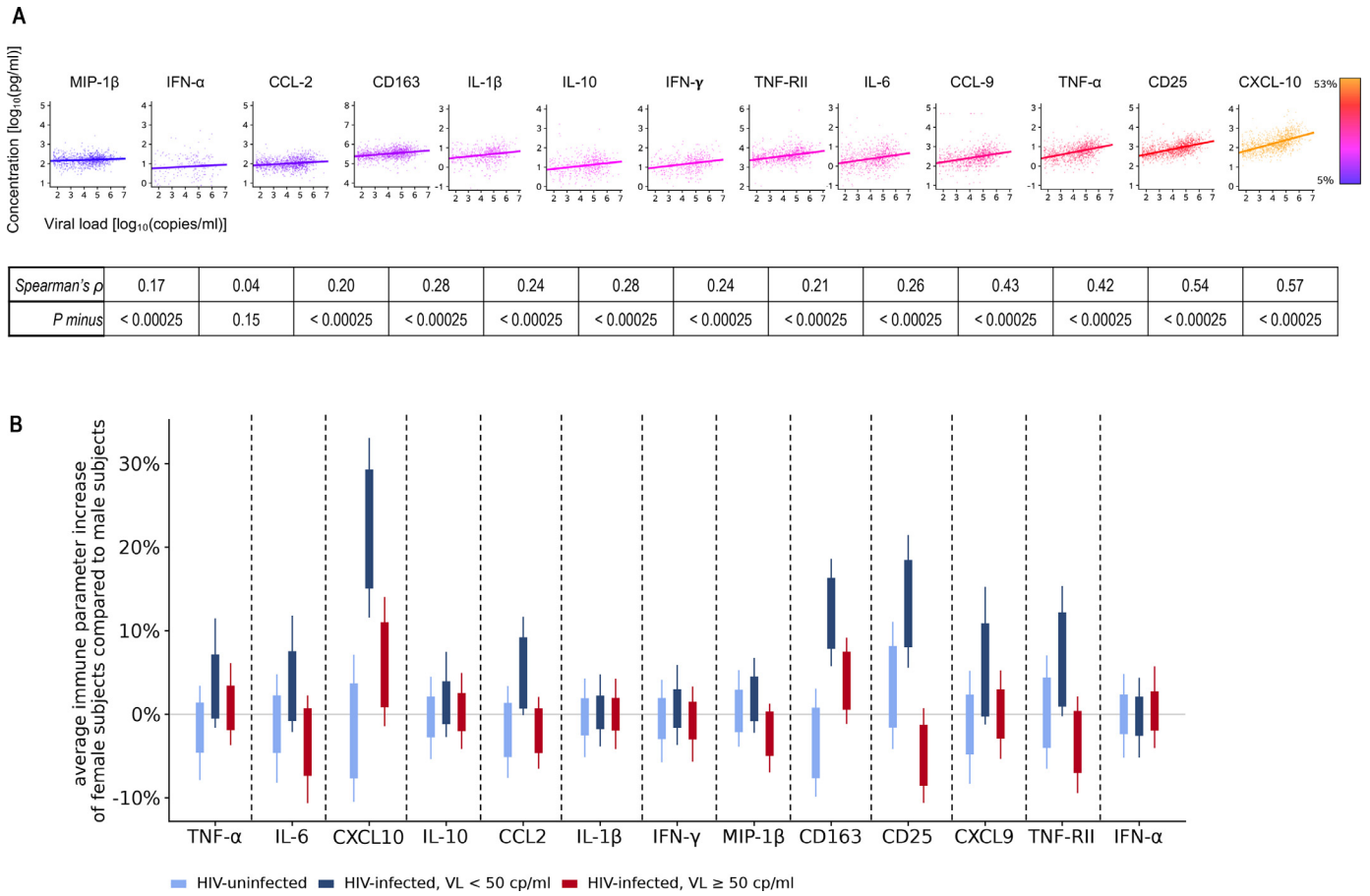
Given the striking inflammatory differences in men and women, we next assessed discriminatory elements of inflammation on a more global level in PLWH and individuals without HIV. Using a machine learning method and quantifying the classification accuracy with Cohen's kappa, we found that geographic differences had the strongest impact on inflammatory profiles (Figure 2A) in individuals without HIV.[21] For example while the average CCL2 concentrations were lower in Kayunga, Uganda, we observed that the MIP-1β concentrations were higher in South Rift Valley, Kenya compared to other countries (Figure 2B). While the region had the strongest effect on immune activation parameters in individuals without HIV, we observed stronger influencing factors in PLWH who had HIV viremia in excess of 50 copies/mL. In these individuals sex, age, non-communicable diseases and communicable diseases are associated with inflammation – either as drivers or consequences of this phenomenon.

Influence of age, CDs and NCDs on inflammatory profiles

To further dissect the factors influencing inflammation identified by the Cohen's kappa coefficient, we next analyzed all cytokines and chemokines individually. First, to understand the effect directionality of age on the inflammatory profile, we correlated the levels of inflammatory markers change as with changes of inflammatory markers in age differences over 10 years (Figure 3). Indeed, we found substantial differences in inflammatory profiles for increasing age. In a time span of 10 years we found an average of 7% increase of CCL2 concentration, while TNF-RII and IL-6 increased by 5%. In contrast, the IL2 receptor CD25 decreased by 5% over the course of 10 years (Figure 3) suggesting that yet unidentified factors in the aging population are contributing to the immune activation profiles.

We next assessed the impact of infectious and noninfectious comorbidities on the inflammatory profile in people living with HIV. In our cohort we identified 63 people living with HIV that were co-infected with pulmonary tuberculosis, 156 individuals with positive laboratory screens for syphilis, 121 individuals screening positives for hepatitis B, and 69 individuals with positive antibody tests for hepatitis C infection. Using regression coefficients we correlated levels of inflammatory markers with communicable diseases (Figure 4). Interestingly, we observed a distinct pattern of immune activation profiles in co-infected individuals compared to individuals with no measurable respective co-infection. Individuals co-infected with active tuberculosis had significantly higher levels of IL-6, CXCL10, IL-1 β, MIP-1β and CXCL9, and lower concentrations of IL-10. In contrast, participants that were co-infected with syphilis showed higher concentration of IL-6, CCL2 and CXCL9 and lower concentration of IL-10, but unlike TB co-infection, not CXCL10, IL-1beta, and MIP-1β. Co-infection with hepatitis B or hepatitis C showed a very distinct pattern. While the perturbation in the inflammatory profiles in hepatitis B co-infection were minor and only showed a significant decrease of CXCL9, we observed that hepatitis C co-infection had an increase of CXCL9 and TNFRII levels. These differences were not attributable to the effects of antiretroviral therapy.

We next evaluated how well immune activation parameters can be used to predict non-communicable



Spearman's ρ	0.17	0.04	0.20	0.28	0.24	0.28	0.24	0.21	0.26	0.43	0.42	0.54	0.57
P minus	< 0.00025	0.15	< 0.00025	< 0.00025	< 0.00025	< 0.00025	< 0.00025	< 0.00025	< 0.00025	< 0.00025	< 0.00025	< 0.00025	< 0.00025

Figure 1. Differences in Immune activation Profiles depending on Gender and Viral Load. (a) Linear regression of viral load vs. immune parameter concentration. R^2 is the squared correlation coefficient (coefficient of determination), Pminus is the posterior probability of the slope exceeding 0. High values of Pminus mean that no association between viral load (VL) and the respective immune parameter is consistent with the observed data. The magnitude of the slope is color coded and corresponds to an expected increase of the immune parameter concentration for a 10-fold increase in viral load, e.g. a 10-fold increase in viral load is associated with an expected increase in immune parameter concentration between 5% and 55%. **(b)** Average immune parameter difference between female and male subjects for different subgroups based on viral load, e.g. given the model and the observed data, female subjects in the HIV infected, VL < 50 cp/ml subgroup have on average around 10% higher CD163 levels than male subjects. Error bars correspond to interquantile intervals of marginal probabilities (bold: 10% and 90% quantiles, thin: 2.5% and 97.5% quantiles).

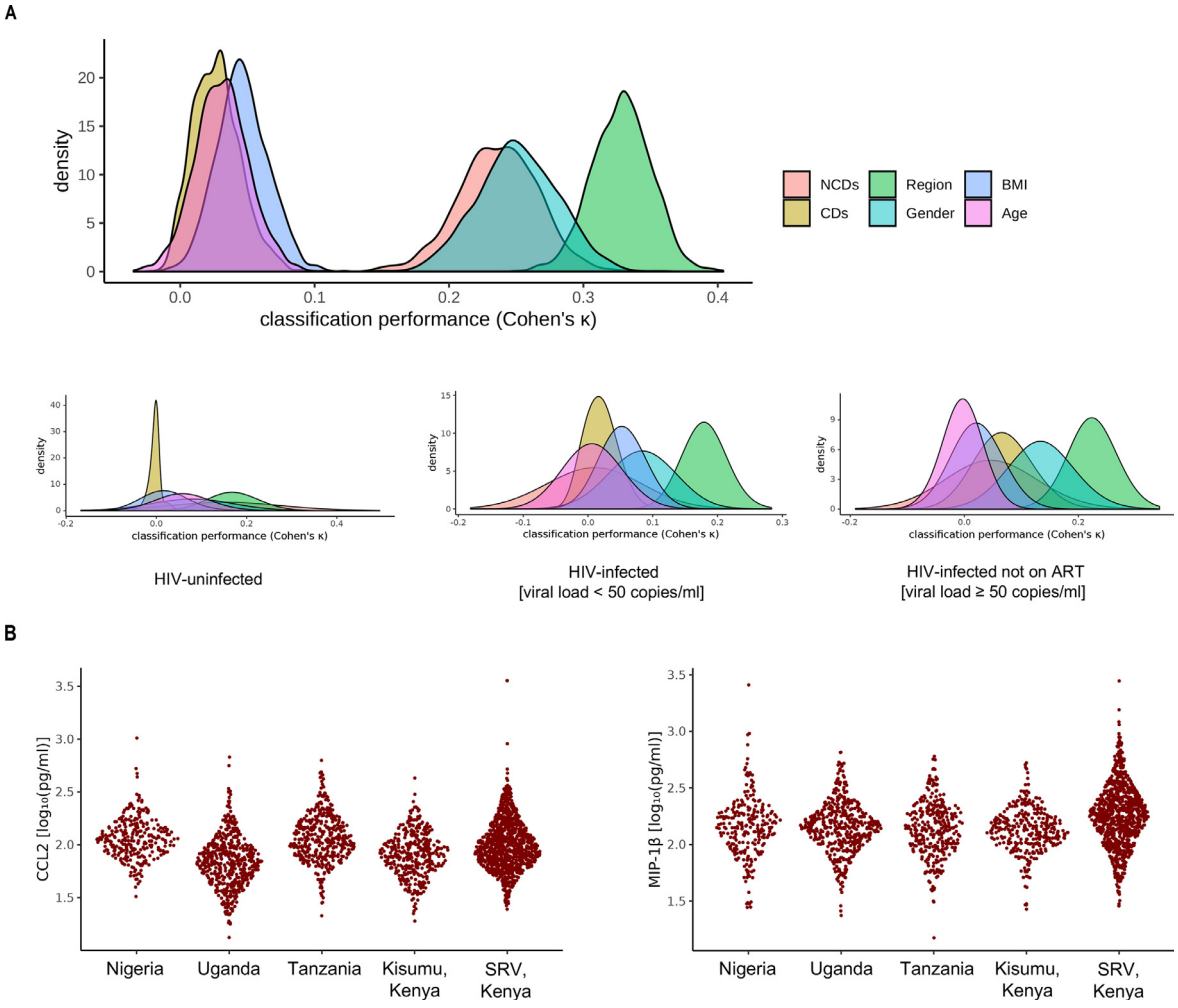


Figure 2. Predictors of Immune activation. The differences are estimated with a model that predicts immune parameter concentrations from gender, viral load, region, education, age, time since HIV diagnosis CD3+ CD4+ cell counts, hepatitis B status, hepatitis C status, tuberculosis, syphilis, hepatitis B and on NRTI (as an interaction term) and HIV-infected and on NRTI (as an interaction term). (**a**) Random forest classification of non-communicable diseases (NCDs), communicable diseases (CDs), region, gender, body mass index (BMI) and age as measured by Cohen's kappa. The explanatory variables are the list of immune parameters, region, education, age category, HIV category, gender, alcohol use, smoker, viral load and active ART therapy. Corresponding explanatory variables are removed when necessary, e.g. region is removed from the predictors when region is the explained variable. **(b)** The plots show that the random forest is able to predict region, gender and NCDs based on the predictors, indicating that there is an association between the predictors and these variables. This analysis was used as a starting point for further modeling, e.g. see Figure 1 for the association between gender and immune parameter values. (**c**) Violin plots of the distribution of CCL2 and MIP-1beta for different regions. The dots represent individual data points. The plots how that CCL2 levels are on average lower in Uganda and Kenya than in the other regions, and that MIP-1beta levels are comparatively higher in SRV, Kenya.

diseases (Figure 5). Among individuals living with HIV, we identified 448 with hypercholesterolemia, 396 individuals with elevated blood pressure, 260 with renal insufficiency and 455 with hyperglycemia. Using regression coefficient analysis we observed that HIV-infected individuals with hypercholesteremia had markedly increased CD25 levels. In contrast IL-6, CXCL10 and CD163 were slightly decreased. Interestingly, individuals with elevated blood pressure had increased levels of IL-6, CXCL10 and MIP-1β and decreased levels of CXCL9. Within the 244 participants presenting with renal insufficiency, TNF-α, CXCL9, TNF-RII and TNF-α concentrations were reduced and CXCL10 was increased. Taken together, our data indicates that inflammatory profiles are associated with age, communicable and non-communicable diseases and may be even used to predict the presence of some of the measured diseases.

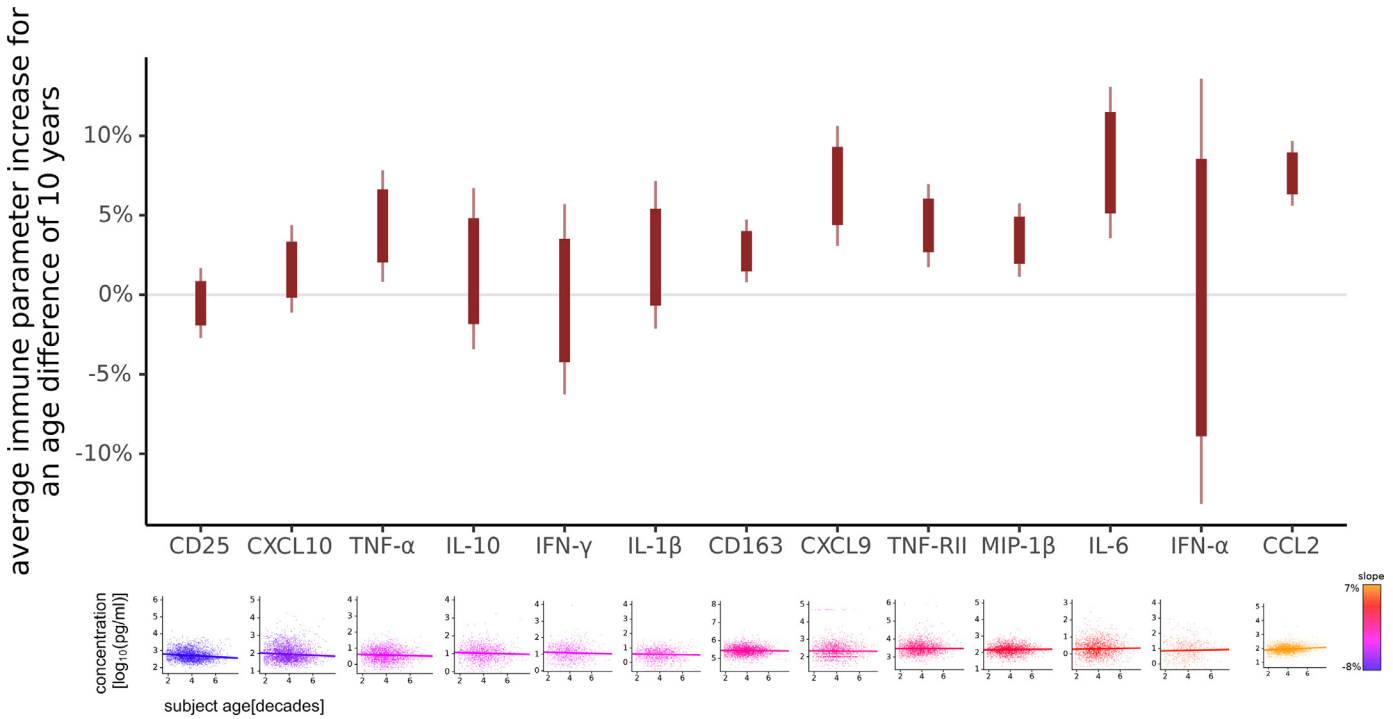


Figure 3. Association of Age and Immune activation. Average immune parameter increase for an age difference of 10 years, e.g. given the model and the observed data, subjects that are 10 years older have CCL2 levels that are around 7% higher compared to younger subjects. Error bars correspond to interquantile intervals of marginal probabilities (bold: 10% and 90% quantiles, thin: 2.5% and 97.5% quantiles). Estimates are computed from a model that predicts immune parameters and includes gender, viral load, region, education, age, time since HIV diagnosis, CD3+ CD4+ cell counts, hepatitis B status, hepatitis C status, tuberculosis, syphilis, hepatitis B and on NRTI (as an interaction term) and HIV-infected and on NRTI (as an interaction term).

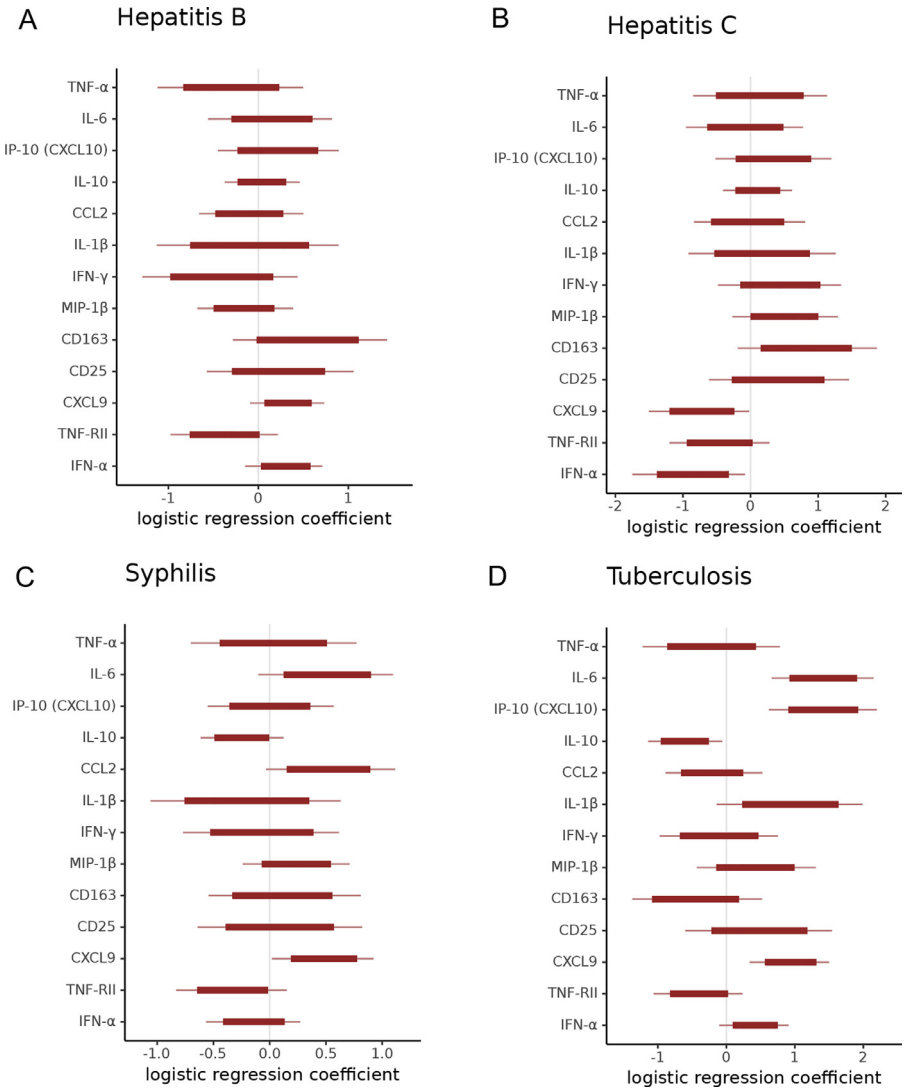


Figure 4. Differences in Immune activation and Co-Infections. Logistic regression model predicting the presence of hepatitis B, hepatitis C, syphilis and tuberculosis with explanatory variables gender, HIV category (uninfected, VL $<$ 50 cp/ml, VL $\geq$ 50 cp/ml), VL, region, education, age, and the list of immune parameters. Regression coefficients for the different immune parameters are shown. Immune parameters that have a high probability of being associated with the respective communicable disease are marked with an asterisk. The immune parameters are measured on a log10 scale, e.g. a 10-fold increase in CXCL9 levels is associated with an increased probability of the subjects being infected with hepatitis B. The model does not make a statement on causality or the direction of the effect and is purely a summary of the observed data, e.g. subjects with hepatitis B have higher CXCL9 levels on average, but it does not say that hepatitis B induces higher CXCL9 levels or that subjects with high CXCL9 levels are more likely to contract hepatitis B. Positive regression coefficients correspond to a positive association with the respective communicable disease, negative regression coefficients correspond to a negative association with the communicable disease, e.g. for tuberculosis, higher CXCL9 levels are associated with an increased probability of observing tuberculosis in this subjects, whereas increased IL-10 levels are associated with a decreased probability of observing tuberculosis in this subject. The regression coefficients are in units of log-odds, which are defined as log(p/1-p).

Discussion

Since the beginning of the AIDS epidemic in the 1980s, 77.5 million people have contracted HIV in the world, and 32.7 million people died due to the disease. In 2018, an estimated 38 million people were living with HIV, and 1.5 million people became newly infected; 45% of these new infections were from sub Saharan Africa.[21] The HIV prevalence rate in Africa has mostly been declining, among adults aged 15–49 years old to around 4%, but remains high compared to the 0.1–0.5% prevalence rates in other WHO regions.

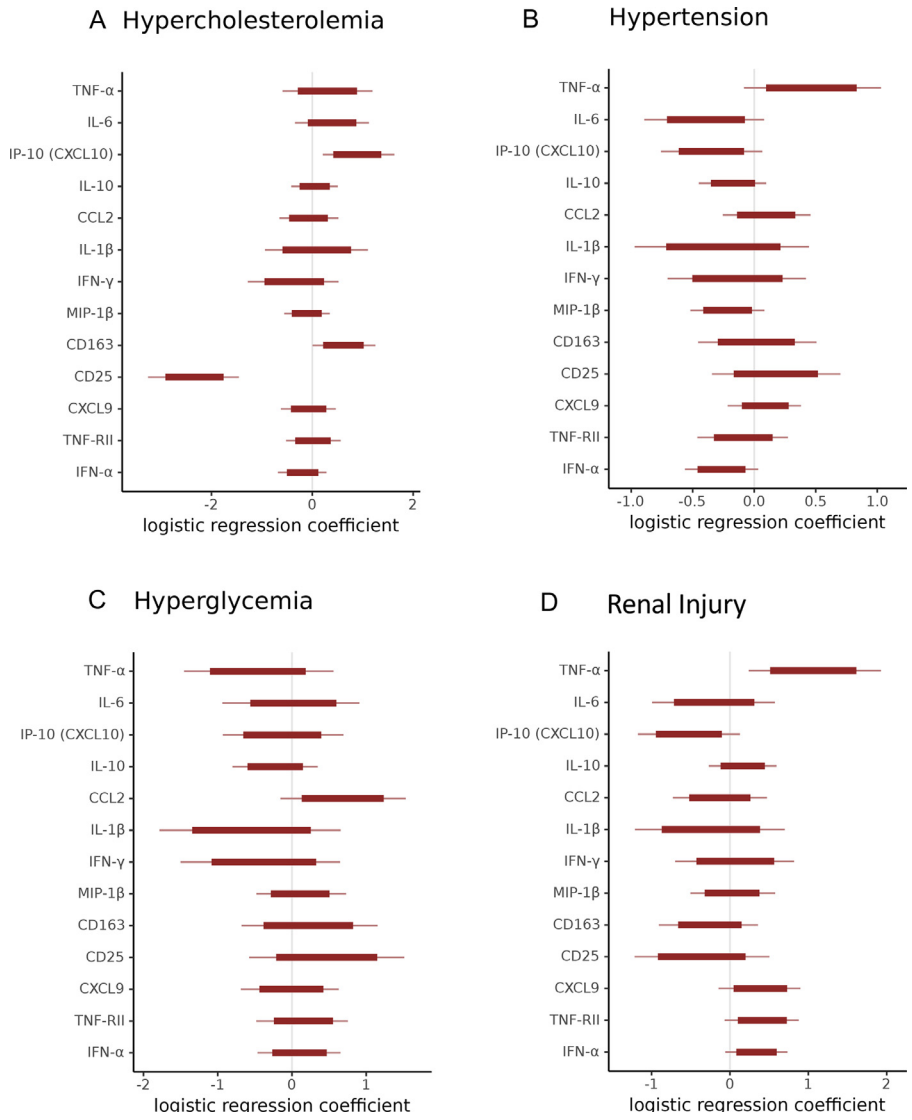


Figure 5. Perturbation of Immune activation profiles in non-communicable diseases. Logistic regression model predicting the presence of Hypercholesterolemia, Hypertension, Hyperglycemia and Renal injury based on the explanatory variables gender, HIV category (uninfected, infected VL $<$ 50 cp/ml, infected VL $\geq$ 50 cp/ml), viral load, region, education, age and the list of immune parameters. Regression coefficients for the different immune parameters are shown. Immune parameters that have a high probability of being associated with the respective non-communicable disease are marked with an asterisk. The immune parameters are measured on a log10 scale, e.g. a 10-fold increase in TNF-alpha levels is associated with an increased probability of the subjects having renal injury. The model does not make a statement on causality or the direction of the effect and is purely a summary of the observed data, e.g. subjects with renal injury have higher TNF-alpha levels on average, but it does not say that high TNF-alpha levels are the result of renal injury or that TNF-alpha is a risk factor for renal injury. Positive regression coefficients correspond to a positive association with the respective non-communicable disease, negative regression coefficients correspond to a negative association with the non-communicable disease, e.g. for Hypercholesterolemia, higher IL-6 levels are associated with an increased probability of observing hypercholesterolemia in this subjects, whereas increased CD25 levels are associated with a decreased probability of observing hypercholesterolemia in this subject. The regression coefficients are in units of log-odds, which are defined as log(p/1-p).

Despite successful antiretroviral therapy and full suppression of viremia, there is substantial evidence for low level immune activation in individuals living with HIV.[22,23] In addition, the influence of immune activation on comorbidities is not well described in the African context. Indeed, reasons for immune activation and development of comorbidities is multifactorial including lifestyle factors such as nutrition, co-infections, or tobacco use.[24] However, understanding the influence of low level immune activation on the

development of comorbidities can inform the development therapeutic options to mitigate excess risk.[25] We therefore studied the influence of low level immune activation in a large African cohort with substantial geographic and socioeconomic diversity. We observed strong associations of immune parameters, differently regulated in HIV infection to be associated with age, sex, co-infections, and comorbidities such as hypercholesteremia as well as high blood pressure.

We correlated the immune parameters with select variables to determine the influence of the predictors, and the changes in concentration of the 13 biomarkers measured. In our analyses, region, communicable and noncommunicable diseases (NCDs) and sex exhibited some influence in the immune parameters analyzed. The country of origin of the participants was the most statistically important variable among assessed factors. These data suggest that different expression of CCL2 and MIP-1β in countries, may be caused by unmeasured environmental or genetic factors or HLA background. Indeed, certain regions were associated with higher cytokine production. In particular, populations from Lake Victoria that mainly consists of fishermen had higher levels of inflammation. This may be due to poor health of the population or potentially infection with schistosomiasis, a parasitic infection we were not able to measure in this cohort. In addition, genetic polymorphisms may affect the inflammatory response and therefore cancer incidence and mortality, and it is already known that certain cytokine gene polymorphisms are strongly associated with ethnicity.[26,27]

Association between age of the participants and plasma biomarker levels was in our study most pronounced for CCL2, TNF-RII, IL-6, MIP-1β, and CD25, which is consistent with results in other studies in other regions of the world and highlights the universal effect of age on the immune system.[28,29] However, reasons for these differences have only been poorly investigated. One effect may be potential comorbidities in the elderly population as we observed for example increased TNF-α levels in individuals with increased blood pressure. Previous studies have shown that both cytokines, TNF-α and IFN-α, increase as soon as blood pressure increases, contributing to adverse renal effects.[30] Unique genetic components to comorbidities and aging in different regional contexts must also be considered as confounders, as they may also shape inflammatory profiles.[31,32]

There are limitations to this study. Please note that all the provided estimates of averages between groups are conditioned on the observed data and the statistical model. The usual caveats of causal inference apply, especially the treatment of these estimates as estimates for the larger population, which implies the additional assumption that the sampled individuals provide enough overlap with that population. Our inferences also rely on the assumption that all relevant covariates have been measured (and have a similar distribution between groups). One example of relevant covariates that have not been measured are genetic factors, which may influence inflammatory profiles. Furthermore, data on tribe and ethnicity were not included; this may be important to consider in future analyses, given that previous studies from Western cohorts suggest ethnicity could influence cytokine production and distribution in HIV infection. Lastly, participants in this well-controlled cohort study may receive more intensive clinical and laboratory evaluations compared to the general population accessing routine PEPFAR-supported services,

In summary, we investigated markers of chronic immune activation in a cohort of HIV infected, treated and HIV uninfected African participants. We demonstrate the effect of HIV viral load on chronic immune activation, considering sex, age, region and ART use, revealing that these predictors, as well as the positive association among some biomarkers and coinfections, influence some markers and are associated with noncommunicable diseases. Further research is needed to clearly understand the mechanisms involved in these complex correlations.

Contributors

HS, DH, CSP, and JAA contributed to study conceptualization, funding acquisition, data collection, data analysis and interpretation, and writing. AM, DH, GS, PAH, and DH contributed to laboratory data collection and analysis, statistical analysis, figures, project administration, study supervision, and writing. TAC, ALE, LAE, MAE, APP contributed to literature search, data cleaning, data analysis, project administration, and study supervision. LM, EB, YA, FK, JM, and JO contributed to study design, project administration, and study supervision. NLM and MLR contributed to study conceptualization, funding acquisition, and study supervision. All authors reviewed and edited the manuscript. All authors read and approved the final version of the manuscript. All authors had direct access and verified the underlying study data. HS and JAA had full acccess to study data and had final responsibility for the decision to submit for publication.

Data sharing statement

The data from this study cannot be made publicly available owing to restrictions in the study's informed consent documents; public availability might compromise participant confidentiality. However, data are available to all interested researchers on request (requests should be directed to the corresponding author) with the agreement of the US MHRP, AFRICOS study investigators, and collaborators.

Declaration of interests

All authors declare no conflicts of interest.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ebiom.2022.104182.

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