Immunopathology of hypertension in HIV-infected adults

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A thesis submitted to the University of Zambia School of Health Sciences in fulfilment of the requirements for the degree of Doctor of Philosophy in Immuno-Pathology

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DECLARATION

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ABSTRACT

Background

Hypertension is a risk factor for stroke, end-organ damage and death. HIV positive individuals are more likely to have hypertension compared to the HIV-negative population, however, the underlying mechanisms are still unknown. Recently, researchers have discovered that chronic immune-activation/inflammation induces and propagates hypertension in humans. Excess dietary salt and infections among others, have been implicated as contributors to hypertension in the HIV-negative population but have not been well studied in HIV-positive individuals. This study was therefore conducted to determine the risk factors, and inflammation markers associated with hypertension as well as to confirm the effect of salt intake on blood pressure and immune cell activation in HIV positive patients.

Methods

This study was a mixed research designs namely cross-sectional, interventional and systematic studies. Six cohorts were recruited at Livingstone Central Hospital (Zambia) and Vanderbilt University (USA) to achieve the objectives. Flow cytometry was employed to analyse cell markers and quantify cytokines. Cells were stimulated using lipopolysaccharide and Phorbol-12-Myristate-13-Acetate (PMA) in Roswell Park Memorial Institute (RPMI) media. Ion-selective electrode technology was used to quantify electrolytes in plasma and urine. For data collection, the international physical activity questionnaire (IPAQ), 24-hour recall form and the World Health Organisation STEPwise approach to surveillance form was adapted and used. Wilcoxon rank-sum test, Kruskal Wallis test, Chisquare and Dunn's multiple comparisons test were used for data analyses. Finally, regression models to predict the impact of selected variables on hypertension, salt sensitivity and all the response variables was used.

Results

In multivariate logistic regression, age, body mass index, employment status, table salt consumption, fasting blood sugar, waist circumference, minutes of moderate physical activity, and sedentary hours, were risk factors significantly associated with hypertension in a Zambian cohort of 226 HIV positive participants, $p<0.05$. Neutrophil count, neutrophil-to-lymphocyte ratio, erythrocytes and creatinine, and a low glomerular filtration were also associated with hypertension. In a cohort of 43 HIV positive participants and 42 HIV negative control group, hypertensives in the HIV positive group had higher IL-6, IL-10, CD80+ expression on total leucocytes and isolevuglandin expression inside monocytes adjusted for sex, age, body mass index and duration on antiretroviral therapy. In a cohort of 70 HIV positive participants from the United States of America (USA), eosinophil count was associated with hypertension and this report was consistent in two HIV-negative control cohorts comprising of 50 and 81, 039 participants respectively. In a systematic study comprising 45 African countries, Norway and the USA, studies revealed that IL-17A, interferon-gamma, and CD4 cells were associated with hypertension in HIV-infected adults. In a Zambian cohort of 85 participants, salt sensitivity was associated with higher levels of IL-6, monocytes, isolevuglandin-adducts inside monocytes, 24-hour sodium and chloride excretion.

Conclusion

Hypertensive HIV positive individuals exhibit high inflammatory (IL-6, IL-10, Isolevuglandin-laden monocytes) and immune-activation markers (CD45 CD80+) compared to normotensives. Eosinophilia is a likely feature of inflammation associated with hypertension in virally suppressed HIV positive individuals. Salt-sensitive hypertension was highly prevalent. Hence, high dietary salt is likely to be a risk factor for the development of, or may worsen already existing hypertension. Salt sensitivity testing in clinical practice is therefore, recommended.

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LIST OF ABBREVIATIONS

3TC	Lamivudine
ABC	Abacavir
ABPM	Ambulatory blood pressure monitor
ABCA1	ATP-binding cassette transporter A1
ACC	American college of cardiology
ACE	Angiotensin converting enzyme inhibitors
ADMA	Asymmetric dimethylarginine
AHA,	American heart association
ALT	Alanine amino transferase or alanine transaminase
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
AOR	Adjusted odds ratio
APC	Allophycocyanin
APCs	Antigen presenting cells
ART	Antiretroviral therapy
ARVs	Antiretroviral drugs
ASCVD	Atherosclerotic cardiovascular disease
AST	Aspartate transaminase
AZT	Azidovudine (also known as zidovudine, or ZDV)
BD	Becton Dickinson
BMI	Body mass index
BMPR-2	Bone morphogenic protein receptor 2
BP	Blood pressure
cART	Combinational antiretroviral therapy
CCR5	C-C chemokine receptor type 5
CNS	Central nervous system
CVD	Cardiovascular diseases
CXCR 4	C-X-C chemokine receptor type 4
CXCR5	C-X-C chemokine receptor type 5
d4T	Stavudine
DBP	Diastolic blood pressure
DCs	Dendritic cells
DDI	Didanosine

DM -2	Diabetes Mellitus type 2
DMIS2	Zambian Health Management Information System
DPBS	Dulbecco's phosphate-buffered saline
EDTA	Ethylenediaminetetraacetic acid
EFV	Efavirenz
FBS	Fasting blood sugar
eNOS	Endothelial nitric oxide synthase
EPCs	Endothelial progenitor cells
F2-isops	F2-isoprostanes
FACS	Flow-activated cell sorting
FITC	Fluorescein isothiocynate
FPG	Fasting plasma glucose
FTC	Emtricitabine
GGT	Gamma-glutamyl transferase
Gp-120	Glycoprotein 120
HDL	High-density lipoprotein
HAART	Highly active antiretroviral therapy
HBsAg	Hepatitis B virus (HCV) surface antigen
HCV	Hepatitis C virus
HIV	Human Immuno-deficiency virus
HIV pVL	HIV plasma viral Load
HLA-DR	Human Leukocyte Antigen – antigen D Related
hs-CRP	High-sensitivity C-reactive protein
HTN	Hypertension or hypertensive
ICAM-1	Intercellular adhesion molecule-1
ISE	Ion selective electrode
IDF	International diabetes federation
IFG	Impaired fasting glucose
IFN-γ	Interferon gamma
IGT	Impaired glucose tolerance
IPAQ	International physical activity questionnaire
IQR	Interquartile range
IRIS	Immune reconstitution inflammatory syndrome
IsoLGs	Iso-levunglandins

JNC 7	Seventh Report of the Joint National Committee on Prevention Detection, Evaluation, and Treatment of High Blood Pressure
LAMP2	Lysosome-associated membrane protein 2
LCH	Livingstone Central Hospital
LDL	Low density lipoprotein
LILRA4-ILT7	Leukocyte immunoglobulin-like receptor subfamily A member 4
LPV	Lopinavir
MACS	Magnetic-activated cell sorting
MAP	Mean arterial pressure
MC	Medical Clinic
MCP-1	Macrophage chemotactic protein 1
M-CSF	Monocyte/macrophage growth factor
mDC	Myeloid DC
MDDC	Monocyte-derived DC
MET	Metabolic Equivalent of Task
MIFlowCyt	Minimum Information about a Flow Cytometry Experiment
MIP-1α	Macrophage inflammatory protein-1 alpha
MSU	Monosodium urate
NADPH	Nicotinamide adenine dinucleotide phosphate
NADH	Nicotinamide adenine dinucleotide hydrogen
NAFLD	Non-alcoholic fatty liver disease
NCC	Sodium (Na+)–chloride (Cl–) cotransporter
NCDs	Non-communicable diseases
Nef	Negative factor
NHE3	Sodium hydrogen exchanger 3
NK	Natural killer cells
NNRTI’s	Non-nucleoside reverse transcriptase inhibitors
NO	Nitric oxide
NT	Normotensive
NSAIDs	Nonsteroidal anti-inflammatory drugs
NVP	Nevirapine
OGTT	Oral glucose Tolerance Test
OPD	Outpatient department
OR	Odds ratio

PAH	Pulmonary arterial hypertension
PBMCs:	Peripheral blood mononuclear cells
PD-1	Programmed death-1
pDC	Plasmacytoid DC
PDGFs	Platelet-derived growth factors
PE	Phycoerythrin
PerCP	Peridinin chlorophyll protein
PG	Plasma glucose
PWH	Persons with HIV
PP	Pulse pressure
PMA	Phorbol 12-Myristate 13-Acetate
PRISMA-P	Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols
PRRs	Pathogen recognition receptors
RPMI	Roswell Park Memorial Institute
ROS	Reactive oxygen species
SBP	Systolic blood pressure
SIRP	Signal-regulatory protein
SLS	Sodium lauryl sulphate
SNAEs	Serious non-AIDS events
SOP	Standard operating procedures
STEPS	STEPwise approach to surveillance
STROBE	STrengthening the Reporting of OBservational studies in Epidemiology
Tat	Transcription proteins
TB	Tuberculosis
TDF	Tenofovir disoproxil fumarate
TLR	Toll-like receptor
TNF-α	Tumor necrosis factor-alpha
TNFR	Tumor necrosis factor receptor
Treg	Regulatory T cells
UNZAHSREC	University of Zambia Health Sciences Research Ethics Committee
VCAM-1	Vascular cell adhesion molecule-1
WB	Whole blood

WC	Waist circumference
WHO	World Health Organisation
WHO STEPS	World health organization stepwise approach to surveillance
WHR	Waist-to-hip ratio

1.0 CHAPTER ONE: INTRODUCTION

Susceptibility to non-communicable diseases (NCDs), such as hypertension, is a rising challenge facing persons with HIV (PWH) especially in low-income countries (Bloomfield et al., 2011). Collection and usage of comprehensive baseline information that elucidates the underlying health risk factors associated with hypertension comorbidities such as diet, lifestyle, associated clinical and laboratory data, identification and management of associated cardiovascular risk factors is critical for patient quality care (Bloomfield et al., 2011; Maher et al., 2011). Such data is lacking in routine practice especially in Sub-Saharan Africa where efforts to combine HIV treatment with vascular disease risk factor prevention and management are urgently needed to address NCD morbidity in PWH (Kemp et al., 2018; Mateen et al., 2013).

Human Immunodeficiency Virus (HIV) remains a global burden. While the advent of antiretroviral therapy (ART) has significantly increased life expectancy among affected individuals, there is now increased risk for the development of NCDs like hypertension including cardiovascular disease (Wada et al., 2015). The global prevalence of hypertension in PWH ranges from 4-57% (van Zoest et al., 2017) with an average prevalence being 25%; 35% for ART-experienced, and 13% for ART-naïve participants respectively (Xu et al., 2017). In the general older population, the overall prevalence of hypertension in Africa is 55% (Kaze et al., 2017) and in PWH living in Sub-Saharn Africa it can range from 28 – 50% (Todowede et al., 2019). In a Zambian survey study, the prevalence of hypertension in PWH was 6.4% where increasing age, male sex and overweight were significant correlates of hypertension (Bauer et al., 2017). Adherence to antihypertensive drugs was low (21%)(Bauer et al., 2017). In a separate study, the prevalence of hypertension in rural Zambians was double (46.9%) that of urban Zambians (22.9%) (Rush et al., 2018). No published data is available, that reports the prevalence of hypertension among PWH in Livingstone or Southern province as a whole.

Generally, PWH live longer due to ART. At the time of the study, in Zambia, three drug classes were used as treatment therapy for HIV infection namely; Non-nucleoside reverse transcriptase inhibitors (NNRTIs) such as nevirapine and efavirenz, Nucleoside reverse transcriptase inhibitors (NRTIs) such as emtricitabine, lamivudine, and abacavir and protease inhibitors such as Lopinavir/ritonavir. In western countries and the USA, newer drugs with less side effects are in use such as entry inhibitors and integrase inhibitors, as older NRTIs were associated with mitochondrial toxicity (Margolis et al., 2014). Some ART regimens especially protease inhibitors have been associated with incident hypertension among PWH as they have been implicated in contributing to adipose tissue dysfunction, dyslipidemia, vascular endothelial damage and kidney injury (Fahme et al., 2018; Xu et al., 2017).

Apart from the effects of HIV and ART-related outcomes, several traditional factors play a critical role in enhancing inflammation and increasing the risk for cardiovasvular disease (CVD) development. Some of the traditional risk factors associated with hypertension are cigarette smoking, sedentary lifestyle, alcohol intake, body mass index (BMI), comorbid conditions such as diabetes and dyslipidemia (Pantell et al., 2019; Whelton et al., 2017). In fact, increasing age, smoking, alcohol intake and a high salt diet were risk factors for uncontrolled hypertension among hypertensives on ART in an African cohort (Magande et al., 2017).

The development of hypertension is multifactorial through mechanisms involving genetic predisposition, excess salt intake, obesity, microbial infections, and sympathetic activity (Elijovich et al., 2016). These factors may induce small raises in blood pressure (BP) that stimulate the immune cells to create an inflammatory milieu for the pathogenesis of hypertension (Schiffrin, 2014). Activation of the sympathetic activity in HIV positive individuals leads to elevations in BP by unknown mechanisms (Fahme et al., 2018).

Potassium intake is inversely related to high BP and stroke in some studies (Kieneker et al., 2014; Mente et al., 2014; Zhang et al., 2013). When potassium levels are higher than normal (25 to 125 mEq/day), the effect of sodium chloride on BP is blunted and a low sodium-potassium ratio has been found to be associated with a lower level of BP for the corresponding levels of sodium or potassium on their own (Rodrigues et al., 2014). The mechanisms behind sodium/potassium intake in regulating BP are not well established. However, murine model studies suggest that the renal sodium (Na^+)–chloride ($Cl^–$) cotransporter (NCC), expressed in the distal convoluted tubule, plays a role in BP modulation such that a diet low in potassium and high in sodium chloride activates the

NCC which in turn activates the sympathetic nevous system and increases BP (Nomura et al., 2019). Whether this occurs in humans, remains unknown.

In Western countries (Bonequi et al., 2013; McCartney et al., 2015) and Sub-Saharan Africa (Oyebode et al., 2016) including Zambia (Oelke et al., 2016) dietary salt (sodium chloride) intake exceeds the 5 g/day recommended by the World Health Organisation (WHO). Excess intake of salt above 5 g/day is positively associated with high BP (Mente et al., 2014; Takase et al., 2015), and increased risk of stroke (Strazzullo et al., 2009; Whelton, 2014), CVD and other adverse outcomes (Whelton et al., 2012). However, the effects of salt on BP may vary among individuals due to salt sensitivity and salt resistance phenotypes (Sanada et al., 2011; Strazzullo et al., 2009). Salt sensitivity is a quantitative trait in which an increase in sodium load disproportionately increases BP (Elijovich et al., 2016). Salt-sensitive BP, is defined as a change in BP greater than 10% in response to either increased or reduced salt intake (Kirabo, 2017). This phenomenon is more common in individuals with higher BP, of African decent, the elderly, and is seen in certain comorbidities such as chronic kidney disease or metabolic syndrome (Whelton et al., 2017). However, though there is currently no agreed and established existing technique to measure salt sensitivity in routine clinical practice (Felder et al., 2013), several methods exist for detecting salt sensitivity. In the Genetic Epidemiology Network of Salt Sensitivity (GenSalt) study comparing continuous distribution of BP responses to 7-day changes in dietary salt where participants were fed low salt (3g NaCl/day) and high salt (18g NaCl/day), reported that majority exhibited increases and decreases in BP with high and low salt respectively (Elijovich et al., 2016).

Salt has been implicated to initiate an inflammatory process that could result in hypertension. Evidence from both murine models and human studies has revealed some of the mechanisms implicating activation of dendritic cells (DCs) and monocytes that in turn activate T cells and produce IL-17A, interferon gamma, tumor necrosis factor-alpha and IL-6 resulting in hypertension (Kirabo et al., 2014; McMaster et al., 2015; Zhang and Crowley, 2015). Yet still, other studies have found that salt intake does not increase BP with some, reporting that it actually has a lowering effect (DiNicolantonio and O’Keefe, 2016). Thus, there is a need to study the mechanisms of salt-induced hypertension. It is evident from several reviews that the issue of salt sensitivity and salt resistance is a fact that must be considered before making any conclusion on salt and blood pressure matters.

BP is a result of the cardiac output and peripheral resistance as blood moves through vessels. Baroreceptors located at strategic points in the body sense the 'quality' of flow and pressure with which blood moves and communicate through the nervous system to the brain (autonomic nervous system) to make adjustments (vasoconstriction or vasodilation) in order to maintain an optimal blood pressure that will not cause harm to blood vessels and blood contents and consequently vital organs (Joyner and Limberg, 2014). Several organs and biomolecules help in the control and maintenance of normal BP, the major ones being but not limited to a healthy heart, healthy vasculature, healthy kidneys and optimally present molecules such as hormones, sodium chloride and albumin (Riet et al., 2015). One of the most important systems regulating BP is the renin–angiotensin–aldosterone system (RAAS). Renin is an enzyme secreted by the kidneys which hydrolyze (breaks down) angiotensinogen secreted from the liver into the peptide angiotensin I, which is further cleaved in the lungs by endothelial-bound angiotensin-converting enzyme (ACE) into angiotensin II, a potent constrictor of all blood vessels (Riet et al., 2015). Aldosterone, is a mineralocorticoid hormone produced by the zona glomerulosa of the adrenal cortex in the adrenal gland and regulates plasma sodium (Na^+), the extracellular potassium (K^+) and arterial BP (Riet et al., 2015).

There are many ways of measuring BP, however, the gold standard is ambulatory blood pressure (AMBP), a home-based interval measurement in 24-hours. However, due to patient uncomfortability with AMBP monitoring, BP can be taken confidently at the hospital with a number of readings made (≥ 2) with intervals in between and an average is taken (Peixoto, 2015). Classification and diagnosis guidelines for high BP and essential hypertension are well elaborated in the new guidelines American Heart Association guidelines (Whelton et al., 2017). In Zambia and Africa in general, the guidelines used are those from the seventh joint national committee (Chobanian et al., 2003).

Common drugs used for the treatment of hypertension in Zambia include but not limited to beta blockers such as atenolol and propranolol, angiotensin converting enzyme inhibitor enalapril, loop diuretic such as furosemide, vasodilator hydralazine, thiazide and potassium-sparing diuretic hydroclorothiazide and calcium channel blocker nifedipine (Yan et al., 2015). Hypertension control in the general Zambian population is generally poor due to non adherence to antihypertensive medications (Yan et al., 2015). The management of hypertension in PWH is not well integrated in some countries including Zambia (Bauer et al., 2017; Fiseha et al., 2019)

The pathogenesis of essential hypertension is not well understood. However, as already stated, a combination of factors such as genetic, demographic, environmental, metabolic, renal handling of salt, sympathetic nervous system and the rennin-angiotensin system all play an important role in the pathogenesis of elevated BP with sustained vasoconstriction or volume overload. Consequently, sustained BP induces structural and functional changes, leading to vascular remodeling and permanent increased peripheral resistance (Peixoto, 2015). However, new insights reflecting on the contribution of the cells of the immune system on hypertension are well elaborated as discussed under sections 2.3 and 2.4. Low-grade chronic inflammatory mechanisms have been found to be important enhancers in the genesis and development of hypertension due to the effect they have on the vasculature and kidney (Mian et al., 2014). The most studied enhancers include increased oxidative stress, innate and adaptive immune cell activation, increased expression of chemokine and adhesion molecules, cellular infiltration and cytokine release that synergize in altering endothelial function and contribute to the development of hypertension (Mian et al., 2014).

Hypertension is a major risk factor for mortality due to stroke, heart failure, myocardial infarction and end-organ damage among HIV patients on antiretroviral therapy (Peck et al., 2014). These consequences are gradual and accelerated by uncontrolled high BP (Carnevale and Lembo, 2016; Potthoff and Vonend, 2017). Moreover, inflammation contributes to hypertension in the HIV negative population (Carnevale and Lembo, 2016; Peck et al., 2014) but the inflammatory milieu that may predispose HIV positive patients to hypertension is poorly understood. In PWH, the case may be different and the inflammation that contributes to hypertension may begin with the HIV infection itself if no existing traditional risk factors are present in which case (with existing traditional risk factors) it may exacerbate and accelerate the progression (Fahme et al., 2018).

Local data available in the Zambian Health Management Information System (HMIS, 2017) (the combination of HIV positive and HIV negative individuals; specific data distinguishing the two groups is unknown) at Livingstone Central Hospital showed rising trends of hypertension for both the in-patient and out-patient departments. In 2014, the hospital recorded 1,010 cases of hypertension. In 2015, there were more than 1,316 cases of hypertension, reflecting both in-patient and out-patients reflecting diagnosis or first case reports, excluding follow-up. Data for 2018 shows that cases have doubled.

This study was conducted to elucidate the immunopathology of hypertension in HIV and to test the effect of dietary salt on immune-cell activation.

1.1 Statement of the problem

PWH generally have a higher inflammatory milieu compared to the general population and thus, are predisposed to develop hypertension (Fahme et al., 2018). Moreover, the prevalence of hypertension is almost double in HIV infected patients on combinational antiretroviral therapy (cART) compared to HIV negative (29% versus 16%) (Peck et al., 2014). However, there is paucity of information accounting for the mechanistic link between inflammation/immune-activation and hypertension in HIV. A recent study (Fahme et al., 2018) reported that chronic inflammation in PWH as characterized by production of IL-6, soluble CD14, and expression of CD163 were among the underlying cytokines and markers influencing kidney disease and activating the sympathetic nervous system resulting in hypertension.

BP increases early after cART initiation correlating in some cases with elevated markers of inflammation (highly sensitive C-reactive protein and soluble vascular cell adhesion molecule), which may involve mechanisms distinct from traditional risk factors (Gleason et al., 2015; Okello et al., 2015). However, T cell immune markers were not assessed. In Zambia, there is lack of data on the linkage between hypertension, inflammation and HIV. Similarly, several studies conducted in other African populations involving PWH (Bloomfield et al., 2011; Mutevedzi et al., 2013), aside from reporting that HIV status was associated with increased inflammatory markers, did not elucidate the exact linkage involving the innate and adaptive immune cells. An exception to this is a recent study by

Fahme *et al* 2018. As hypertension is becoming, not only a challenge in Zambia, but a global concern, research that focuses on insights into the pathological mechanisms involving immune cells are instrumental for proper future management and treatment target of this non-communicable disease.

In addition, high dietary salt intake correlates positively with high BP (Mente et al., 2014; Takase et al., 2015). Though the mechanisms are not clear in PWH, there is substantial evidence from murine models and human studies reporting that high dietary salt initiates an inflammatory process that results in hypertension and some of the mechanisms have been revealed (Kirabo et al., 2014; McMaster et al., 2015; Zhang and Crowley, 2015). The levels of salt intake in Sub-Saharan Africa including Zambia is higher than that recommended by WHO (Oelke et al., 2016; Oyebode et al., 2016). With this relationship between salt and hypertension, it remained warrantable to investigate further on how immune cells and BP were influenced by a high salt diet in PWH.

1.2 Justification of the study

It is now well established as reported from several studies (Barbaro et al., 2017; Itani et al., 2016; Kirabo et al., 2014; Loperena and Harrison, 2017; McMaster et al., 2015; Norlander et al., 2017; Wu et al., 2014; Xiao et al., 2015) that inflammation contributes to the development of hypertension in both mouse models and human studies. However, this phenomenon and the underlying mechanism is not clear in PWH, hence the need to conduct this study.

At the time of commencement of the study, it was unknown whether inflammation was associated with hypertension in PWH. Prodded by the known effect of high dietary salt in modulating the innate and adaptive immune cells in the HIV negative population, the study was aimed to determine if the same effect occurred in PWH. Furthermore, determining the risk factors associated with hypertension in the HIV population in Livingstone would provide new information to compare with what other authors internationally had reported in their studies, this study will add knowledge to the existing literature in the understanding of hypertension in PWH.

This study is in line with the National Health Strategic Plan of Zambia 2017 -2021 (www.moh.gov.zm/docs/ZambiaNHSP.pdf) that supports strategies, interventions and innovations seeking to strengthen the mitigation of hypertension, other NCDs and HIV-related complications.

Routine laboratory data captured from the laboratory information system's reports (data unpublished) suggested that the HIV positive patients seemed to be affected much more by hypertension than HIV negative patients as seen from various elevated viral load, glucose, BP, plasma creatinine levels, reduced liver function and low CD4 counts.

This study will provide a link between inflammation, HIV and hypertension and reveal a potential therapeutic target for the treatment of hypertension in PWH.

1.3 Novelty and implications of this study

This study has several clinical implications and impacts on patient management such as importance of using ambulatory blood pressure monitoring to monitor blood pressure for 24 hours, the importance of including salt assessment and other modifiable risk factors in hypertension management.

Immunosuppression of mice produces blunted hypertension to stimuli such as angiotensin II, high dietary salt, and norepinephrine and this phenomenon is of clinical interest for certain human diseases such as acquired immunodeficiency syndrome (AIDS). Identification of specific immune-activation and inflammatory markers associated with hypertension in this study can, therefore, be potential targets for future interventional studies in the management of hypertension. Thus, studies of immune cell activation and inflammation in PWH are clearly useful in understanding hypertension.

As murine models mimicking human hypertension has revealed that adoptive transfer of activated T cells induces hypertension in previously normotensive mice, this may pose serious implications on blood transfusions and stem cell transplants. Thus, research into such areas will consequently be necessitated by the novelty of this study.

Salt sensitivity tests in PWH has scarcely been explored as evidenced from literature review, therefore, this study is key in providng information about how salt modulates BP in PWH.

1.4 Research questions

1. Is hypertension associated with inflammation in HIV-infection?
2. Does high salt contribute to immune-activation, inflammation and blood pressure in persons with HIV?

1.5 General objective

To understand the immune mechanisms of hypertension in HIV infected individuals.

1.6 Specific objectives

1.6.1 To determine the risk factors and inflammatory surrogates associated with hypertension in HIV positive adults in relation to the American Heart Association hypertension diagnostic criteria.

1.6.1.1 To determine traditional risk factors associated with hypertension and compare the impact of diagnostic criteria between the Seventh Joint National report (JNC 7) and the American Heart Association new (2017) diagnostic criteria.

1.6.1.2 To compare inflammatory surrogates in a full blood count test between hypertensive and normotensive individuals.

1.6.2 To determine the relationship between hypertension and inflammation in HIV

1.6.2.1 To compare ant- (IL-10) and pro-inflammatory markers (monocytes, IsoLGs, IL-17A, IL-6, TNF-α, IFN-γ) among HIV positive hypertensives, HIV positive without hypertension, hypertensive HIV negative, and normotensive HIV negative adults as the control.

1.6.2.2 To determine the relationship between ant- and pro-inflammatory markers and exposure variables (such as blood pressure, HIV status, ART duration, age, sex, Body Mass Index, waist circumference etc.) between hypertensive and normotensive individuals living in Livingstone and compare data with findings from Sub-Saharan Africa and Western country studies.

1.6.3 To quantify and compare the effect of high dietary salt on immune cell activation and blood pressure

1.6.3.1 To compare IsoLGs, and ant- and pro-inflammatory markers (IL-17A, IL-6, TNF-α, IFN-γ, IL-10) produced by PBMCs from HIV positive hypertensive, HIV positive without hypertension, hypertensive HIV negative, and normotensive HIV negative adults as controls.

1.6.3.2 To determine the relationship between high dietary salt and IsoLGs, ant- (IL-10) and pro-inflammatory markers (IL-17A, IL-6, TNF-α, IFN-γ) and, immune activation markers (CD80, CD86) in HIV positive with hypertension, HIV positives without hypertension, hypertensive HIV negative, and normotensive HIV negatives as controls.

2.0 CHAPTER TWO: LITERATURE REVIEW

2.1.0 Synopsis of the link between HIV, immune activation/inflammation and hypertension

HIV remains a global challenge. The advent of antiretroviral therapy (ART) has changed the natural course and progression of HIV infection. Persons with HIV (PWH) are living longer, however, non-communicable diseases (NCDs) such as hypertension have now become common (Deeks, 2011). Besides traditional risk factors for developing hypertension, immune-activation resulting in the release of inflammatory cytokines by cells of the innate and adaptive immune system causes gradual vascular damage and end-organ damage that leads to or exacerbates hypertension (McMaster et al., 2015). Chronic immune-activation through the effects of the HIV virus results in poor immunological outcomes (Papagno et al., 2004).

2.1.1 A brief Overview of the Immune System

The immune system is an organized defense network of the body that ensures that there is no invasion and harm from living and inanimate foreign substances. It is divided into innate and adaptive/acquired immune system. The innate immune system is non-specific, rapid and is the first line of defense against foreign invaders. Adaptive immunity, on the other hand, is acquired over time and is very specific (Hato and Dagher, 2015; Lubbers et al., 2017; Yatim and Lakkis, 2015). Components of innate immunity (Figure 1) include but not limited to the skin and mucous membranes, natural killer (NK) cells, phagocytic cells such as macrophages, neutrophils, dendritic cells (DCs) and complement proteins. The adaptive immunity is composed of B cells (humoral immunity) and T cells (cell-mediated immunity) (Hato and Dagher, 2015; Lubbers et al., 2017; Yatim and Lakkis, 2015). Most of the immune cells have subpopulations, for example, the major T cell subpopulation includes CD8+ cytotoxic T cells, CD4+ T regulatory cells and CD4+ T helper cells such as T follicular helper cells, T helper 1, T helper 2 and T helper 17 (Hirahara and Nakayama, 2016; Yatim and Lakkis, 2015; Zhu et al., 2010).

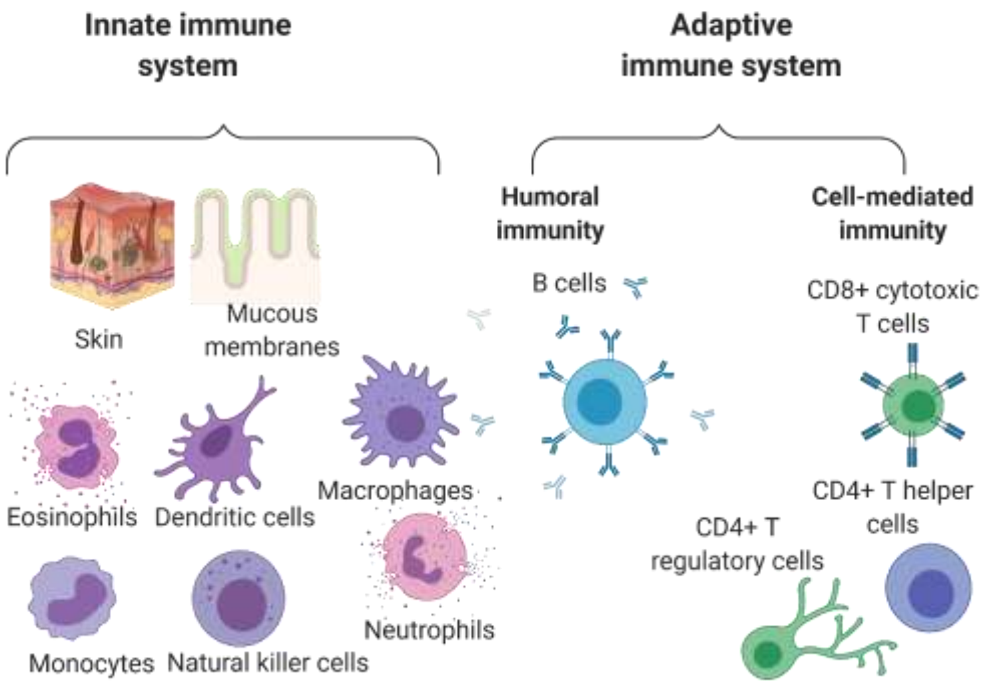


Figure 1. Components of the immune system
The immune system is divided into innate and adaptive immune system. The adaptive immune system is further divided into humoral consisting of plasma B cells and cell-mediated immunity consisting of T cell population. Apart from cells, the innate immune system also consists of physical barriers such as skin and mucous membranes (Yatim and Lakkis, 2015).

Monocytes are precursors for macrophages and dendritic Cells (DCs). Human monocytes are divided into three subtypes based on the differential expression of cluster of differentiation 14 (CD14) and CD16: classical CD14++CD16- (also referred as CD14+ or CD14+CD16-), intermediate CD14++CD16+ (also referred as CD14+CD16+ or CD14+CD16int/low) and non-classical CD14+CD16++ (also referred as CD14LowCD16+ or CD14dimCD16+) monocytes (Wacleche et al., 2018). The classical CD16- and intermediate/non-classical CD16+ monocytes represent ~90% and 10% of total monocytes, respectively (Collin et al., 2013; Collin and Bigley, 2018; Rhodes et al., 2019; Wacleche et al., 2018).

Human DCs are divided into three major subsets: plasmacytoid DC (pDC), myeloid DC (mDC) and monocyte-derived DC (MDDC). Plasmacytoid DC (pDC) express blood DC antigen 2 (BDCA-2) and -4, leukocyte immunoglobulin-like receptor subfamily A member 4 (LILRA4-ILT7) and IL-3 receptor α-chain CD123 at the cell surface. The mDCs can be detected in the peripheral blood and are classified into two major populations based on the expression of CD141 (or BDCA-3) and CD1c (or BDCA-1): CD141+ DC (or cDC1) and CD1c+ DC (or cDC2) (Wacleche et al., 2018). Human MDDC express CD16- and CD16+. Additionally, human MDDC express at the cell surface CD11b, CD11c, MHC-II, Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin (DC-SIGN), cell adhesion molecule CD24, signal-regulatory protein (SIRP) and macrophage markers CD107b and lysosome-associated membrane protein 2 (LAMP2). The MDDC can be generated in-vitro from human monocyte precursors in the presence of IL-4 and granulocyte-macrophage colony-stimulating factor (GM-CSF) (Collin et al., 2013; Collin and Bigley, 2018; Rhodes et al., 2019; Wacleche et al., 2018).

Antigen-presenting cells (APCs) of the innate immune system present signals to activate the adaptive immunity. Of note, excessive and prolonged stimulation of both the innate and adaptive immune system inevitably leads to these cells directly damaging other cells, tissues, organs and the vasculature and indirectly, by secreting inflammatory cytokines and injurious substances such as reactive oxygen species (Yatim and Lakkis, 2015).

2.2 Inflammation and HIV

2.2.1 Inflammation

Inflammation is a complex vascular and cellular response of living cells and tissues to an injurious agent or harmful stimuli such as pathogens, damaged cells and biological chemicals that serve to eliminate both the cause of cell injury and the result of such injury (Bordoni et al., 2017; Serhan and Levy, 2018). This response involves immune cells, blood vessels and molecular mediators. Fundamental to this process is the fact that this response when sustained or persistent has been documented to cause more damage to blood vessels and tissues (Serhan and Levy, 2018).

Inflammation can either be acute (minutes to a few days) or chronic (weeks to months) and the factors that trigger inflammation include infections, tissue injury, tissue stress and tissue malfunction (Medzhitov, 2008). The consequences of an inflammatory response can be physiological, pathological or both. The physiological response includes the host's defense against infection, tissue repair, adaptation to stress and restoration of the homeostatic state. Pathological consequences include autoimmunity, inflammatory tissue damage, sepsis, fibrosis, metaplasia, shift in homeostatic set points and development of diseases (Medzhitov, 2008).

The factors that trigger an inflammatory process leads to a production of numerous inflammatory mediators, which in turn alter the functionality of many tissues and organs (Medzhitov, 2008). Inflammatory mediators include vasoactive amines (i.e histamine and serotonin), vasoactive peptides (i.e kinins, fibrinopeptide A, substance P), fragments of complement components (i.e C3a, C4a and C5a), lipid mediators (i.e eicosanoids and platelet-activating factors), cytokines (i.e tumour-necrosis factor-α (TNF-α), IL-1 and IL-6), chemokines (i.e CCL2, CCL3 and CCL5, CXCL1, CXCL2 and CXCL8) and proteolytic enzymes (elastin, cathepsins and matrix metalloproteinases) (Chovatiya and Medzhitov, 2014; Medzhitov, 2008).

Cytokines play a major role in inflammation. Although the categorization of cytokines is variable, cytokine production and their effects can be grouped into those that are mononuclear phagocytic-derived or T-lymphocyte derived, those that mediate cytotoxic antiviral or anticancer effects, or those that mediate humoral, cell-mediated and immunosuppressive effects (Borish and Steinke, 2003). Mononuclear phagocytic-derived cytokines include TNF-α, IL-1, IL-6, IL-8, IL-12, IL-15, IL-18, and IL-23. Cytokines with cytotoxic effects include IL-2, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12, IL-15, TNF-α, TNF-β and IFN-γ. Cytokines that mediate humoral immunity are IFN-γ, IL-1, IL-2, IL-5, IL-6, IL-12, IL-15, and IL-21. Cytokines mediating cellular immunity include IFN-γ, IL-16, IL-17, IL-21, TNF-α and TNF-β. Cytokines having predominantly anti-inflammatory effects include transforming growth factor–β, IL-10, IL-19, IL-20, IL-22, and IL-24 (Borish and Steinke, 2003).

2.2.2 HIV and its linkage to inflammation

The Human Immunodeficiency virus (HIV) is a virus that attacks the cells that help the body to fight infection, making a person more vulnerable to other infections and diseases. HIV causes systemic T cell destruction, directly damages tissues such as the gut, brain, and blood vessels (Lucas and Nelson, 2015). Through immune activation and HIV effects on the vasculature, it can cause subtle systemic organ damage to the cardiovascular and central nervous system (Lucas and Nelson, 2015).

HIV infection has about three stages. The first is the acute HIV infection phase followed by chronic HIV infection, and finally the acquired immunodeficiency syndrome abbreviated as AIDS (Doitsh and Greene, 2016; Goodsell, 2015). Following HIV infection, a generalized systemic immune-activation resulting in inflammation begins (Mohan et al., 2014), which subsides but still persists at low-grade following initiation of combinational antiretroviral therapy (cART) (Wada et al., 2015). The use of ART helps to slow or prevent HIV from advancing to the next stage. The acute phase is characterized by high HIV viral load and low CD4 count reflecting cell death and increased viral replication (Doitsh and Greene, 2016; Goodsell, 2015). The second phase is characterized by reduced viral replication and CD4 count approaching normal due to viral control by the immune system. In the third stage, CD4 T cells undergo rapid depletion, HIV viral load is high and results in detrimental inflammatory processes (Doitsh and Greene, 2016; Goodsell, 2015). In the first and second stage, CD4+ T cells are activated, as they are the majority of targeted cells for HIV infection (Corneau et al., 2017; Vega and Espinoza, 2018). High levels of CD38 expression on CD8+ T-lymphocytes is seen along with other T-cell activation markers such as Human Leukocyte Antigen–D Related (HLA-DR) along with pro-inflammatory cytokines tumor necrosis factor-alpha (TNF-α), interleukin 6 (IL-6) and interleukin 1 beta (IL-1β) (Corneau et al., 2017; Mohan et al., 2014; Vega and Espinoza, 2018). Increased expression of CD38 alone or in concert with HLA-DR+ in T-cells is a marker of disease progression and mortality in some cases correlating directly with HIV viral load and indirectly with CD4+ count which is also evident in stage 3 of HIV infection (Corneau et al., 2017; Djoba Siawaya et al., 2007; Mohan et al., 2014).

Immune activation resulting in inflammation in PWH is influenced by several factors. There is the direct binding of the HIV envelope protein gp120/41 to CD4 and/or C-C chemokine receptor type 5 (CCR5) which down-modulates the expression of CD3+ T-cell receptor (TCR) in the infected cells resulting in cell death (Anand et al., 2018; Mohan et al., 2014). Also, the interaction between HIV viral particles and plasmacytoid DCs through Toll-like receptor (TLR) stimulation activates the adaptive immune system and the effect of CD8+ cytotoxic cells can be deleterious to vascular cells and fuel chronic inflammation once the virus escapes immune response (Mohan et al., 2014; Zevin et al., 2016). Microbial translocation from a leaky gut into the circulation due to CD4+ T cell depletion and increased viral replication that lyses mucosal cell lining also leads to a generalized systemic inflammatory process (Zevin et al., 2016). Also important is the role of CD4+ regulatory T cells (Treg) that helps to suppress immune-activation through the production of cytokines and inhibition of DCs that are most predominant in activating T cells (Xiao et al., 2019). In PWH, dysfunction of Treg due to unknown reasons, is common resulting in an uncontrolled over-activation of immune cells and increased inflammation (Méndez-Lagares et al., 2014). It should be noted that increased immune-activation as a result of increased viral duplication is associated with disease progression (Mohan et al., 2014). There is also a tendency towards immune-senescence, a term referring to an exhausted immune response that exhibits ageing characteristics. Thus, a low-grade chronic inflammatory process is sustained and leads to CVDs and poor disease outcomes even with ART (D'Abramo et al., 2014; Tsoukas, 2014).

IL-6 is an important pro-inflammatory cytokine secreted by cells of the innate and adaptive immune system and is positively associated with acute and chronic inflammation in HIV infection (Borges et al., 2015). IL-6 production by monocytes and macrophages is increased and although reduced after initiation of ART, remains higher when compared to HIV uninfected persons (Borges et al., 2015; Nixon and Landay, 2010). IL-6, when elevated can propagate deleterious systemic effects in the vasculature as it affects the recruitment of other immune cells. IL-6 production also helps to activate T cells and can be a valuable prognostic marker of AIDS disease progression (Borges et al., 2015; Nixon and Landay, 2010).

CD14 is a myeloid differentiation marker found primarily on monocytes and macrophages (Shive et al., 2015) and undergoes enzymatic shredding from the plasma membrane following activation of monocytes and macrophages producing sCD14 (Anzinger et al., 2014). Soluble CD14 (sCD14) is a biomarker that can be measured in plasma. It is elevated in PWH regardless of treatment status and is an independent predictor of mortality (Anzinger et al., 2014; Wang et al., 2019). It is important to note though, that hepatocytes also produce this biomarker in acute phase reactions, therefore, plasma sCD14 levels must be interpreted with caution. A key inducer of monocyte activation resulting in higher sCD14 is increased microbial translocation in the gut that persists even after ART (Anzinger et al., 2014). CD69 is another marker expressed early in T cell activation and is also upregulated in DCs. This marker has been implicated in inflammatory processes (Mohan et al., 2014).

Biomarkers of 'immune exhaustion', a term used here to refer to reduced or unresponsive immunity in chronic HIV related immune-activation, have emerged in assessing the progression of disease (Aberg, 2012; Mojumdar et al., 2011). The expression of Programmed death-1 (PD-1) is rapidly increased on CD4+, CD8+ T cells, B-cells, natural killer cells, and monocytes as they interact with PD-L1, its ligand on antigen presenting cells (APCs) (Day et al., 2006; Liu et al., 2019). The engagement on virus-infected cells leads to impaired generation of effector T cell responses (Mohan et al., 2014). Other markers include increased expression of CD28-, CD57+ and reduced expression of CD127 on CD4+ and CD8+ T cells (Aberg, 2012; Mojumdar et al., 2011). CD28- and CD57+ expression on CD4+ and CD8+ T cells are possibly reflective of immunosenescence, however, their role and mechanism is still unknown (Larbi and Fulop, 2014; Strioga et al., 2011). High expression of CD127 has an important role in maintaining the naïve T cell proportion, therefore, reduced expression is reflective of the loss of the naïve CD4 T cells in PWH on ART (Xu et al., 2017).

The hallmark of a continuous immune-activation and inflammation as occurs in PWH is an accelerated genesis and progression of atherosclerosis and endothelial dysfunction that may result in morbidity and mortality (Nemeth et al., 2015). HIV infection is responsible for excessive inflammation that results in a majority of CVD events irrespective of treatment and in some cases ART exacerbates the inflammatory milieu where as in some cases reduces it (Nemeth et al., 2015).

2.3 Inflammation and Hypertension

2.3.1 Hypertension

Hypertension is defined as systolic/diastolic blood pressure (SBP/DBP) above 140/90 mmHg according to the seventh report of the Joint National Committee on prevention, detection, evaluation, and treatment of high blood pressure (JNC 7) (Chobanian et al., 2003). More recently, the committee led by the American Heart Association (AHA) and the American College of Cardiology (ACC) lowered the threshold in the definition of hypertension to a systolic/diastolic BP above 130/80 mmHg (Whelton et al., 2017).

According to the JNC categories of BP, normal BP constitutes SBP and DBP below 120/80 mmHg. SBP ranging from 120-139 mmHg and DBP ranging from 80-89 mmHg is termed pre-hypertension. Hypertension is classified into two stages namely stage 1 and stage 2. Stage 1 hypertension is categorized by a SBP ranging from 140-159 mmHg or DBP ranging from 90-99 mmHg and stage 2 hypertension is categorized by a SBP that is equal to or above 160 mmHg or DBP equal to or above 100 mmHg (Chobanian et al., 2003). The AHA/ACC criteria on the other hand categorises normal BP as SBP and DBP of less than 120 mmHg and less than 80 mmHg respectively. SBP of 120-129 mmHg and DBP less than 80 mmHg is termed elevated BP. Stage 1 hypertension is a SBP of ranging from 130-139 mmHg or DBP ranging from 80-89 mmHg. Stage 2 hypertension is SBP that is equal to or exceeding 140 mmHg or DBP that is equal to or exceeding 90 mmHg (Whelton et al., 2017). A detailed guideline and classification of hypertension based on the JNC and AHA/ACC criteria is found in appendix I.

Based on the etiology of hypertension, there are two types of hypertension namely primary (essential) and secondary (non-essential) hypertension. Essential hypertension is usually a gradual rise in BP of unknown cause while non-essential hypertension occurs secondary to an underlying condition or disease (Messerli et al., 2018, 2007).

In the early phase of hypertension, subtle target-organ damage may occur such as left-ventricular hypertrophy, microalbuminuria, and cognitive dysfunction although severe adverse events such as stroke, myocardial infarctions and kidney failure may develop after long periods of uncontrolled hypertension (Messerli et al., 2007).

2.3.2 The role inflammation plays in contributing to hypertension

Chronic activation of the cells of the innate immunity by infection or tissue injury, results in secretion of reactive oxygen species (ROS) and inflammatory cytokines by these cells. In the long-term, ROS production increases causing oxidative stress and injury to endothelial cells which leads to endothelial dysfunction (Agita and Alsagaff, 2017). As the endothelia cells regulate blood vessel tone and structure through nitric oxide (NO) production, ROS react with NO and decreases its bioavailability resulting in disruption of blood vessel relaxation and vasodilation (Agita and Alsagaff, 2017; Guzik and Touyz, 2017). This creates a disturbance in blood flow that is exacerbated by more recruitment of cells of the innate immunity such as monocytes and activation of the adaptive immunity that secrete more cytokines and ROS to directly injure blood vessels. The healing process by fibrosis hardens arteries and constricts them leading to high blood pressure (Agita and Alsagaff, 2017; Guzik and Touyz, 2017).

Additionally, inflammation activates the sympathetic nervous system and RAAS that increases BP and when this activation is prolonged and no remedy is sought for then hypertension develops (Dinh et al., 2014).

2.3.2.1 The role of Isolevuglandins (IsoLGs) in the development of hypertension

The specific cell types and cytokines contributing to hypertension including the role of Isolevuglandins (IsoLGs) is well studied in both murine and human models (Caillon and Schiffrin, 2016; Dinh et al., 2014; Kirabo et al., 2014; McMaster et al., 2015; Nguyen et al., 2013; Wade et al., 2016).

Chronic inflammation is the hallmark underlying the patho-immunology of hypertension (Mian et al., 2014). In hypertensive mice models studies, activated DCs, through the intracellular production of ROS that react with components of the F2-isoprostane (F2-Isops) pathway produce IsoLGs (also termed isoketals) which adduct to intracellular proteins forming a complex neo-antigen that is processed in major histocompatibility molecules and presented to T cells leading to T cell proliferation and the production of interferon-gamma (IFN-γ) and IL-17A. This contributes to the development of hypertension (Barbaro et al., 2017; Kirabo et al., 2014). Plasma and urine concentrations of F2-Isops can be used as an index of endogenous oxidant stress (Kirabo et al., 2014; Salomon and Bi, 2015). Moreover, plasma IsoLGs and IsoLG-protein adducts inside circulating monocytes and DCs are elevated in treated hypertension and markedly elevated in patients with resistant hypertension (Kirabo et al., 2014). Increased formation of IsoLG-protein adducts has been found in several diseases associated with oxidative stress, including alcohol-induced liver damage, atherosclerosis, Alzheimer's disease, and asthma (Kirabo et al., 2014). When DCs are activated, they secrete pro-inflammatory cytokines IL-6, IL-1β, and IL-23 and their interactions with T-cells is exhibited by the expression of costimulatory proteins CD80 and CD86 as illustrated in Figure 2 (Barbaro et al., 2017; Kirabo et al., 2014).

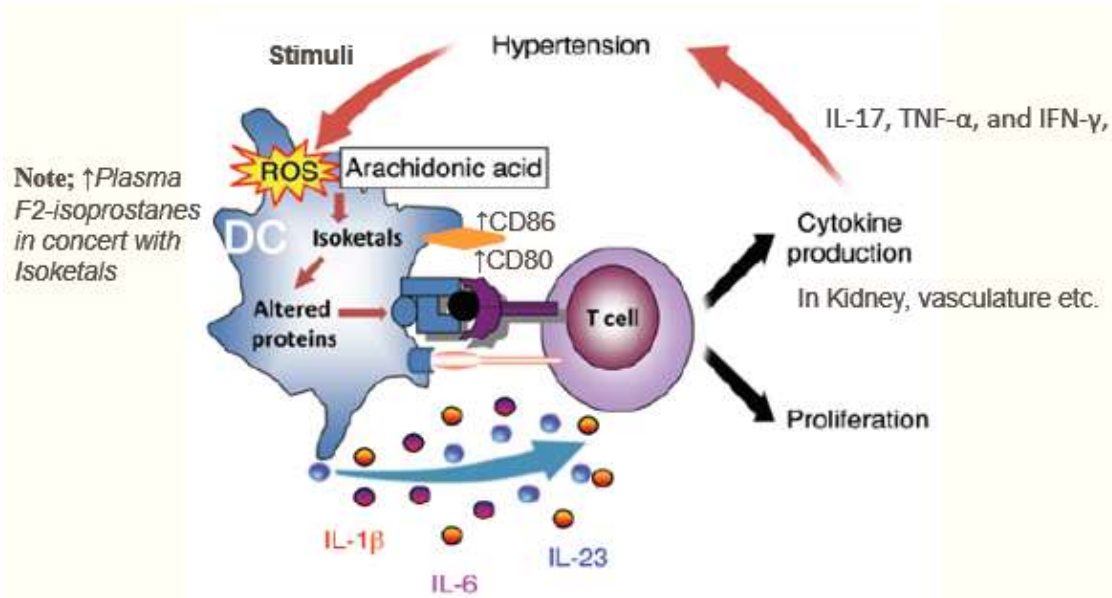


Figure 2. Isolevunglandin model of immune activation and hypertension
IsoLGs formed from peroxidation of arachidonic acid, adduct to lysine residues on proteins and extensively crosslink proteins, leading to alteration of protein function and activation of DCs which activate the adaptive immune system and contribute to hypertension. Hypertensive stimuli include: angiotensin II, aldosterone, endothelin-1, genetic susceptibility and salt. *Reproduced and modified with permission (Kirabo et al., 2014)*

2.3.2.2 Other inflammatory mediators involved in hypertension

Monocytes, macrophages and lymphocytes secrete a wide range of cytokines including IL-1, IL-8, TNF-α and platelet-derived growth factors (PDGFs) that cause endothelial injury and predispose to hypertension in PWH (Bigna et al., 2016; Devadas et al., 2004; Kedzierska and Crowe, 2001). However, the exact mechanisms are not yet clear apart from the fact that they cause an imbalance in nitric oxide (NO) homeostasis. More specifically, IL-6 upregulates vascular endothelial growth factor receptor II and promotes cell attachment, proliferation, and migration through matrix metalloproteinase-9 activity (Bigna et al., 2016). Although the mechanisms are still unknown, the proinflammatory cytokines TNF-α and IL-1 which are produced by macrophages may induce BP elevation and contribute to target organ damage (Justin Rucker and Crowley, 2017).

Current evidence now shows that there are specific and different subpopulation of the cells of the innate and adaptive immune system involved in the genesis and progression of hypertension in humans (Caillon and Schiffrin, 2016). T cell subpopulations have an important role in immune regulation, vascular remodeling and kidney injury in hypertension that must be taken into consideration (Caillon and Schiffrin, 2016; da Silva et al., 2016; Nobakht et al., 2016). An example are effector T cells from CD4+ precursors which include T helper 1 (Th1) (interferon-γ –producing, also IL-2, TNFα), Th2 (interleukin-4 producing, also IL-5, IL-10, IL-13), Th17 (which produce interleukin-17 and IL-22) and T-suppressor lymphocytes such as Treg-cells (regulatory T-cells), which express the transcription factor Foxp3 (forkhead box P3) that controls the expression of IL-10 and express the IL-2 receptor α-subunit (CD25) (Caillon and Schiffrin, 2016; Schiffrin, 2014). IL-4 and Treg-cells have anti-inflammatory effects whereas Th17 and Th1 have pro-inflammatory effects (Caillon and Schiffrin, 2016; Schiffrin, 2014). Among these, IL-17 has been shown to have very potent effects in the genesis and maintenance of hypertension due in part to its direct effect in causing endothelial dysfunction (Schiffrin, 2014; Wenzel et al., 2019). Under the stimulus of angiotensin II, IL-17 is associated with T cell infiltration in the kidney, perivascular fat and superoxide generation by activating Rho-kinase, which leads to phosphorylation of the inhibitory endothelial nitric oxide synthase (eNOS) residue, threonine 495 (Dinh et al., 2014). The inhibition of eNOS increases vascular tone (Dinh et al., 2014). Moreover, Th1 and Th17 cells promote inflammation and oxidative stress by exerting their injurious effects on the vasculature, leading to remodeling and endothelial dysfunction (Fahme et al., 2018; Schiffrin, 2014). In humans, a study assessing prehypertension and plasma levels of IL-17 reported that elevated IL-17 was associated with increased BP (Yao et al., 2015). Current evidence revealed that IL-17 is secreted by more than one cell type, CD4+, and these include γ/δ T-cells, T_C17 subset of CD8+ T cells, some B cells and natural killer T-cells and several other cells (McMaster et al., 2015).

Neutrophils have been implicated in antiretroviral therapy-controlled HIV to exhibit hyper activation and production of IL-17 (Campillo-Gimenez et al., 2014). Six isoforms of IL-17 (A-F) exist (Schiffrin, 2014). IL-17A and IL-17F are the most studied with the former being predominantly correlated with inflammatory injurious processes (Schiffrin, 2014). Treg cells help to suppress inflammation especially through the effects of IL-10 and therefore tend to be protective against a raised BP although other mechanisms exist (Schiffrin, 2014; Wang et al., 2016). In short, DCs create a chronic hypertensive response by activating T cells and T cells raise BP by causing vascular dysfunction and sodium retention and it is particularly the Th17 subpopulation of cells that augments BP elevation where as IL-10 serves to suppress inflammation and its effects (Zhang and Crowley, 2015).

The association between specific cytokine concentrations and hypertension was evaluated (Mirhafez et al., 2014) in 155 hypertensive patients and 148 control subjects. It was found that hypertensive subjects had higher serum concentrations of IL-1α, IL-2, IL-8, vascular endothelial growth factor, IFN-γ, TNF-α, MCP-1, and epidermal growth factor; and lower concentrations of the anti-inflammatory cytokine, IL-10 ($P < .05$), compared with the controls. Univariate and multivariate analyses revealed that IL-1α and IFN-γ were independent predictors of high systolic blood pressure, while IFN-γ, IL-1α, TNF-α, and MCP-1 remained statistically significant for diastolic blood pressure after correcting for age, gender, body mass index, smoking, fasting blood glucose, and triglycerides (Mirhafez et al., 2014). These associations may either have been related to common underlying factors that cause hypertension and /or the inflammatory cytokines might directly be involved in the etiology of hypertension (Mirhafez et al., 2014).

2.4 HIV and hypertension – the underlying role of inflammation

Infection with HIV and the use of ART for more than 2 years have the potential to induce an inflammatory milieu likely to induce hypertension (Fahme et al., 2018) as illustrated in Figure 3. Additionally, traditional risk factors alone or in concert with HIV/ART and immune activation induces end-organ damage that may result in the development or exacerbation of hypertension and CVD events (Figure 4) (Fahme et al., 2018).

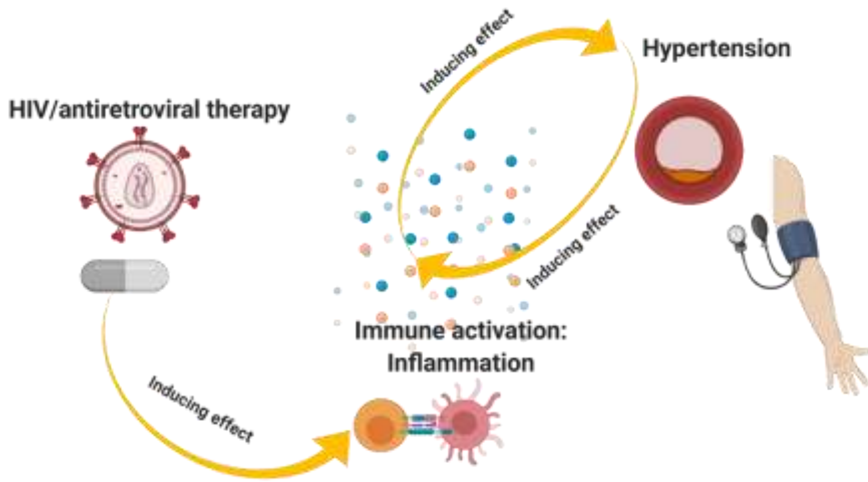


Figure 3. The relationship between HIV, Immune activation and hypertension relationship

HIV/ART results in systemic inflammation that leads to the development of hypertension especially after 2-years on antiretroviral therapy. Hypertension can lead to immune-activation independent of HIV infection and ART use. Biorender.com was used to create this figure.

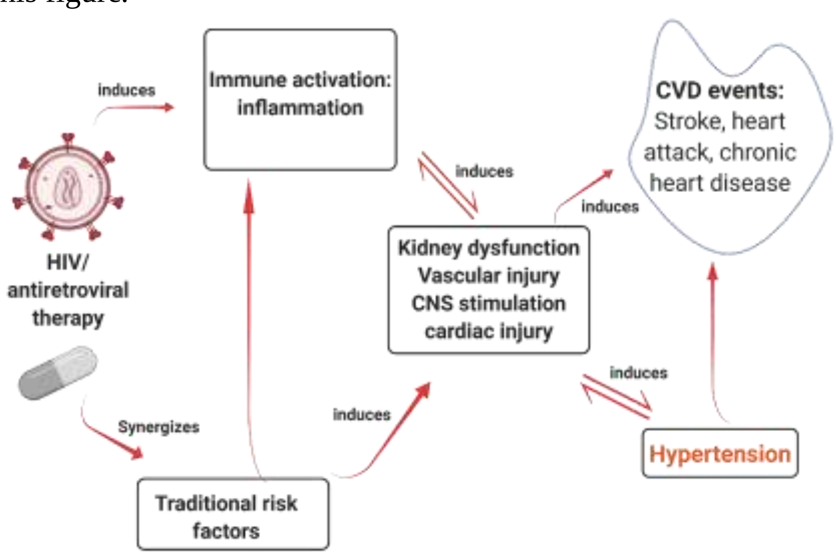


Figure 4. The interplay of HIV, traditional risk factors and hypertension illustration

HIV/ART may increase the risk to develop kidney, vascular and heart-related injury and CNS stimulation via existing traditional risk factors and lead to hypertension which may result in various CVD complications. CNS, central nervous system; CVD, cardiovascular disease. Biorender.com was used to create this figure.

The prevalence of hypertension has been reported to be higher in PWH (29%) compared to the HIV negative people (16%) (Peck et al., 2014). The overall global prevalence of hypertension is estimated to be 25.2% (21.2%, 29.6%; 95% confidence interval). The contributions of HIV infection and especially cART (Xu et al., 2017) to increasing blood pressure (BP) in PWH is well documented for specific treatment regimens such as use of protease inhibitors (PIs) and non-nucleoside reverse transcriptase inhibitors (NNRTI's) (Palacios et al., 2006; Tripathi et al., 2015). Contributing risk factors to the incidence of hypertension included longer duration on ART, female sex, higher body mass index (BMI), increasing age, elevated plasma cholesterol and microalbuminuria (Tripathi et al., 2015). In Zambia, the prevalence of hypertension is estimated to be 34% in the general population (Goma et al., 2019) and ranging from 6.4% (Bauer et al., 2017) to 19% in PWH (Deckert et al., 2017). However, as a limitation to the study, only 58% of patients with high blood pressure were confirmed to have hypertension in the study conducted by Bauer *et al* 2017. Male sex, increasing age and being overweight or obese was associated with high BP (Bauer et al., 2017).

The underlying pathology in PWH that contribute to hypertension are HIV related inflammation, HIV-related proteins and genetic predisposition (Bigna et al., 2016) which are discussed in section 2.4.1. HIV treatment too may play an important role as discussed in section 2.4.3.

2.4.1 The Role of HIV-related proteins in hypertension

Three HIV viral proteins namely glycoprotein 120 (GP-120), transcription proteins (Tat) and negative factor (Nef), have been implicated in contributing to hypertension through mechanisms that are not well established (Masenga et al., 2019b). Briefly, they induce vascular oxidative stress in the vasculature leading to smooth muscle proliferation and migration, and endothelial dysfunction that worsens or initiates an atherosclerotic process especially in patients with high HIV viral load and this contributes to the development of hypertension or accelerate adverse outcomes related to hypertension (Bigna et al., 2016).

The envelope glycoprotein Gp120 is a 120-kD protein expressed on the surface of the HIV envelope and subsequently undergoes viral shedding enabling it to be quantified in plasma (Shahbaz et al., 2015). Gp120 interacts with monocytes and macrophages which get activated and thereby produce pro-inflammatory cytokines, and stimulating the secretion of a potent vasoconstrictor endothelin-1, as well as inducing cell death in infected cells predominantly by direct interaction with the co-receptor, CXCR4 (Anand et al., 2018; Correale et al., 2015; Shahbaz et al., 2015). The hallmark of endothelial dysfunction leading to artherosclerosis and arterial stiffness is contributed in part to the synergy of Gp120 and TNF-α in decreasing eNOS and NO in human coronary artery endothelial cells (Masenga et al., 2019b; Shahbaz et al., 2015).

The second viral protein, HIV Tat (trans-activator of transcription) is a 16-kD regulatory protein encoded by the *tat* gene that enhances viral transcription. It is found in circulation as it is secreted extracellularly by infected cells (Anand et al., 2018; Hofman et al., 1993). Tat has been linked to various pathologies of endothelial cells, such as enhanced ROS production through activation of NADPH oxidase and through decreased antioxidant capacity, increased endothelial cell permeability and adhesion molecule expression (Anand et al., 2018; Marincowitz et al., 2019). Tat stimulates the release of pro-inflammatory cytokines and induces expression of endothelial- leukocyte adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin which induce initial binding of leukocytes to the blood vessel wall (Anand et al., 2018). The increased expression of ICAM-1 and VCAM-1 in the vasculature results in firm adhesion and transmigration of leukocytes that exacerbates artherosclerosis or endothelial injury (Shahbaz et al., 2015). The levels of soluble ICAM-1 concentration correlate with HIV disease as well as a reduction in CD4 count. Tat also induces IL-1β, macrophage chemotactic protein 1 (MCP-1), and IL-6 production which increases endothelial permeability. It can induce macrophage and foam cell apoptosis, causing the release of their lipid content into the intima of the artery resulting in the formation of a lipid plaque (Anand et al., 2018; Marincowitz et al., 2019). Tat can also suppress the bone morphogenic protein receptor 2 (BMPR-2) responsible for regulating endothelial cell proliferation and survival. This results in increased vascular smooth muscle proliferation and activation of endothelial cells leading to pulmonary arterial hypertension (PAH) (Correale et al., 2015). In PWH, Tat has been shown to increase the transcription of IL-17 and secretion by T cells causing a pro-inflammatory milieu and has been associated with a devastating immune reconstitution inflammatory syndrome (IRIS) in the brain (Johnson et al., 2013). In addition, Tat also acts indirectly on endothelial function by activation of protein kinase C, MAP kinase, and NFκB signalling, enhancing cell adhesion interactions with human monocytes that become activated and secrete tumour necrosis factor-alpha (TNF-α)(Marincowitz et al., 2019; Masenga et al., 2019b).

HIV Nef is a 27-kD, n-myristoylated accessory protein containing multiple domains essential for interaction with host cell signaling molecules (Anand et al., 2018). Nef enters endothelial cells by interacting with the C-X-C chemokine receptor type 4 (CXCR 4) (James et al., 2004). Nef impaires macrophage cholesterol transport resulting in lipid deposition in the vasculature that contributes to artherosclerosis and hypertension (Anand et al., 2018) as Nef is able to block the ATP-binding cassette transporter A1 (ABCA1) pathway, a cholesterol efflux pump in the cellular lipid removal pathway (Masenga et al., 2019b; Shahbaz et al., 2015). A summary of the involvement of Gp-120, Tat and nef is shown in Figure 5.

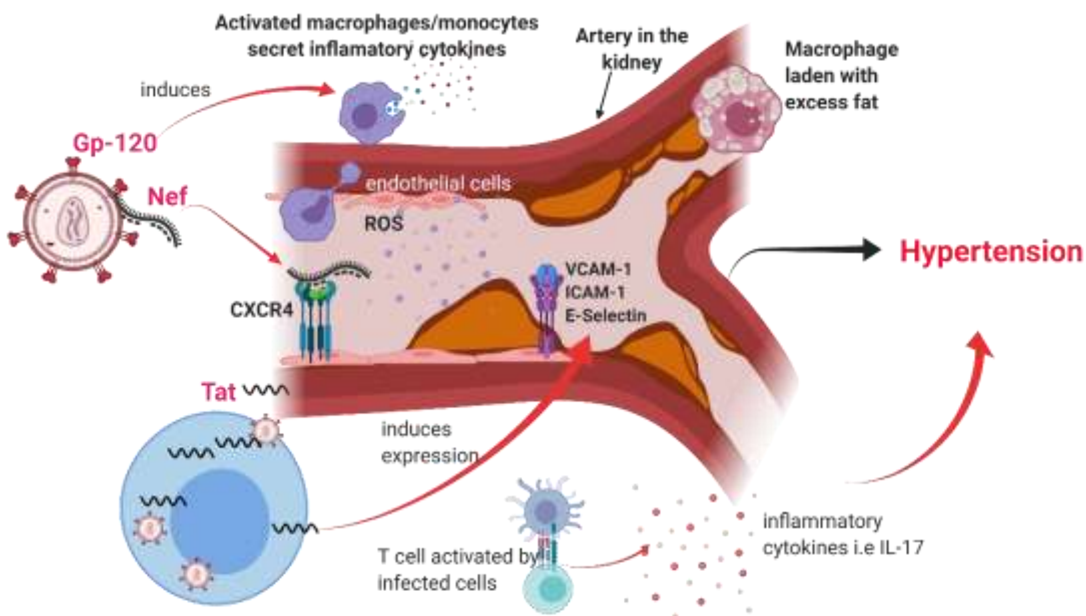


Figure 5. Contribution of Nef, Tat and Gp-120 in contributing to hypertension
Nef, Tat and Gp120 activate the immune system leading to inflammation and hypertension through interactions with vascular cells. Biorender.com was used to create this figure.

2.4.2 The role of HIV related inflammation in hypertension

Infection with HIV creates a paradoxical generalized immune activity environment condusive for vascular injury and exacerbates existing metabolic derangements. In most cases, an inflammatory condition though injurious to cells and blood vessels may exist without noticeable clinical changes in an individual, and becomes only detectable following a subsequent event such as a myocardial infarction, opportunistic infection, stroke or any serious vascular condition (Nemeth et al., 2015). The presence of both increased HIV viral replication and continued immune activation augments and creates a vicious inflammatory activation cycle that is a risk factor for many non-communicable conditions.

Many studies have reported an increased incidence of hypertension in PWH compared to the general population. This has been attributed to both the infection and cART (Nemeth et al., 2015). HIV associated atherosclerosis, myocardial infarction, systemic and pulmonary hypertension have been reported in both treated and treatment naïve patients (Barbaro et al., 2001). It is the excessive inflammation that is partially responsible for CVD development including hypertension in PWH (Nemeth et al., 2015). The exact mechanisms are not clearly understood, however, known processes for the pathogenesis of atherosclerosis may play a role.

2.4.2.1 The role of dendritic cells and their role in hypertension

Dendritic cells (DCs), the most potent APCs of the innate immune system have been shown to interact with components of HIV-1 both intrinsically (following fusion of cellular and viral membrane) and extrinsically (prior to being infected) through pathogen recognition receptors (PRRs) resulting in activation of the adaptive immune system (Iwasaki and Medzhitov, 2010). DCs express high levels of the HIV-1 co-receptors CCR5 and CXCR4 and low levels of CD4. This facilitates DC infection by HIV via the viral glycoprotein GP120 and spreading the infection to CD4+ T-cells. However, HIV infection and maturation of DCs are very low due to restricted viral replication in these cells and this impedes their immunogenic potential. The frequency of DCs infected is inversely associated with viral load (Wacleche et al., 2018).

Through their CD28/B7 ligands, CD80 and CD86 (also expressed on monocytes and B cells), DCs provide a costimulatory signal necessary for T cell activation and survival. In hypertension, there is an increase in B7 ligand expression and therefore T cell activation. DCs, in murine models, were shown to undergo maturation in hypertension by expressing CD11c+CD86+ as a signal of antigen presentation to T cells (Mian et al., 2014).

An important element in the activation of T cells by DCs and other cells in hypertension is antigen presentation. It is unclear what antigens are presented to T cells in relation to hypertension. An existing and most studied is the neoantigen, some form of modified self-proteins that arise inside APCs under a hypertensive stimuli. One such neo-antigen that others have studied are Isolevuglandins (IsoLGs) (Kirabo et al., 2014). These are produced following hypertensive stimuli such as oxidative stress and an excess salt environment. In DCs, Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activation creates the formation of IsoLGs through superoxide formation and action, and these IsoLGs adduct to self-proteins. This new complex (self-modified protein-IsoLGs) having become foreign to the cell is presented to T cells which then become activated. Elevated levels of IsoLGs, formed through this and other stress conditions creates a chronic immune activation and it is this chronic inflammation that is responsible for vascular and kidney injury and therefore the development of hypertension (Kirabo et al., 2014).

2.4.2.2 Monocytes in HIV

Initially, following HIV infection, HIV replication promotes activation of the endothelial cells lining the vasculature resulting in endothelial dysfunction (Fourie et al., 2015, 2011; Fourie and Schutte, 2017). Endothelial cells secret chemotactic mediators in the process, recruiting circulating monocytes that differentiate into macrophages in the sub-endothelial lining (Anzinger et al., 2014). Macrophages produce several pro-inflammatory mediators and recruit other monocytes. In the presence of elevated low-density lipoprotein (LDL) cholesterol, macrophages take up cholesterol-rich particles resulting in their inability to evacuate the atherosclerotic plaque leading to further injury of the endothelium (Almodovar et al., 2017; Anzinger et al., 2014; Loonam and Mullen, 2012). This process is exacerbated by the effect of ART especially protease inhibitors that directly impair lipid metabolism resulting in greater cholesterol accumulation (Anzinger et al., 2014).

Though monocyte activation is reduced when ART is introduced, macrophages continue to produce neopterin, a molecule involved in maintaining reactive oxygen and nitrogen products that injure the vasculature and contribute to the genesis of hypertension (Anzinger et al., 2014). Though not well established, macrophage 1 (M1), may likely be involved in mediating hypertensive vascular effects whereas macrophage 2 (M2) subset, may be protective (Mian et al., 2014).

Monocytes and macrophages are important drivers of pathogenesis and disease progression in PWH (Wong et al., 2019). Monocytes are chronically activated in PWH on cART as evidenced by increased monocyte activation markers soluble (s)CD14 and sCD163 (Reid et al., 2019). This affects their susceptibility to HIV-1 infection and their ability to migrate into the tissues, thus contributing to HIV-1 pathogenesis (Kruize and Kootstra, 2019; Nabatanzi et al., 2019). Activated monocytes secrete inflammatory cytokines especially IL-6 (Reid et al., 2019) and are associated with serious non-AIDS events (SNAEs) in virologically suppressed HIV-infected persons (Anzinger et al., 2014; Nabatanzi et al., 2019).

HIV mostly infects CD16+ expressing monocytes and not the classical (CD16-) monocytes consistent with the fact that CD16+ and not CD16- monocytes express higher levels of CCR5, which may facilitate HIV entry (Wacleche et al., 2018). HIV infected monocytes, especially intermediate monocytes expressing CD14+ and CD16+ produce more pro-inflammatory cytokines as compared to their counterpart (Prabhu et al., 2019). They adhere to endothelial cells and transmigrate into the sub endothelial area where their pro-inflammatory activity is increased. It is evident that in HIV, monocytes and macrophages have a reduced phagocytic activity and they age prematurely (Shahbaz et al., 2015). Monocytes expressing CD16+ (intermediate) are elevated in HIV infection and increases range from 30 to 50% in HIV-chronically infected and AIDS patients, as opposed to 5–10% observed in HIV negative individuals have been reported (Prabhu et al., 2019; Wacleche et al., 2018). This increase may be due to high levels of monocyte/macrophage growth factor (M-CSF) observed in HIV-infected subjects, which induces monocytic maturation including the acquisition of CD16 expression and is related to activation of the renin-angiotensin aldosterone system that results in increased BP (Bogorodskaya et al., 2019; Wacleche et al., 2018). However, this increase is reported in ART naïve HIV viremic individuals and not in those receiving ART (Wacleche et al., 2018). The intermediate monocytes have been reported to be high in hypertension, correlating positively with plasma levels of IL-6, sCD14 and high-sensitivity C-reactive protein (hs-CRP) (Justin Rucker and Crowley, 2017; Loperena et al., 2018; Manner et al., 2013; Wacleche et al., 2018).

Several murine model studies (Karbach et al., 2016; Kirabo et al., 2014; Lu and Crowley, 2018) and human studies (Justin Rucker and Crowley, 2017; Loperena et al., 2018) have found that monocytes promote hypertension. Monocytes/macrophages express functional Angiotensin II and mineralocorticoid receptors which are essential for their function, and when changes or disruptions in angiotensin II regulation occur, they produce excess ROS through the activation of reduced NADPH oxidase resulting in vascular injury (Loperena et al., 2018; Mian et al., 2014). In murine models, activated monocytes/macrophages, through IL-12 production, can also activate NK cells. NK cells are activated through the T-bet/TBX21-dependent mechanism (Mian et al., 2014). When they are activated, they produce IFN-γ and other pro-inflammatory cytokines that contribute to angiotensin II-induced vascular injury in hypertension (Mian et al., 2014).

2.4.2.3 The role of eosinophils and inflammation

Eosinophils, known for their involvement in allergic hypersensitivity reactions, are part of the innate immune system and can enter extravascular tissues and participate in various immunologic reactions relevant to innate immunity following their inducement by IL-3, granulocyte-macrophage colony stimulating factor (GM-CSF) and especially IL-5 (Gauckler et al., 2018). Eosinophils play a protective role by secreting several bioactive molecules that have specific effects on other cells, on the vasculature and in fighting against pathogens (Gauckler et al., 2018). For example, major basic protein granules, eosinophil cationic proteins and eosinophil peroxidase have cytotoxic and antiviral effects on other cells and pathogens (Gauckler et al., 2018). While eosinophils are key regulators of perivascular adipose tissue and vascular functionality (Withers et al., 2017), they have been implicated in contributing to inflammation resulting in kidney injury (Daniel et al., 2019; Gauckler et al., 2018).

Eosinophils play a role in adipose tissue in the induction of a brown adipocyte phenotype in white adipocytes, a process known as browning or beiging (O'Sullivan and Bochner, 2018). In obese persons where inflammation is an important pathogenic mediator of the development of obesity-induced insulin resistance, eosinophils and other cells, such as regulatory CD4 T helper cells (Tregs), and Th2 CD4 T cells, infiltrate the adipose tissue where they exert anti-inflammatory effects (Lee and Lee, 2014). Obesity is therefore responsible in counter balancing between pro- and anti-inflammatory immune cell homeostasis by skewing/tilting this balance towards a more pro-inflammatory status (Lee and Lee, 2014).

Recent data shows that eosinophils play a protective role in inhibiting HIV viral replication in PWH (Ramirez et al., 2018), however, there is insufficient information about their role in hypertension. Most of the studies reporting on eosinophils in PWH do not report on hypertension but report on other cormobid conditions and infections with HIV such as dengue virus infection (Pang et al., 2015), hepatitis viral infection (Demiraslan et al., 2017), asthma (Adrish et al., 2019) and parasitic infections (Marchese et al., 2019).

2.4.3 HIV treatment and hypertension

The introduction of highly active antiretroviral therapy (HAART) has given rise to various CVDs including hypertension. But the effect of specific regimens on BP has not been well established except for their low to moderate increase attributed to NNRTI's and PIs (Bigna et al., 2016; Calò et al., 2013). Also, it is now known that patients only become hypertensive at least after two years of cART and systolic pressure increases further after five years of cART(Calò et al., 2013). Treatment with lopinavir/ritonavir is associated with BP increase compared to other drugs in combination with increasing body mass index (BMI) (Calò et al., 2013). Indinavir containing regimens are associated with arterial hypertension (Shankar and Dubé, 2004). However, some studies that have reported contrary findings and have found no association between HIV status, cART and hypertension. This was on account of short term follow-up (less than two years) and non-usage of cART known to associated with hypertension (Ogunmola et al., 2014; Okello et al., 2015).

The hallmark of inducement of hypertension lies in endothelial dysfunction from the effects of HIV infection and ART in the long term (Calò et al., 2013; Dubé et al., 2008). PIs induce endothelial damage through induction of oxidative stress, reduced expression of endothelial nitric oxide synthase, metabolic dysregulation that leads to lipid deposition along vessel walls, increased leukocyte cell adhesion exacerbated by increased immune activation and chronic inflammation (Calò et al., 2013; Dubé et al., 2008).

2.5 The role of genetic predisposition to hypertension

With regards to genetic predisposition, there are many genes or gene combinations that influence elevated BP. Several rare mutations (more than 25) and more than 120 single nucleotide polymorphisms that contribute to high BP and hypertension have been identified (Lifton et al., 2001). However, even with the associated variants at present, the collective effect of all of BP loci identified through genome-wide association studies accounts for only 3.5% of BP variability (Dominiczak and Kuo, 2017). The genotypes associated with hypertension include *CYP11B2, PRKG1, ADRB2, FGF5, SLC8A1* and *BCAT1* (Liu et al., 2017). Another study reported evidence of a relationship between rare variants in the endothelial sodium channel pathway and salt sensitivity of BP (Gu et al., 2018). A protective, aggregate effect of rare variants in the *SCNN1A* gene on mean arterial pressure (MAP) response to the sodium intervention was identified (Gu et al., 2018). In addition, several common variants in the *SCNN1A* gene were observed to be associated with salt sensitive BP (Gu et al., 2018).

It is reported that mutations in the Human Leukocyte Antigen – antigen D Related type 6 and 5 (HDL-DR6 and HLA-DR5 respectively) histocompatibility complex alleles have been implicated in PAH (Bigna et al., 2016). Additionally, mutations involving the renin-angiotensin-aldosterone system such as the *ACE I/D rs4340* and *ACE A2350G rs4343*, and those involving the alpha-aducin (*ADD1 G460W rs4961*) and intracellular signalling (*GNB3 C825T rs5443*) were associated with hypertension (Sousa et al., 2018). Although some individuals of African descent may be more susceptible to the development of hypertension (Rayner and Spence, 2017), there is insufficient evidence on the specific polymorphisms associated with the risk for hypertension (Yako et al., 2018).

2.6 Sodium chloride intake and hypertension

The review by DiNicolantonio and O'Keefe, 2016 showed that salt increases BP in some, while having no effect on BP in others for both normotensive and hypertensive HIV negative individuals. They also highlight a paradox where salt loading was associated with BP reduction in salt-resistant individuals (those whose BP does not rise with higher salt intake). Mostly those who were salt sensitive and therefore had their BP increased following salt loading were more likely to have had a poor kidney function and/or insulin resistance. Also, a higher salt intake does not appear to lead to an over retention of water but something else may be doing this, sugar. Additionally, there is no evidence of increased plasma sodium following a high salt intake (DiNicolantonio and O'Keefe, 2016).

However, mechanistic studies on salt loading revealed that dietary salt can be stored in tissues above normal plasma concentrations in excess of 40 mmoles (mM) and activate immune cells such as DCs, monocytes and T cells (Schatz et al., 2017). This challenges the current dogma that interstitial sodium readily equilibrates with plasma and that renal excretion and reabsorption is sufficient to regulate extracellular fluid volume and control BP. Recent discovery now shows that sodium accumulates in tissues without commensurate volume retention and activates immune cells, leading to hypertension and autoimmune disease (Kirabo, 2017).

Using ^{23}Na-Magnetic resonance imaging (MRI) technology allows for non-invasive visualization and the quantification of tissue sodium stores in humans. Studies using this technology revealed that high salt levels accumulate in the medulla of the kidney, skin and skeletal muscles without commensurate water retention at approximately 40 mM higher than concentrations in the plasma. Furthermore, salt tends to accumulate in infected skin and activate immune cells such as monocytes, macrophages and T cells. However, this concept and the mechanisms of inflammation-driven salt accumulation upon the site of infection and/or inflammation are not very clear in humans (Schatz et al., 2017).

Figure 6 summarizes the interactions or links between HIV/ART, immune activation/inflammation, and traditional risk factors in contributing to hypertension.

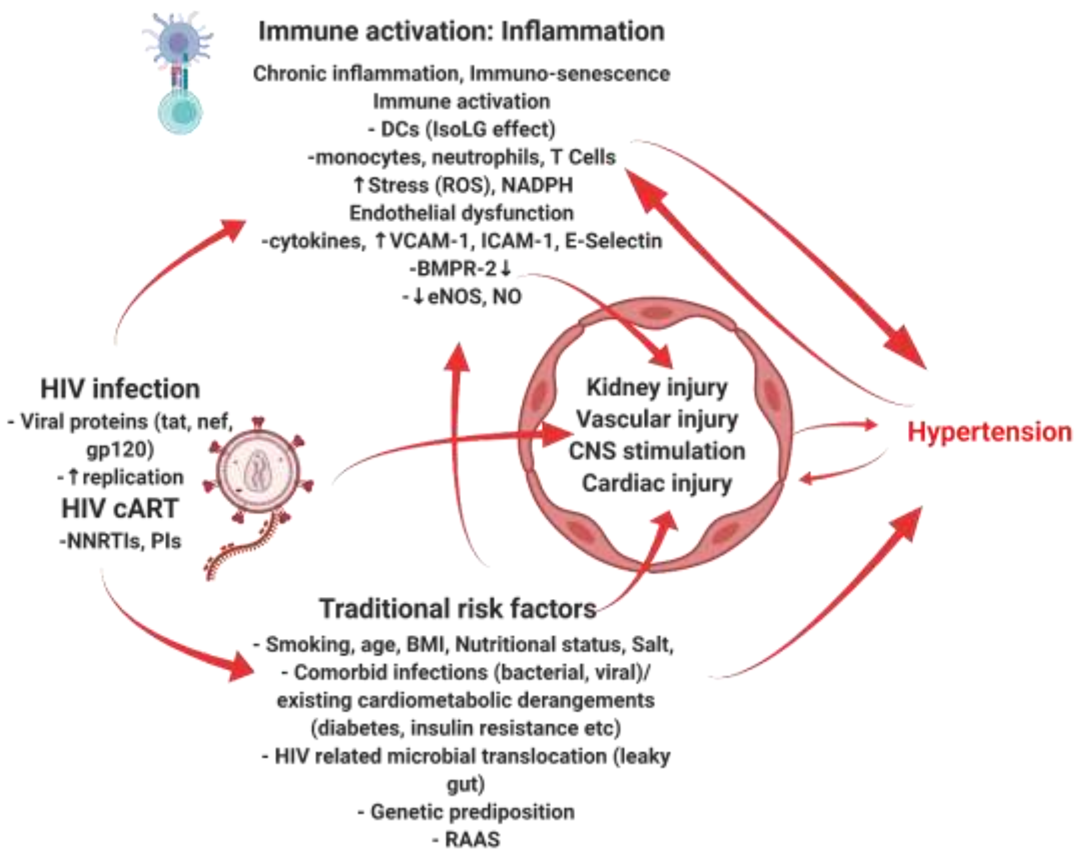


Figure 6. Summary of the conceptual framework linking HIV, inflammation and hypertension

HIV interacts with the immune system to synergistically cause kidney damage, vascular injury, and lead to hypertension while the presence of traditional risk factors including excess dietary salt exacerbates this. DCs, dendritic cells; IsoLGs, isolevuglandins; ROS, reactive oxygen species; NADPH, nicotinamide adenine dinucleotide phosphate; VCAM, vascular cell adhesion molecule; ICAM, intracellular cell adhesion molecule; BMPR-2, bone morphogenetic protein receptor type II; eNOS, endothelial nitric oxide synthase; NO, nitric oxide; cART, combinational antiretroviral therapy; NNRTI's, non-nucleoside reverse transcriptase inhibitors; PIs, protease inhibitors; BMI, body mass index; RAAS, renin-angiotensin- aldosterone system; CNS, central nervous system. *Biorender.com was used to create this figure.*

2.7 Laboratory methods and principles

2.7.1 **Biochemistry tests**

The Pentra C200 chemistry automated analyser uses absorption photometry for determining the amount of absorbance in plasma. Samples are measured using bi-chromatic light absorbance in 8 different wavelengths ranging from 340 to 700 nm. The color intensities for each analyte is proportional to the concentration (www.horiba.com).

For glucose analysis, the reagents are formulated with peroxidase (Duxbury, 2004). This procedure, takes advantage of the specificity of glucose oxidase and is based upon the conversion of glucose to gluconic acid and hydrogen peroxide by glucose oxidase and the subsequent oxidation of o-dianisidine to its oxidized form, measurable at 420 nm, by hydrogen peroxide with peroxidase (Duxbury, 2004). The absorbance is used to calculate the concentration in the solution.

For the determination of total cholesterol, a series of coupled reactions are employed to hydrolyze cholesteryl esters and oxidize the 3-OH group of cholesterol (Li et al., 2019). Hydrogen peroxide, one of the by-products is then measured quantitatively in a peroxidase catalyzed reaction that produces color. Absorbance is measured at 500 nm (Li et al., 2019).

For tryglyceride analysis, a series of coupled reactions are initiated in which tryglycerides are hydrolyzed to produce glycerol, which is oxidized by glycerol oxidase and hydrogen peroxide is one of the by-products whose absorbance is measured at 500 nm (Sardesai and Manning, 1968).

High density lipoprotein (HDL) is measured by mixing the plasma with a blocking reagent (sulfated alpha-cyclodextrin in the presence of Mg^{+2}) that excludes apoB containing lipoproteins (Miller et al., 2010). The blocking reagent also forms complexes with polyethylene glycol-coupled cholesteryl esterase and cholesterol oxidase for HDL-cholesterol measurement. The absorbance in the color change is measured at 600 nm.

LDL-cholesterol is calculated from measured values of total cholesterol, triglycerides and HDLcholesterol according to the relationship: [LDL-$_{chol}$] = [total $_{chol}$] - [HDL-$_{chol}$] - [TG]/5 (Nauck et al., 2002).

For the creatinine test, the reagent contains an enzyme creatininase which hydrolyzes creatinine in the sample to form creatine. Creatine is in turn hydrolyzed by creatinase to sarcosine and urea. Sarcosine is then oxidized by sarcosine oxidase in the reagent to glycine and formaldehyde, with the concomitant production of hydrogen peroxide. The hydrogen peroxide reacts with 4-aminoantipyrine and N-ethyl-N-sulfopropyl-m-toluidine in the presence of peroxidase to yield a quinoneimine dye. The resulting change in absorbance at 548 nm is proportional to the creatinine concentration (Peake and Whiting, 2006).

To measure urea, the reagents for urea quantification are formulated with urease which reacts with urea to yield ammonia and carbon dioxide. The ammonia and α-oxoglutarate are converted to glutamate in a reaction catalyzed by L-glutamate dehydrogenase (GLDH) (Francis et al., 2002). In this process, two molecules of nicotinamide adenine dinucleotide (NAD+) hydrogen (NADH) are oxidized for each molecule of urea hydrolyzed and the rate of change in absorbance due to the disappearance of NADH, is directly proportional to the concentration of urea in the sample. The absorbance for the urea test is measured at 340 nm (Francis et al., 2002).

For Alanine amino transferase (ALT) test, the reagents for ALT quantification are formulated with substrates L-alanine and α-ketoglutarate to measure the enzymatic rate activity of ALT in plasma (Henley, 1980). When ALT in the sample mixes with the substrates, ALT catalyzes the reversible transamination of L-alanine and α-ketoglutarate to pyruvate and L-glutamate. The pyruvate is then reduced to lactate in the presence of lactate dehydrogenase (LDH) with the concurrent oxidation of NADH to NAD. The rate of change in the absorbance at 340 nm is directly proportional to the ALT concentration in the sample (Henley, 1980).

2.7.2 Haematology tests

Automated haematology analysers uses three principle technologies in sample analysis. The first principle is Hydrodynamic focusing (electric resistance detection method). This conducts direct count of cells based on the resistance generated as they pass through an electrically charged aperture (Golden et al., 2012; Zelmanovic et al., 2000). This method generates the red blood cell and platelet counts. The second principle is flow cytometry which measures white blood cells and differential counts (Zelmanovic et al., 2000). Briefly, the cell nuclei are stained and then are subjected to a laser beam as they pass through the flow cell. The cells are determined by scatter gram (forward and side scatter) as cells scatter differently according to size and shape (Zelmanovic et al., 2000). The third principle is sodium lauryl sulphate (SLS) haemoglobin method (Oshiro et al., 1982). This is used in the determination of haemoglobin by photometry. Briefly, the Sulfolyser lyses red blood cells in the sample by altering the globin and then oxidising the haeme group to allow conversion of haemoglobin from ferrous (Fe^{2+}) to ferric (Fe^{3+}) state. The SLS hydrophilic groups bind to the haeme group and form a stable, coloured compound SLS-Hb-hemi chrome molecule. The colour intensity is equivalent to haemoglobin concentration (Oshiro et al., 1982).

2.7.3 CD4 Count test

For the CD4 test, the Becton Dickinson (BD) FACSCount analyser was used. The BD FACSCount System is an automated instrument designed specifically for enumerating the absolute cell counts of CD4 and T lymphocytes in unlysed whole blood. When whole blood is added to the reagents, fluorochrome-labelled antibodies in the reagents bind specifically to lymphocyte surface antigens (Givan, 2001). After a fixative solution is added to the reagent tubes, the sample is run on the instrument. Here, the cells come in contact with the laser light, which causes the fluorochrome-labelled cells to fluoresce (Givan, 2001). This fluorescent light provides the information necessary for the instrument to count the cells. In addition to containing the antibody reagent, the reagent tubes also contain a known number of fluorochrome-integrated reference beads. These beads function as a fluorescence standard for locating the lymphocytes and also as a quantitative standard for enumerating the cells. The instrument automatically calculates the absolute T lymphocyte populations (Givan, 2001).

2.7.4 Viral Load test

Several machines are used to quantify HIV viral load and in Zambia the COBAS AmpliPrep /COBAS TaqMan HIV-1 Test v 2.0 is used. The COBAS AmpliPrep /COBAS TaqMan HIV-1 Test is a nucleic acid amplification test for the quantitation of Human Immuno-deficient Virus Type 1 (HIV 1) RNA in human plasma (Pas et al., 2010; Pyne et al., 2010). The COBAS AmpliPrep /COBAS TaqMan HIV test, v 2.0 is based on three major processes namely: HIV 1 RNA Isolation; Reverse Transcription of the Target RNA to generate complementary DNA (cDNA); Simultaneous PCR amplification of Target cDNA and detection of cleaved dual labelled oligonucleotides detection probe specific to the target (Pas et al., 2010; Pyne et al., 2010).

3.0 CHAPTER THREE: METHODOLOGY

3.1 Ethical consideration, approval and dissemination

This study was undertaken after ethical approval was granted from the University of Zambia Health Sciences Research Ethics Committee (UNZAHSREC), under protocol ID 20180726003, the University of Zambia Biomedical Research Ethics Committee (UNZABREC) under reference number 005-03-17 and the Vanderbilt Institutional Review Board under VICTR Study #1952.1. Permission to conduct the study was granted by the Livingstone Central Hospital Administration.

The information obtained in the data collection forms was treated with total confidentiality. No patient identifiers were extracted in the data collection forms that were used for data analyses and only a unique study number was used. Restriction of data access to the third party was maintained throughout the study. Data obtained from this study and the records were kept in a private locked cabinet and only the principal investigator had access to it. Biological samples (plasma and serum) awaiting reagents or other tests that were not available at the time of specimen collection were stored at -20 ^{0}C until the time of analysis and participants were made aware of this.

For volunteers to be used as controls, the counselling and treat service was used. Individuals included were those attending routine medicals for employment and other reasons as well as volunteer health workers who were counselled and tested for HIV to confirm their status through existing service.

The benefits of the study were explained to the participants and were contained in the information sheet (see appendices F). The benefits of the study to the participants were that the aggregated results will provide valuable information that will assist how patients with hypertension are managed. Since the participants were screened before inclusion/exclusion, new medical problems discovered were referred for standard medical attention and attended to appropriately and routinely as per hospital care. The participants also benefited from the health talks and personal advice based on any findings during the interview and clinical examinations.

Clinical and laboratory data were collected by the Principal investigator (PI) who is a clinical Biochemist with assistance from a medical doctor and nurses to ensure credibility and accuracy of information.

Dissemination of research results will be done by the PI at the Hospital's weekly scientific and clinical presentations after the thesis has been marked and all necessary corrections made.

3.2 Description of study sites and study cohorts

3.2.1 Study sites

Two study sites were used for recruitment of particpants namely Livingstone Central Hospital (LCH) and Vanderbilt university (USA). Livingstone Central Hospital is the largest referral hospital in Southern Province of Zambia and the antiretroviral therapy (ART) clinic is the largest out-patient clinic that offers ART and general medical services to the community with approximately 3776 PWH enrolled in ART. The Vanderbilt university Vanderbilt Comprehensive Care Center (VCCC), is the largest HIV provider in Central Tennessee and a principal study site for the AIDS Clinical Trials Group, which has enrolled over 7000 patients since 1994.

Two sites were used in this study mainly for the purposes of widening the depth of knowledge on how inflammation is linked to hypertension by using separate patient groups differing by race and geographical location.

3.2.2 Conceptual design of cohorts

This study used mixed study designs to inference and extrapolate linkages between HIV-infection, inflammatory- and immune-activation markers, salt sensitivity and hypertension. A total of six cohorts was recruited to achieve the study objectives and fill specific knowledge gaps created by results from the two main cohorts. Thus, experimental, interventional and analytical cross-sectional designs were employed as illustrated in Figure 7 below:

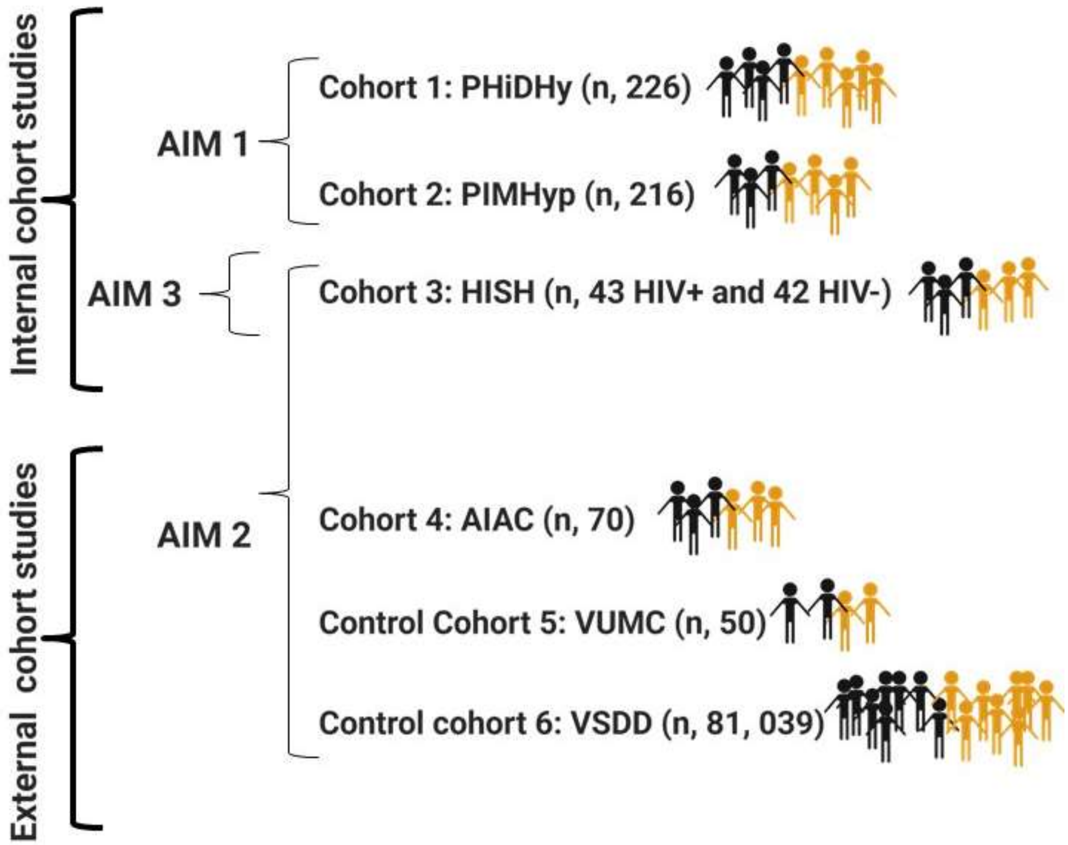


Figure 7. Conceptual design of study cohorts

Cohort 1-3 were internal participants recruited at Livingstone Central Hospital (LCH) while 4-6 are cohorts from the USA. Orange denotes hypertensives; Black denotes normotensives. PHiDHy, Profiling HIV positive patients with or without hypertension cohort; PIMHyp, Patho-immune mechanisms of hypertension cohort; HISH, HIV Immune-activation and salt-sensitive hypertension; VUMC, Vanderbilt University Medical centre cohort; AIAC, Adiposity and Immune Activation Cohort; VSDD, Vanderbilt Synthetic Derivative Database

3.3.0 Methods for risk factors and inflammatory surrogate study

3.3.1 Specific objective 1: cohort 1 and sub-cohort 2

3.3.1.1 Study design and Setting

This was a cross-sectional analytical study

3.3.1.2 Participants and setting

Participants were enrolled from the ART clinic at LCH during their regular routine visits.

3.3.1.3 Eligibility criteria

3.3.1.3.1 ***Inclusion criteria***

The study included all HIV-1 positive patients above 18 years of age. Study participants were only recruited after verbally consenting and signing a consent form.

3.3.1.3.2 Exclusion criteria

The study excluded participants with diabetes mellitus and comorbidity patients who were seeking healthcare due to an illness rather than routine ART clinic reviews. This exclusion was to avoid confounding of risk factors contributing to both diabetes mellitus and hypertension.

3.3.1.4 Sample size estimation

OpenEpi online software (sample size for a proportion or descriptive study) was used to compute a total sample size of 226 using an estimated prevalence of hypertension of 19.3% (local quarterly monthly records) at 95% significance level and 80% power in an ART population of 3776. The formula is stipulated below:

Sample size (n) = [DEFF*Np(1-p)]/ [(d2/Z21-α/2*(N-1)+p*(1-p)];

N - is the population size.

p - is hypothesized % frequency of outcome factor in the population.

d - is Confidence limits as % of 100 (absolute +/- %, which is 5%).

α – is error of detection at 0.05

DEFF - is the design effect.

3.3.1.5 Study variables

The primary response variable was hypertension. The diagnosis of hypertension for participants was based on BP readings of 140/90 mmHg or higher (Chobanian et al., 2003) and the new American Heart Association/ American College of Cardiology (AHA/ACC) criteria which was based on BP readings of 130/80 mmHg or higher (Whelton et al., 2017).

3.3.1.5.1 Explanatory variables

***3.3.1.5.1.1* Social demographic characteristics**

Age, gender, marital status, employment status, highest education.

***3.3.1.6.1.2* Physical activities and dietary lifestyle factors**

Physical activity (dichotomized), minutes of weekly vigorous and moderate activity, minutes of weekly walking activity, minutes spent seated every weekend and weekday, days of weekly fruit intake, days of weekly vegetable intake, addition of salt at the table and while cooking, processed salt intake, alcohol consumption, former smoker or previous smoking activities.

***3.3.1.6.1.3* Clinical factors**

BMI, WC, duration on ART, ART regimen, use of antihypertensive medication, pulse, pulse pressure [defined as systolic BP (SBP) minus diastolic BP (DBP)], mean arterial pressure (MAP) (defined as DBP plus one third pulse pressure), mid-BP (sum of SBP and DBP, divided by 2), CD4 counts, HIV RNA viral load, fasting blood sugar (FBS), diabetes risk scores, diabetes risk category. Diabetes risk scores were calculated using the International Diabetes Federation (IDF) risk assessment form as previously described (Masenga et al., 2019c). The current ART regimen in use at the time of the study using the Zambia consolidated guidelines of 2018 were TDF (tenofovir disoproxil fumarate), 3TC (Lamivudine), EFV (efavirenz), NVP (nevirapine), LPV/r, (lopinavir/ritonavir), ABC (abacavir), AZT (azidothymidine also called zidovudine), DTG (dolutegravir). The combinations used are stipulated below:

TDF/3TC/EFV; TDF/3TC/NVP; TDF/3TC/LPV/r; ABC/3TC/NVP; ABC/3TC/EFV; AZT/3TC/LPV/r; AZT/3TC/NVP; TDF/3TC/DTG

3.3.1.6 Blood Pressure measurements

The WGNBPA 730 (USA) and SBM 67 (Germany) BP monitors for measurements were used. For standard measurements the new AHA/ACC guidelines (Whelton et al., 2017) were adapted. The patients were required not to have been exercising, smoking or drinking beverages with caffeine, had their bladders emptied and were seated for more than 30 minutes before measurements were taken in a still position. The limb used to measure BP was supported ensuring that the BP cuff was at heart level. During the measurements, the participants were asked to sit upright, back straight with feet flat on the floor without legs crossing each other. Three readings were taken at two (2) minute interval and averaged. The average BP was used to reflect the person's BP. Routine BPs taken by attending nurses did not take into account the consideration explained above. Furthermore, we also compared previous BP readings from the participant's medical history.

To diagnose hypertension or classify individuals as hypertensive, history on the use of antihypertensive drugs was employed except for four participants, of whom, we used BP readings taken on 2 or more occasions (from records) which were consistent with the standard measurements taken.

3.3.1.6.1 Antihypertensives

The hypertensive patients at the medical clinic were taking two antihypertensive drugs at the time of data collection, a calcium channel blocker (amlodipine or nifedipine) and either an angiotensin-converting enzyme inhibitor (ACE) (enalapril or losartan) or a diuretic (furosemide or moduretic).

3.3.1.7 Data sources/measurement

For data collection, the interviewer structured questionnaire adapted from the World Health Organization's STEPwise Approach to Surveillance (WHO STEPs)(Riley et al., 2016), the International Physical Activity Questionnaire (IPAQ) (Appendix H) and the IDF type 2 diabetes risk assessment forms described in our previous study (Masenga et al., 2019c). These were translated into the local language Tonga for participants that could not comprehend English. (See appendix H for the data collection forms).

3.3.1.8 Data analysis

Descriptive statistics such as means, medians, percentages were used to summarise the data collected. Since data were not normally distributed, the non-parametric test Mann-Whitney was used to compare medians (interquartile range) of continuous variables such as age, minutes of weekly activities, minutes spent seated, body mass index, duration on ART as shown in Tables 4-9 between normotensive and hypertensive groups. For categorical variables, Chi-square or fishers' exact test were appropriately used to determine associations between hypertension and all categorical variables. A careful selection of known risk factors for developing hypertension and considering variables significant in univariate logistic regression were included in the multivariate logistic regression model. Odds ratios (OR), adjusted odds ratios (AOR) and confidence limits at 95% were reported. Descriptive statistics to compare the proportion of participants in each BP category and hypertension status between JNC 7 and new AHA/ACC criteria were employed. To compare routine BP measurements taken by attending nurses and standard BP measurements, a Mann-Whitney and non-parametric Spearman correlation coefficient to assess the strength of association was used. Median differences and their confidence limits (95%) were reported. Age, waist circumference, BMI and diabetes risk were also categorized to ease interpretation. P values less than 0.05 was the criteria used to inference statistical significance and they are shown in bold in all the tables.

3.3.1.9 Testing concepts and rationale

The objective of this study cohort was to determine the risk factors and inflammatory surrogates associated with hypertension in HIV in relation to the new American Heart Association hypertension guidelines**.** To achieve this aim, data was collected on "traditional risk factors" using the WHO STEPS and IPAQ data collection tools and associations made with hypertension comparing the impact between using the Seventh

Joint National report (JNC 7) diagnostic criteria and the American Heart Association new (2017) diagnostic criteria.

Secondly, a comparison was made of inflammatory surrogates in a full blood count between hypertension and normotensive individuals to ascertain the use of a full blood count to postulate an existing inflammation.

3.3.1.9.1 Laboratory methods

3.3.1.9.1.1 **Biochemistry tests**

All blood samples for biochemistry analyses were collected in lithium heparinized green top 5 ml blood collecting containers. They were centrifuged at 350 x g for 5 minutes at room temperature and assayed on an automated Pentra C200 auto-analyser.

3.3.1.9.1.2 **Haematology tests**

For all haematology tests, the Sysmix XT1800i was used. The Sysmex XT -1800i measures 24 haematological parameters to analyse a complete blood count. All samples were collected in Ethylenediaminetetraacetic acid (EDTA) tubes and mixed thoroughly prior to analysis

3.3.1.9.1.3 **CD4 Count test**

For the CD4 test, the Becton Dickinson (BD) FACSCount analyser was used. All blood samples were collected in EDTA tubes. Whole blood was added to the reagents and incubated for 30 minutes and then assayed on the instrument.

3.3.1.9.1.4 **Viral Load test**

The COBAS AmpliPrep /COBAS TaqMan HIV-1 Test v 2.0 was used to quantify HIV viral load. Blood samples collected in EDTA were centrifuged immediately and assayed on the analyzer.

3.4.0 Methods for inflammation and hypertension studies

3.4.1 Specific objective 2 and 3: Sub-cohort 3

3.4.1.1 Study design

This study used cross-section analytical design

3.4.1.2 Study site and population

This study was conducted at the Livingstone Central Hospital ART clinic. The study comprised HIV positive patients attending ART and HIV negative controls from volunteer health workers matched for age and sex.

3.4.1.3 Selection of participants and sampling methods

For selection of hypertensive and normotensive study participants, a simple random sampling assisted by an online random number generator was used. A random selection of HIV positive patients coming for their routine medical check-ups was done. The matched controls were selected conveniently to match the HIV positive group.

For selection of hypertensive and normotensive HIV and HIV negative study participants, randomization in blocks of size four and six was used to create equal groups in the strata using an online randomization software. Below is an illustration.

Block sizes: 4, 6

Actual list length: 44

The first digit is the block identifier . The second digit is the block size. The third digit is the sequence within the block and the hypertension status is a reference to the subgroup.

1, 4, 1, Normotensive	4, 6, 2, Hypertensive	7, 4, 1, Normotensive
1, 4, 2, Hypertensive	4, 6, 3, Hypertensive	7, 4, 2, Normotensive
1, 4, 3, Hypertensive	4, 6, 4, Normotensive	7, 4, 3, Hypertensive
1, 4, 4, Normotensive	4, 6, 5, Normotensive	7, 4, 4, Hypertensive
2, 4, 1, Normotensive	4, 6, 6, Normotensive	8, 4, 1, Normotensive
2, 4, 2, Hypertensive	5, 6, 1, Normotensive	8, 4, 2, Hypertensive
2, 4, 3, Normotensive	5, 6, 2, Normotensive	8, 4, 3, Hypertensive

2, 4, 4, Hypertensive
3, 6, 1, Normotensive
3, 6, 2, Hypertensive
3, 6, 3, Hypertensive
3, 6, 4, Normotensive
3, 6, 5, Hypertensive
3, 6, 6, Normotensive
4, 6, 1, Hypertensive
5, 6, 3, Hypertensive
5, 6, 4, Hypertensive
5, 6, 5, Normotensive
5, 6, 6, Hypertensive
6, 4, 1, Hypertensive
6, 4, 2, Normotensive
6, 4, 3, Normotensive
6, 4, 4, Hypertensive
8, 4, 4, Normotensive
9, 6, 1, Hypertensive
9, 6, 2, Normotensive
9, 6, 3, Normotensive
9, 6, 4, Hypertensive
9, 6, 5, Normotensive
9, 6, 6, Hypertensive

3.4.1.4 Eligibility criteria

3.4.1.4.1 Inclusion criteria

Study participants were only recruited after verbally consenting and signing a consent form. The study cohort included all adults (aged 18 and above) attending the medical clinic for both general clinics and ART and thus, were allocated groups as described below.

1. HIV+ hypertensive on ART
2. HIV+ normotensives on ART
3. HIV negative with hypertension
4. HIV negative normotensive individuals (these served as control)

All HIV positive patients were HIV-1 (subtype) positive and tested negative for HIV-2. Hypertension was defined as systolic and diastolic BP of equal to or more than 140 and 90 mmHg respectively. Medical history on the use of antihypertensive medication in addition to BP readings on record were used to diagnose hypertension.

3.4.1.4.2 Exclusion criteria

Participants in the group with hypertension were excluded if found to have diabetes mellitus, a record of alcohol consumption, and smoking. In the HIV positive group, those with existing and recent past opportunistic infections such as syphilis infection and tuberculosis infection were excluded. Sick patients or clients seeking healthcare due to an illness rather than routine ART clinic reviews were also excluded.

In all groups, participants who had other chronic conditions such as cancer or communicable conditions such as Hepatitis C and B virus, and those mentioned above in the individual groups were excluded from the study by reviewing files, inquiring from the participants (self-report), and through laboratory testing.

3.4.1.5 Sample size

An inflammatory marker (TNF-α) with the minimal detectable difference between hypertensives versus controls (Mirhafez et al., 2014) of 0.16 and a standard deviation difference of 0.18 was used.

In this study (Mirhafez et al., 2014), the response within each subject group was normally distributed with a standard deviation of 0.18. If the true difference in the exposure and control means was 0.16, we would need to study 21 hypertensive subjects and 21 control subjects to be able to reject the null hypothesis that the population means of the exposed and control groups are equal with probability (power) 0.8. The Type I error probability associated with this test of this null hypothesis is 0.05. DuPont's software, PS (Dupont, 1998) was used to calculate the sample size for this group as shown in Figure 8:

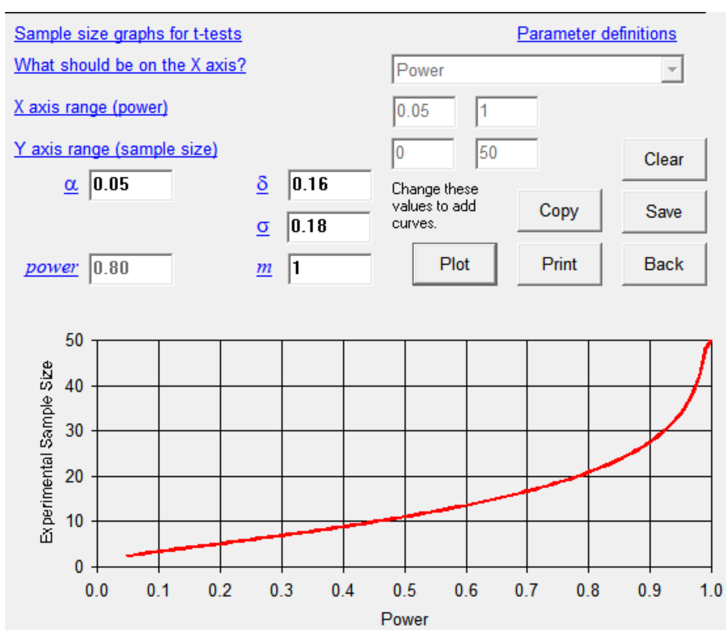


Figure 8. Sample size graphing using PS software for the HIV negative group
α, type I error probability for a two sided test (0.05); δ, difference in population means (0.16); σ is the within or between group standard deviation (0.18); m is the ratio of control to experimental patients.

For the HIV positive group, due to non-available information that specifically provides data on TNF-α levels between hypertensives and normotensives, based on the assumption that HIV infection has a double increasing effect on TNF-α levels comparing with the HIV negative. This was derived from murine model experiments and the few experiments performed in the laboratory at Vanderbilt, USA. So, the minimal detectable differences between hypertensives versus normotensives in HIV expected was 0.32 with a standard deviation of 0.36, and thus, a sample size of 21 per group using DuPont's software as shown in Figure 9 was computed.

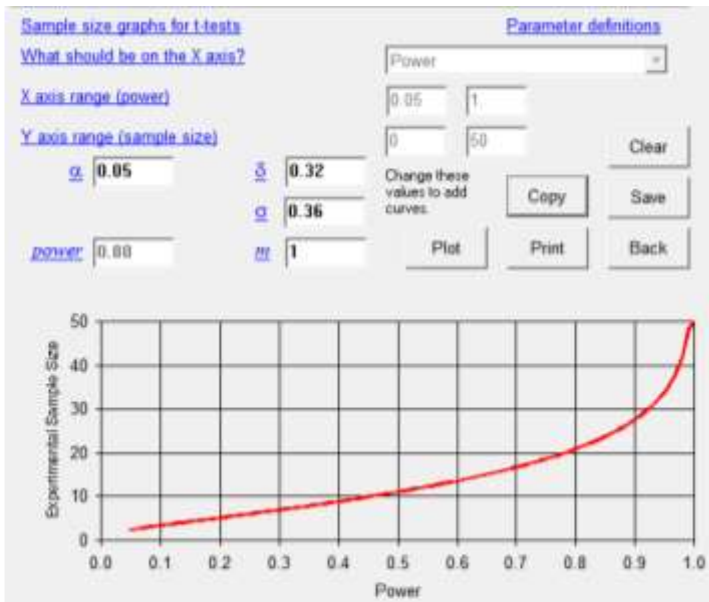


Figure 9. Sample size 2 graphing using PS software for the HIV positive group
α, type I error probability for a two sided test (0.05); δ, difference in population means (0.32); σ is the within or between group standard deviation (0.36); m is the ratio of control to experimental patients.

Below are the groups alongside the sample size required for each.

No.	**Group**	**Sample size**
1	HIV positive without HTN	21
2	HIV positive with HTN	21
3	HIV Negative control without HTN	21
4	HIV Negative control with HTN	21
	Total sample size	**84**

3.4.1.6 Testing Concepts and Rationale

The objective in this Sub-cohort was to determine the relationship between hypertension and inflammation in PWH and HIV negative individuals. To achieve this objective, a series of experiments were performed to evaluate the isolated inflammatory cells and the cytokines they produce in the supernatant between hypertensive and normotensive persons. Generally, a comparison was made of ant- (IL-10, IL-4) & pro-inflammatory markers (IsoLGs, IL-2, IL-9, IL-13, IL-17A, IL-17F, IL-21, IL-22, IL-6, TNF-α, IFN-γ) of PBMCs produced by HIV positive hypertension group, HIV positive without hypertension group, hypertensive persons without HIV, and normotensive HIV negative individuals that served as controls. This was followed by determination for the relationship between inflammatory markers and exposure variables (HIV status, ART duration, age, sex, BMI, waist circumference etc.) between groups.

For the determination of cytokine production, the LEGENDplex™ Human Inflammation Panel (13-plex) #740722 (Biolegend) was used. The panel has 13 cytokines namely: IL-2, IL-4, IL-5, IFN-γ, TNF-α, IL-6, IL-9, IL-10, IL-13, IL-17A, IL-17F, IL-21, and IL-22. Refer to Appendix A for the full protocol. Briefly, activated samples were added to the V-bottom plate along with matrix B, assay buffer, diluted standard and mixed beads in their appropriate wells. The plate was then incubated for two (2) hours on a shaker at room temperature. Then the supernatants were removed and detection antibodies were added to all wells and then the plate was incubated for one (1) hour at room temperature on a shaker. After the incubation period, streptavidin-phycoerythrin was added to the wells and the plate was then incubated for thirty (30) minutes at room temperature on the shaker after which, the plate was washed with a wash buffer and samples were immediately analysed on a flow cytometer (BD FACSCalibur).

As opposed to studies examining plasma cytokine levels, for this type of study that assesses cytokine production of PBMCs in cell culture supernatant, the reference range used is that of the health controls as shown in the figure legends (Figure 15 and 16). The median and interquartile range data for health normotensive controls is shown in appendix C together with data from the patients.

Oxygen saturation using oxillometric technology was used to measure hemoglobin oxygen saturation levels. Expected normal levels range from 95 to 100%.

3.4.1.6.1 Flow cytometry for Isolevuglandins (IsoLGs):

For IsoLGs detection, intracellular staining with the single-chain antibody D-11 to detect IsoLG-protein adducts was conducted. The D11 ScFv antibody was labeled with a fluorochrome using the APEXTM Alexa Fluor 488 Antibody Labeling kit (Invitrogen). Cells were then fixed and permeabilized for intracellular detection of IsoLGs using a cell permeabilization kit (Invitrogen).

Briefly, whole blood was suspended in Roswell Park Memorial Institute (RPMI) medium supplemented with 5% fetal bovine serum and stimulated with 2 µl of BD leukocyte activation cocktail (ionomycin and phorbol myristic acetate (PMA) along with the golgi inhibitor, brefeldin A) at 37°C for 6 hours. Surface staining was performed followed by intracellular staining. For each experiment, a gating on specific cell populations was performed aided by flow minus one (FMO) and compensation controls used for each fluorophore.

Flow cytometry data analysis was done using FlowJo software (Tree Star, Inc.). STATA version 15 and SPSS version 22 were used for statistical inferences and to determine if hypertension was associated with inflammation in HIV patients. To compare inflammation (inflammatory cells and cytokine quantification) between hypertensive and normotensive patients with or without HIV, a Wilcoxon rank-sum test was used as data was not approximately normally distributed and for comparison of more than two groups, Kruskal Wallis test was used.

3.4.1.7.1 Whole blood assays:

3.4.1.7.1.1 Unstimulated whole blood

4 *ml* of heparinized whole blood (WB) was drawn from participants and flow cytometry was perfomed to assess inflammatory markers that were activated.

3.4.1.7.1.2 stimulated whole blood

Heparinized WB was diluted in a 1:1 ratio in RPMI 1640 medium containing 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), and 2 mM of L-glutamine (Thermo Fisher Scientific). A cell activation cocktail (Biolegend USA) containing 40.5 µM of phorbol-12-myristate 13-acetate (PMA), ionomycin (669.3 µM), and Brefeldin A (2.5 mg/ml) in dimethyl sulfoxide (DMSO) (500X) was added to each millilitre of cell suspension and incubated for 4-6 hours in 5% CO_2 at 37°C. Following this, red blood cell lysis buffer was added and incubated for 5 minutes at room temperature. The samples were then centrifuged at 3000 RPM at room temperature and the supernatant stored between 2-8 ^{0}C for cytokine measurement using the LEGENDplex™ kit. A Cell Staining Buffer (Biolegend USA) was then added and mixed with the cell pellet, then centrifuged at 3000 RPM room temperature for 1 minute and supernatant discarded and cell surface immunofluorescent staining performed.

3.4.1.7.1.2.1 Fixation

Cells were fixed in 0.5 ml/tube fixation buffer in the dark for 20 minutes at room temperature then centrifuged at 350 x g for 5 minutes and the supernatant discarded

3.4.1.7.1.2.2 Permeabilization

Fixed cells were resuspended in intracellular staining permeabilization wash buffer and centrifuge at 350 x g for 5-10 minutes. This step was repeated twice

3.4.1.7.1.2.3 Intracellular staining

Fixed/permeabilized cells were resuspended in residual intracellular staining perm wash buffer and a predetermined optimum concentration of fluorophore-conjugated antibody of interest (i.e. IsoLGs (D11) or negative control for 20 minutes in the dark at room temperature. The cells were then washed twice with intracellular staining permeabilization wash buffer and centrifuged at 350 x g for 5 minutes. The cells were then suspended in 0.5ml cell staining buffer and analyzed with appropriate controls.

3.4.1.7.1.3 Staining panels and control

Several panels for the experiments were used as illustrated in Table 1: Additionally, for compensation, Fluorescence minus one (FMO) control tubes and BD Calibrite beads were used (Table 2).

Table 1. Staining Panels and tube preparations (per patient)

Tube #		**FITC**	**PE**	**PerCP**
1	Unstained control	-	-	-
2	Monocytes	CD14	CD16	CD45
3	CD4 Cocktail	CD3	CD4	CD45
4	Activated leucocytes	CD80	CD86	CD45
5	Activated monocytes	CD80	CD16	CD14
6	IsoLG Panel$_1$	IsoLG	CD16	CD14
7	Activated monocyte panel	CD86	CD16	CD14
8	Eosinophil	CD45	Siglec-8	CD80

FITC, Fluorescein isothiocyanate; PE, Phycoerythrin; PerCP, Peridinin Chlorophyll Protein Complex

Table 2. Fluorescence minus one (FMO) control tubes (for monocytes only shown)

Tube #		**FITC**	**PE**	**PerCP**
5	**FITC negative control**	**-**	**CD16**	**CD45**
6	**PE negative control**	**CD14**	**-**	**CD45**
7	**PerCP negative control**	**CD14**	**CD16**	**-**

One sample per experiment used. FITC, Fluorescein isothiocyanate; PE, Phycoerythrin; PerCP, Peridinin Chlorophyll Protein Complex

3.4.1.7.2 IsoLG Preparation

The APEXTM Alexa Fluor 488 Antibody Labelling kit (Invitrogen) was used to prepare IsoLGs as follows: To the reactive dye (component A: Alexa fluor 488 5-SDP ester), 2 µL of DMSO (component D) was added and mixed by pipetting up and down to dissolve. 18 µL of labelling buffer (component E) was added and mixed appropriately. 20 µL of D11 (already prepared from Vanderbilt, US) was added and incubated at room temperature overnight (more than hours). The 200 µL of wash buffer (component C:0.1 PBS) was added and the reagent stored at 4^{0}C**.**

3.4.1.7.2.1 IsoLG-protein adduct detection in monocytes

Intracellular staining with the single-chain antibody D-11 to detect IsoLG protein adducts was used and analysed by flow cytometry. D-11 is a single-chain antibody that was developed by phage display screening of literally millions of single chains to identify one that reacts with IsoLGs adducted to any peptide backbone. The D11 ScFv antibody was labelled with a fluorochrome using the APEXTM Alexa Fluor 488 Antibody Labelling kit (Invitrogen). The cells labelled with surface antibodies were then fixed and permeabilized for intracellular detection of IsoLGs.

3.4.1.7.3 Gating strategy for cell populations and immune activation markers

On flow cytometry, cell populations were visualized using side-scatter and forward scatter. Side scatter versus CD45 marker was used to gate on leucocyte populations. In order to identify monocytes expressing IsoLG (D11). A gate was created on monocytes and a CD14 marker versus D11 was employed to identify monocyte expressing D11 (CD14+ D11+). To identify monocytes expressing CD86, a gate was created on monocytes visualized using side-scatter and CD14. Using CD86 versus CD14 visualization, activated monocytes were identified as CD14+ CD86+. For identification of eosinophils, a gate was created on leucocytes using side-scatter versus CD45 marker. A gate was created on the granulocyte population and using siglec-8 marker versus CD45, eosinophils were identified as CD45+ siglec-8+. To identify activated leucocytes expressing CD80, a gate was set on the leucocyte population using side-scatter versus

CD45 marker. Activated leucocytes were then identified using CD80 versus CD45 markers as cells expressing CD45+ CD80+.

3.4.2 Specific objective 2: Adiposity and Immune Activation Cohort (AIAC) and HIV-negative control cohorts

A paper has since been published (Masenga et al., 2020) on this data and appended at the end of the thesis (Appendix J).

3.4.2.1 Study design

This was a cross section analytical study

3.4.2.2 Study sample and setting

The study sample included 70 PWH from the Immune Activation Cohort (AIAC) which has been previously described (Bailin et al., 2018; Koethe et al., 2016a, 2016b) who were part of the clients enrolled into the ART services from the Vanderbilt Comprehensive Care Clinic beginning in the years 2013-2014.

3.4.2.3 Selection of participants and sampling methods

HIV positive participants were recruited from the Vanderbilt Comprehensive Care Center (VCCC). Study information sheets which briefly described the study was given to primary care providers. The provider discussed the study with the patients during routine clinic visits and invited them to participate. Eligibility was determined either by the patient's primary care provider prior to referral to the study, and/or by screening assessment administered by the principal investigator or a study nurse. Informed consent was obtained by research personnel (a research nurse, physician, or social worker) in one of the research clinic exam rooms prior to any screening procedures being performed.

3.4.2.4 Eligibility criteria

3.4.2.4.1 Inclusion criteria

Participants were included if they were older than 18 years, on ART for more than 2 years, with a CD4+ nadir of >100 cells/µl prior to starting ART, a CD4 >350 cells/µl at the time of enrollment, viral load less than 50 copies/ml (at visit closest to enrollment and within past 6 months). Both males and females were included but for females, only pre-menopausal females were included (This inclusion was for women only - due to effects of estrogen deficiency on T cells). All participants were on efavirenz, tenofovir, and emtricitabine (i.e., the combination pill Atripla) for at least the 6 months prior to enrollment, and had been on ART with persistent HIV-1 RNA measurements <50 copies/ml for at least the previous 2 years

3.4.2.4.2 Exclusion criteria

All eligible participants were excluded if diabetic, current use of anti-diabetic medications or statins., with history of acute coronary events, stroke, known rheumatologic or inflammatory conditions (aside from HIV), or concomitant comorbidities that might modify eosinophil counts (allergic rhinitis, asthma, dermatoses and parasitic disease) and other inflammatory cells of the innate immune system. Pregnant women were also excluded (confirmed with serum pregnancy screening prior to any study interventions).

3.4.2.5 Control cohorts settings

A cohort of 50 HIV-negative persons, also from Vanderbilt University Medical Center (VUMC), served as controls. Data on this cohort were obtained by review of medical records of volunteers who provided consent for participation in research conducted at the Division of Clinical Pharmacology. Data on normotensive and hypertensive HIV-negative persons with available differential counts of white blood cells (WBCs) were collected eliminating participants with comorbidities known to modify eosinophil counts as stated above. Each subject could have one or more eosinophil counts over time (mean 3.9 ±0.4 x 10^3µl, median 3 x 10^3µl). If counts were >1, values were averaged.

As an additional HIV-negative control cohort, individuals of interest were sought in the Synthetic Derivative, a VUMC database of ~2.5×10^6 de-identified electronic medical records. The search strategy consisted of identifying subjects without HIV/AIDS and putting them into two groups namely, hypertension and normotensive using system diagnostic codes. Only subjects containing one or more datapoints on eosinophil counts and BMIs were employed. All persons with diseases known to produce eosinophilia, including but not limited to respiratory and cutaneous allergic disorders, hematologic malignancies, some infections (such as helminths, ascariasis, and trichinosis), and collagen vascular disorders were excluded. Those below 30 years and above 60 were also excluded to match the AIAC cohort. For subjects with more than one measurement of eosinophils or BMI, their values were averaged.

A systematic study was also performed, comprising of cohorts from Sub-Saharan Africa (Kenya, Tanzania, South Africa, Uganda, Senegal, Cameroon, Nigeria, Zimbabwe, Botswana, Zambia, Malawi, Burkina Faso, Republic of Congo), European (Norway) and United states to identify and explore the possible mechanistic interaction between inflammatory and immune activation markers and hypertension in PWH. Studies included were only those reporting HIV, hypertension and inflammation. Search strategies using Boolean terms was used. When comparing findings to other authors, consideration was made for how the results of other studies might be affected by factors such as methodological differences between studies, or variable characteristics of the populations studied or interventions investigated. Conceptual models were used to explore relationships and patterns in the studies so as to provide a simple schematic understanding of the findings. Findings from other studies were evaluated for the quality of the evidence in a thorough and consistent manner using the GRADE approach (Guyatt et al., 2011a, 2011b, 2011c, 2011d). For the criteria used to assess the internal validity of included studies in the systematic study, the Cochrane Risk of Bias tool (Cochrane, 2017) was used. Attention was paid to only certain parts such as completeness of outcome data, selective outcome reporting, reporting bias and any other bias that may affect the exposure-outcome relationship. A narrative synthesis (Thematic or content analysis), that includes investigation of the similarities and the differences between the findings of different studies, as well as exploration of patterns in the data were employed. A published paper (Masenga et al., 2019b) on this study is found in Appendix J.

3.4.2.6 Sample size estimation

From preliminary data, the assumed adjusted correlation coefficients of inflammatory markers was 0.42 after controlling for sex, race, smoking status, CD4+ T cell count, and protease inhibitor usage. With 70 participants there was greater than 90% power to detect a significant association between inflammatory cytokines assuming the relationship in individuals was similar (nQuery Advisor 4.0).

3.4.2.7 Clinical parameters

Use of antihypertensive medication at the time of the study visit and/or >2 sequential outpatient systolic BP measurements >140 mmHg preceding the visit were used to classify hypertension status.

3.4.2.8 Inflammatory biomarker assessment

Venous blood was drawn in the morning between 8 and 11 am and after a minimum of an 8 hour fast. Samples were collected in an EDTA-containing vacutainer, centrifuged at 3000 RPM for 10 minutes at 4°C, and the plasma removed and immediately frozen at -80°C. Plasma levels of soluble CD14 (sCD14) and soluble CD163 (sCD163) and two surface markers released into circulation by activated macrophages, were measured using ELISA (R&D Systems, Minneapolis, MN). Other plasma cytokines and immune biomarkers including interleukins, monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1 α and β (MIP-1α/β), tumor necrosis factor-α (TNF-α), and soluble TNF-α receptors 1 and 2 (sTNFR1 and sTNFR2), vascular cell adhesion molecule-1 (VCAM-1) and intracellular adhesion molecule-1 (ICAM-1), were measured in duplicate using a standard multiple immunoassay panel (MesoScale, Rockville, MD).

3.4.2.9 Flow Cytometry

Peripheral blood mononuclear cells (PBMCs) were obtained from fasting whole blood samples as previously described (Koethe et al., 2016a). Flow cytometry was performed using the Fortessa (Becton Dickson Biosciences) flow cytometer to measure activated, senescent, exhausted and memory T cell subsets using previously described fluorochrome panels (Bailin et al., 2018). See section 4.2.2.1 Figure 20 for the gating panels.

3.4.2.10 Eosinophil Counts:

Eosinophil counts and percentages were obtained in normotensive and hypertensive participants with and without HIV from automated differential cell counts performed in the VUMC Clinical Laboratory using Coulter plus IV three instruments.

3.4.2.11 Assessment of fat mass composition

A full-body DEXA (GE Lunar Prodigy; GE Healthcare, Little Chalfont, United Kingdom) measured total body fat mass to calculate fat mass index (FMI; total fat in kilograms divided by height in meters, squared). FMI is a variant of body mass index (BMI) that accounts for individual variability in the ratio of fat to lean mass (VanItallie et al., 1990).

3.4.2.12 Statistical analyses:

Assessement of the normality of data was done using kurtosis and skewness values as well as graphing using Q-Q plots. Medians and interquartile ranges were calculated for continuous variables and percentages for categorical variables. Demographic, clinical characteristics and the outcome variables were compared between hypertensives and normotensives using Mann-Whitney U test or chi-square tests as appropriate. Statistical analysis in R software was conducted (www.r-project.com). Logistic regression was performed to analyze the association between hypertension and inflammation markers, with adjustment of age, gender, and FMI/BMI for the HIV-positive and age, gender and BMI for the HIV-negative participants. Log_2 transformation was performed on variables with highly skewed data distribution to aid visualization. Multiple Imputation was performed to impute missing data with Hmisc package in R (cran.r-project.org/package=Hmisc). No adjustments were made for multiple comparisons for this exploratory study, though results and the potential for false discovery was interpreted in the context of known biological pathways (Savitz and Olshan, 1995). A p-value of 0.05 was used to infer statistical significance.

3.5.0 Methods for the HIV immune-activation salt-sensitive hypertension study

3.5.1 Specific objective 3 Sub-cohort 3: The salt study

3.5.1.1 Study design

This was an interventional study.

3.5.1.2 Study site, population and sampling

This study was conducted at the Livingstone Central Hospital ART clinic. The study participants were sampled from the 84 inflammation and hypertension cohort (section 3.4.1). The study comprised HIV positive patients attending ART and HIV negative controls from volunteer health workers matched for age and sex. Samples were randomly selected from the HIV positive group and controls were conveniently selected to enable matching for age and sex.

3.5.1.3 Sample size estimation

A G*Power (Faul et al., 2009, 2007) version 3.1.9.4 to calculate sample size for each group was used. The input assumptions are detailed in Table 3 and graphed in Figure 10.

Table 3. Sample Size and assumptions for sub-cohort 3

F tests using MANOVA for repeated measures, within-between interaction		
Options	Pillai V, O'Brien-Shieh Algorithm	
Analysis	A priori: Compute sample Size	
Input	Effect size f(v)	1
	α err prob	0.05
	Power (1-β err prob)	0.80
	Number of groups	4
	Number of measurements	3
Output	Noncentrality parameter λ	22
	Critical F	2.8477260
	Numerator df	6.0
	Denominator df	14.0
	Sample size	11 per group
	Lost to follow (10%)	1 per group
	Total sample size	**48**
	Actual power	0.825
	Pillai V	1.0

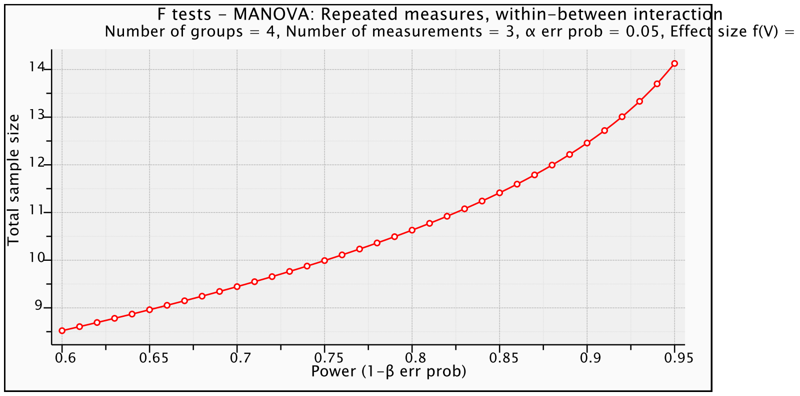


Figure 10. Power and sample size graphing using G* power for each group of sub-cohort 3
Each level of power shows the corresponding sample size

3.5.1.4 Testing Concepts and Rationale

The objective of the study was to quantify and compare the effect of excess dietary salt on immune cell activation. A full protocol is available and published for this aim (Masenga et al., 2019a).

3.5.1.5 Salt sensitivity analysis

This was a 3 weeks intervention study where participants received controlled pre-measured dietary salt in tablet form. Each sodium chloride tablet (research consolidated midland corporation division, New York, USA) was 1 gram containing 394 mg of sodium. In the first week (7 days), they were requested not to put salt in their food as well as to avoid consuming processed food such as ready-made soup powders, processed spices, bread, peanut butter, and all foods containing sodium. They recorded their daily diet using a 24-hour recall form (see appendix H). In the second week, they were given the WHO/AHA recommended low salt (4 g sodium chloride/day = 1, 576 mg sodium/day), and in the third week, high salt (9 g sodium chloride/day = 3,546 mg of sodium/day). The BP changes for low salt were calculated (Elijovich et al., 2016) as BP on days 5 to 7 of low salt minus baseline as well as average BPs on the last day of each phase. Those for high salt (9 g/day) were calculated as BP after high salt minus that after low salt. BP was monitored 24hrs each day using an ambulatory BP monitoring device.

Several experiments were conducted to evaluate the effect of high dietary salt intake on plasma inflammatory cytokine levels. IsoLGs and inflammatory markers such as IL-17A, IL-6, TNF-α, IFN-γ secreted by PBMCs from HIV positive patients with hypertension, HIV positive patients without hypertension, hypertensive persons without HIV, and controls (normotensive HIV negative individuals) were quantified. This was followed by the determination of the relationship between excess dietary salt and IsoLGs, inflammatory markers such as IL-17A, IL-6, TNF-α, IFN-γ and immune activation markers (CD80, CD86) in the four groups.

High salt exposure in HIV negative populations and murine models activates monocyte-dendritic cells via increased production of immunogenic IsoLGs in monocyte/DCs leading to the accumulation of IsoLG-protein adducts (Kirabo et al., 2014) which was associated with DC production of IL-6, IL-1β, and IL-23 and an increase in costimulatory proteins CD80 and CD86. These activated DCs package IsoLGs in MHC molecules and present them to T cells, particularly CD8+ T cells, promoting their proliferation and promoting the production of IFN-γ and IL-17A and hypertension. As this concept was not yet tested in HIV patients with an existing inflammatory milieu that may favour such developments, therefore, the study measured IsoLG production and inflammatory cytokines of participants on low and high dietary salt using the LEGENDplex™ Human Inflammation Panel kit. All laboratory methods were the same with those in section 3.4.1.6.1, 3.4.1.7.2 and 3.4.1.7.3.

Sodium excretion in urine samples was collected and analyzed over 24 hour period as previously described (Rakova et al., 2013). Briefly, a 24-hour urine was collected by participants and transported to the laboratory were electrolytes were measured. Average sodium excretion was calculated and used as a surrogate of dietary intake. Although generally, sodium excretion exhibits circaseptan rhythm and infradian rhythm depending on salt intake, increases or decreases in intake affects blood pressure and usually, a 24-hour urine recovers about 95% of dietary salt intake.

3.5.1.6 Data analysis

Descriptive analyses were used to obtain frequency, median and interquartile range for independent variables across all dependent variables depending on the normality of data. Shapiro-Wilk's test was also used to numerically determine further normality of data. In SPSS, a Shapiro-Wilk's significant (p-value) value > 0.05 was an indication that data is approximately normally distributed (Shapiro and Wilk, 1965). Kurtosis and skewness values with their accompanying standard errors (SE) were obtained and used to calculate normality by dividing for example skewness value by its SE. A value within the critical value ± 1.96 was considered approximately normally distributed.

3.5.1.6.1 Inferential statistical analysis

The collected data was compiled, entered, cleaned and coded then analysed using GraphPad prism 8.3, SPSS version 22 and Flowjo software. Tables and graphs were used to illustrate the results. Non-parametric tests such as Wilcoxon rank-sum test and Kruskal-Wallis were used as briefly stated below:

In the first instance, to compare cell marker expression i.e. the amount of IsoLGs formed between hypertensives and normotensives, Wilcoxon rank-sum test was used to compare medians between two groups while Kruskal-Wallis test was used to compare all four groups. To correct for multiple comparisons, Dunn's test was used for pairwise comparisons between all available pairs as a posthoc test for Kruskal-Wallis. The same statistical tests were used when comparing all other variables. To enable comparison between the relations of two risk factor continuous variables at once such as inflammatory cytokines, spearman correlation was employed to indicate the degree to which two quantitative variables were linearly related.

Logistic regression models were used to examine the relationship between outcome variables (hypertension etc) and selected determinant factors such as age, sex, physical activity, duration on ART, as well as to determine the impact of each variable (univariate) on the outcomes. To control for confounding, multivariate models were used.

3.5.1.6.2 Data analysis interpretation

The interpretation of results was based on calculated statistical analysis significance levels and clinical (biological) significance coupled with recent studies in the literature. The comparison of inflammatory cytokine levels between groups was based on the amount of cytokines secreted by activated cells after they were stimulated by PMA. This was to test the hypothesis that cells from hypertensive participants produce more inflammatory cells as compared to cells from normotensive individuals when stimulated. This was the basis for ‘statistical significance’ where as ‘biological significance’ was also assessed based on the statistically significant effect that has a noteworthy impact on health. This takes into consideration expected reference ranges and time frame. The study findings were compared with the global literature to be able to determine the impact, mechanistic links and significance of hypertension.

4.0 CHAPTER FOUR: RESULTS

4.1.0 Risk factors and inflammatory surrogate study

4.1.0.1 Specific Objective one

To determine the risk factors and inflammatory surrogates associated with hypertension in HIV positive adults in relation to the American Heart Association hypertension diagnostic criteria.

4.1.1 Cohort 1

4.1.1.1 General characteristics of participants

The prevalence of hypertension in the study population, based on the new AHA/ACC criteria (Table 4) and JNC 7 criteria (Table 5) was 42% and 16% respectively. The age range was 18 to 88 years old and 66% (149) of the total 226 participants were female. All participants were HIV positive.

Increasing age and employment status ($p<0.01$) were associated with hypertension in both the new AHA/ACC and JNC 7 diagnostic criteria (Table 4 and 5) while marital status ($p=0.001$) was only associated with hypertension in the new diagnostic criteria (Table 4). Highest education attained was associated with hypertension in the JNC 7 diagnostic criteria as shown in Table 5.

Table 4. Social-demographic characteristics associated with hypertension using New AHA/ACC diagnostic criteria

Variables	Normotensive n(%) 131 (58.0)	Hypertensive n(%), 95 (42.0%)	p-value
Age, *median years (IQR)*	40 (30, 46)	50 (42, 57)	**<0.001**
Age category (years)			
18 - 35	49 (37.4)	8 (8.4)	**<0.001**
36 - 45	45 (34.4)	28 (29.5)	
46 – 55	30 (22.9)	30 (31.6)	
56 – 65	6 (4.6)	25 (26.3)	
66 - 90	1 (0.8)	4 (4.2)	
Gender, n(%)			
Female	89 (67.9)	60 (63.2)	0.454
Male	42 (32.1)	35 (36.8)	
Marital status			
Married	57 (43.5)	44 (46.3)	**0.001**
Widowed	26 (19.8)	37 (38.9)	
Single	38 (29.0)	11 (11.6)	
Divorced	10 (7.6)	3 (3.2)	
Employment Status			
GRZ/Private	28 (21.4)	30 (31.6)	**<0.001**
Self employed	51 (38.9)	24 (25.3)	
Retired	3 (2.3)	17 (17.9)	
Unemployed	49 (37.4)	24 (25.3)	
Highest Education attained			
No formal education	5 (3.8)	4 (4.2)	0.339
Primary	28 (21.4)	20 (21.1)	
Secondary	76 (58.0)	46 (48.4)	
Tertiary	22 (16.8)	25 (26.3)	

Mann-Whitney and Chisquare test used for continuous and categorical response variables respectively. IQR, interquartile range; GRZ, government; n, number of participants; %, percentage

Table 5. Social-demographic characteristics associated with hypertension using previous JNC 7 diagnostic criteria

Variables	**Normotensive** n(%) 191 (84.5)	**Hypertensive** n(%), 35 (15.5%)	**p-value**
Age, *median years (IQR)*	42 (35, 49)	56 (44, 60)	**<0.001**
Age category (years)			
18 - 35	55 (28.8)	2 (25.20	**<0.001**
36 - 45	63 (33.0)	10 (32.3)	
46 – 55	52 (27.2)	8 (26.5)	
56 – 65	18 (9.4)	13 (13.7)	
66 - 90	3 (1.6)	2 (2.2)	
Gender, n(%)			
Female	128 (67.0)	21 (60.0)	0.421
Male	63 (33.0)	14 (40.00	
Marital status			
Married	87 (45.5)	14 (13.9)	0.06
Widowed	48 (25.1)	15 (42.9)	
Single	46 (24.1)	3 (8.6)	
Divorced	10 (5.2)	3 (8.6)	
Employment Status			
GRZ/Private	48 (25.1)	10 (28.6)	**0.001**
Self employed	67 (35.1)	8 (22.9)	
Retired	11 (5.8)	9 (25.7)	
Unemployed	65 (34.0)	8 (22.9)	
Highest Education attained			
No formal education	7 (3.7)	2 (5.7)	**0.018**
Primary	43 (22.5)	5 (14.3)	
Secondary	108 (56.5)	14 (40.0)	
Tertiary	33 (17.3)	14 (40.0)	

Mann-Whitney and Chisquare test used for continuous and categorical response variables respectively. IQR, interquartile range; GRZ, government; n, number of participants; %, percentage.

Daily physical activity (p=0.018), addition of salt at the table (p<0.001) and processed salt intake (p=0.027) were associated with hypertension using using the new AHA/ACC criteria (Table 6) while the rest of the dietary and types of physical activity factors such as smoking, alcohol intake, addition of salt while cooking were not associated with hypertension (Table 6).

Table 6. Dietary and lifestyle factors associated with Hypertension using new AHA/ACC criteria

Variables	**Normotensive** n(%) 131 (58.0)	**Hypertensive** n(%), 95 (42.0%)	**p-value**
Daily physical activity			
Yes	69 (52.7)	35 (36.8)	**0.018**
No	62 (47.3)	60 (63.2)	
Minutes of Weekly vigorous activity, *median (IQR)*	0 (0, 120)	0 (0, 120)	0.893
Minutes of Weekly moderate activity, *median (IQR)*	60 (30, 180)	20 (0, 120)	0.172
Minutes of Weekly walking activity, *median (IQR)*	55 (23, 120)	60 (20, 120)	0.221
Minutes spent seated every weekend, *median (IQR)*	300 (120, 383)	300 (180, 420)	0.327
Minutes spend seated every weekday, *median (IQR)*	180 (120, 360)	240 (120, 360)	0.257
Days of weekly fruit intake, *median (IQR)*	1 (0, 2)	1 (0, 3)	0.490
Days of weekly vegetable intake, *median (IQR)*	7 (5, 7)	7 (7, 7)	0.429
Addition of salt at the table			
Never or rarely	42 (32.1)	11 (11.6)	**<0.001**
Sometimes or always	89 (67.9)	84 (88.4)	
Addition of salt while cooking			
Never	0 (0.0)	1 (1.1)	0.108
Sometimes	1 (0.8)	4 (4.2)	
always	130 (99.2)	90 (94.7)	
Processed salt intake			
Never	21 (16.0)	26 (27.4)	**0.027**
Rarely	26 (19.8)	25 (26.3)	
Sometimes	74 (56.5)	42 (44.2)	
always	10 (7.6)	2 (2.1)	
Alcohol consumption			
No	98 (74.8)	81 (85.3)	0.056
Yes	33 (25.2)	14 (14.7)	
Former smoker			
No	113 (86.3)	81 (85.3)	0.832
Yes	18 (13.7)	14 (14.7)	

Mann-Whitney and Chisquare test used for continuous and categorical response variables respectively. IQR, interquartile range.

In the JNC criteria, minutes of weekly moderate walking activity (p=0.006) and addition of salt while cooking (p=0.001) were associated with hypertension (Table 7) while daily physical activity, minutes spent walking and sitting, weekly fruit intake, weekly vigorous activity, vegetable intake, addition of salt at the table, processed salt intake, alcohol consumption and smoking were not associated with hypertension.

Table 7. Dietary and lifestyle factors associated with Hypertension using JNC 7 criteria

Variables	**Normotensive** n(%) 191 (84.5)	**Hypertensive** n(%), 35 (15.5%)	**p-value**
Daily physical activity			
Yes	92 (48.2)	12 (34.3)	0.130
No	99 51.8)	23 (65.7)	
Minutes of Weekly vigorous activity, *median (IQR)*	0 (0, 112)	0 (0, 120)	0.414
Minutes of Weekly moderate activity, *median (IQR)*	60 (10, 180)	15 (0, 60)	**0.006**
Minutes of Weekly walking activity, *median (IQR)*	60 (20, 120)	60 (20, 150)	0.518
Minutes spent seated every weekend, *median (IQR)*	300 (165, 382)	360 (60, 420)	0.050
Minutes spend seated every weekday, *median (IQR)*	210 (120, 345)	180 (60, 420)	0.058
Days of weekly fruit intake, *median (IQR)*	1 (0, 3)	1 (0, 3)	0.302
Days of weekly vegetable intake, *median (IQR)*	7 (6,7)	7 (5, 7)	0.486
Addition of salt at the table			
Never or rarely	42 (22.0)	11 (31.4)	0.226
Sometimes or always	149 (78.0)	24 (68.6)	
Addition of salt while cooking			
Never	0 (0.0)	1 (2.9)	**0.001**
Sometimes	2 (1.0)	3 (8.6)	
always	189 (99.0)	31 (88.6)	
Processed salt intake			
Never	37 (19.4)	10 (28.6)	0.487
Rarely	42 (22.0)	9 (25.7)	
Sometimes	102 (53.4)	14 (40.0)	
always	10 (5.2)	2 (5.7)	
Alcohol consumption			
No	150 (78.5)	29 (82.9)	0.562
Yes	41 (21.5)	6 (17.1)	
Former smoker			
No	166 (86.9)	28 (80.0)	0.281
Yes	25 (13.1)	7 (20.0)	

Mann-Whitney and Chisquare test used for continuous and categorical response variables respectively. IQR, interquartile range.

Increased BMI, increased WC, longer duration on ART, high pulse pressure,high mean arterial pressure, high mid-BP, high fasting blood sugar, higher diabetes risk scores and risk category were associated with hypertension ($p<0.05$) in the new AHA/ACC criteria (Table 8). The specific ART regimen, pulse, CD4 count and HIVRNA viral load were not associated with hypertension. Of all hypertensives based on the new AHA/ACC criteria, only 33% were using antihypertensives but non of the persons on antihypertenives had controlled BP (Table 8).

Table 8. Clinical factors associated with Hypertension using new AHA/ACC criteria

Variables	**Normotensive** n(%) 131 (58.0)	**Hypertensive** n(%), 95 (42.0%)	**p-value**
Body mass index, kg/m^2	20.3 (18.2, 23.8)	24.2 (21.1, 27.7)	**0.002**
Body mass index, kg/m^2			
<18.5	24 (18.3)	7 (7.4)	**0.001**
18.5 – 24.9	78 (59.5)	45 (47.4)	
25.0 – 29.9	18 (13.7)	25 (26.3)	
≥30	11 (8.4)	18 (18.9)	
Waist circumference, *cm*	73 (69, 83)	85 (76, 95)	**<0.001**
Waist circumference category, *cm*			
<94 men or < 80 women	94 (71.8)	43 (45.3)	**<0.001**
94-102 men or 80-88 women	20 (15.3)	19 (20.0)	
>102 men or >88 women	17 (13.0)	33 (34.7)	
Duration on ART, *months*	102 (51, 129)	108 (72, 144)	**0.008**
ART regimen (n, %)	115 (57.5)	85 (42.5)	
TDF/3TC/EFV	83 (72.2)	59 (69.4)	0.420
TDF/3TC/NVP	8 (7.0)	11 (12.9)	
TDF/3TC/LPV/r	8 (7.0)	7 (8.2)	
ABC/3TC/NVP	1 (0.9)	0 (0.0)	
ABC/3TC/EFV	2 (1.7)	3 (3.5)	
AZT/3TC/LPV/r	4 (3.5)	3 (3.5)	
AZT/3TC/NVP	9 (7.8)	2 (2.4)	
Using Antihypertensive	*Not applicable*	31 (32.6%)	
BP uncontrolled with antihypertensive	*Not applicable*	31 (100)	
Pulse, *beats per minute*	73 (63, 79)	70 (60, 80)	0.300
Pulse pressure, *mmHg*	42 (38, 46)	55 (45, 67)	**<0.001**
Mean arterial pressure. *mmHg*	88 (82, 93)	110 (102, 115)	**<0.001**
Mid BP, *mmHg*	94 (90, 101)	120 (108, 127)	**<0.001**
CD4 Count, *cells/µL*	487 (387, 672)	447 (305, 630)	0.678
HIV RNA Viral Load, *copies per ml*	48 (20, 445)	20 (20, 290)	0.143
Fasting blood sugar, mmol/l	4.9 (4.4, 5.3)	5.2 (4.8, 6.0)	**0.007**
Diabetes Risk scores	3 (1, 7)	7 (4, 10)	**<0.001**
Diabetes risk category			
Low risk (<7)	93 (71.0)	42 (44.2)	**<0.001**
Slightly elevated risk (7-11)	32 (24.4)	35 (36.8)	
Moderate (12-14	5 (3.8)	12 (12.6)	
High (15-20)	1 (0.8)	6 (6.3)	

Mann-Whitney and Chisquare test used for continuous and categorical response variables respectively. ART, antiretroviral therapy; BP, blood pressure; TDF, tenofovir disoproxil fumarate ; 3TC, Lamivudine ; EFV, efavirenz ; NVP, nevirapine ; LPV/r, lopinavir/ritonavir ; ABC, abacavir ; AZT, azidothymidine, also called zidovudine; Antihypertensive drugs used: Calcium channel blockers (Amlodipine, nifedipine); Angiotensin-converting enzyme inhibitors (ACE) (Enalapril, Losartan); Diuretics (Furosemide, Moduretic).

As shown in Table 9, BMI, WC, pulse pressure, mean arterial pressure, mid-BP, diabetes risk scores and risk category were associated with hypertension based on the JNC 7 criteria ($p<0.05$). Duration on ART, specific ART regimen, pulse, CD4 count, HIV RNA viral load and fasting blood sugar were not associated with hypertension. While 89% of hypertensive were on antihypertensive medication, 94% of them had uncontrolled BP (Table 9).

Table 9. Clinical factors associated with Hypertension Using the JNC 7 criteria

Variables	**Normotensive** n(%) 191 (84.5)	**Hypertensive** n(%), 35 (15.5%)	**p-value**
Body mass index, kg/*m*^{2}	21.4 (19.3, 25.6)	56.0 (44.0, 60.0)	**0.010**
Body mass index category, kg/*m*^{2}			
<18.5	29 (15.2)	2 5.7)	**0.003**
18.5 – 24.9	106 (55.5)	17 (48.6)	
25.0 – 29.9	38 (19.9)	5 (14.3)	
≥30	18 (9.4)	11 (31.4)	
Waist circumference, *cm*	77.5 (70.3, 85.8)	95.0 (82.0, 100)	**<0.001**
Waist circumference category, *cm*			
<94 men or < 80 women	125 (65.4)	12 (34.3)	**<0.001**
94-102 men or 80-88 women	35 (18.3)	4 (11.4)	
>102 men or >88 women	31(16.2)	19 (54.3)	
Duration on ART, *months*	102 (63, 132)	120 (72, 156)	0.232
ART regimen (n, %)	171 (85.7)	29 (14.5)	
TDF/3TC/EFV	122 (71.3)	20 (69.0)	0.493
TDF/3TC/NVP	14 (8.2)	5 (17.2)	
TDF/3TC/LPV/r	12 (7.0)	3 (10.3)	
ABC/3TC/NVP	1 (0.6)	0 (0.0)	
ABC/3TC/EFV	5 (2.9)	0 (0.0)	
AZT/3TC/LPV/r	6 (3.5)	1 (3.4)	
AZT/3TC/NVP	11 (6.4)	0 (0.0)	
Using Antihypertensive	*Not applicable*	31 (88.6)	
BP uncontrolled with antihypertensive	*Not applicable*	29 (93.5)	
Pulse, *beats per minute*	73 (62, 79)	68 (60, 80)	0.204
Pulse pressure, *mmHg*	45 (39, 54)	51 (47, 79)	**<0.001**
Mean arterial pressure. *mmHg*	94 (86, 105)	115 (104, 134)	**<0.001**
Mid BP, *mmHg*	102 (93, 115)	126 (113, 146)	**<0.001**
CD4 Count, *cells/µL*	441 (351, 627)	630 (428, 728)	0.172
HIV RNA Viral Load, *copies per ml*	20 (20, 414)	20 (20, 100)	0.249
Fasting blood sugar, mmol/l	5.1 (4.6, 5.5)	5.3 (4.7, 5.7)	0.151
Diabetes Risk scores	5 (2, 7)	9 (6, 14)	**<0.001**
Diabetes risk category			
Low risk (<7)	126 (66.0)	9 (25.7)	**<0.001**
Slightly elevated risk (7-11)	55 (28.8)	12 (34.3)	
Moderate (12-14	9 (4.7)	8 (22.9)	
High (15-20)	1 (0.5)	6 (17.1)	

Mann-Whitney and Chisquare test used for continuous and categorical response variables respectively. ART, antiretroviral therapy; BP, blood pressure; TDF, tenofovir disoproxil fumarate; 3TC, Lamivudine ; EFV, efavirenz ; NVP, nevirapine ; LPV/r, lopinavir/ritonavir ; ABC, abacavir; AZT, azidothymidine, also called zidovudine; Antihypertensive drugs used: Calcium channel blockers (Amlodipine, nifedipine); Angiotensin converting enzyme inhibitors (ACE) (Enalapril, Losartan); Diuretics (Furosemide, moduretic). P values less than 0.05 are shown in bold.

Increasing age, BMI of 25 and above, higher fasting blood sugar using the new AHA/ACC criteria remained significantly associated with hypertension in multivariate logistic regression as shown in Table 10. Unemployment and table salt consumption were negatively associated with hypertension. Sex, WC, moderate physical activity, sedentary lifestyle, smoking status, alcohol consumption and duration on ART did not remain to be significantly associated with hypertension in multivariate analysis (Table 10).

Table 10. Factors associated with hypertension (new AHA/ACC criteria) in logistic regression

Variable	**Odds Ratio (OR) (95%CI)**	**p-value**	**Adjusted Odds Ratio AOR (95%CI)**	**p-value**
Sex				
Female	1		1	
Male	1.24 (0.71, 2.15)	**0.454**	1.14 (0.29, 4.48)	0.850
Age, *Years*	1.09 (1.06, 1.13)	**<0.001**	1.08 (1.03, 1.15)	**0.004**
Body mass index, kg/m^2				
<18.5	1		1	
18.5 – 24.9	1.97 (0.79, 4.95)	**0.145**	5.17 (0.96, 27.91)	0.056
25.0 – 29.9	4.76 (1.68, 13.43)	**0.003**	8.60 (1.07, 69.35)	**0.043**
≥30	5.61 (1.81, 17.32)	**0.003**	23.36 (1.44, 446.46)	**0.027**
Waist circumference category, *cm*				
<94 men or < 80 women	1		1	
94-102 men or 80-88 women	2.07 (1.01, 4.28)	**0.048**	0.56 (0.13, 2.48)	0.443
>102 men or >88 women	4.24 (2.13, 8.44)	**<0.001**	1.99 (0.27, 14.81)	0.503
Employment Status				
GRZ/Private	1		1	
Self employed	0.44 (0.22, 0.89)	**0.023**	0.33 (0.09, 1.23)	0.100
Retired	5.28 (1.39, 20.01)	**0.014**	2.37 (0.26, 21.18)	0.442
Unemployed	0.45 (0.22, 0.93)	**0.031**	0.19 (0.04, 0.95)	**0.043**
Minutes of moderate physical activity	1.00 (0.99, 1.00)	0.973	0.99 (0.99, 1.00)	0.218
Sedentary hours (sitting)	1.00 (1.00, 1.01)	**0.129**	1.00 (0.99, 1.00)	0.148
Smoking status				
No	1		1	
Yes	0.92 (0.43, 1.96)	**0.832**	1.99 (0.43, 9.30)	0.382
Table salt consumption				
Never or rarely	1		1	
Sometimes or always	0.27 (0.13, 0.57)	**0.001**	0.17 (0.04, 0.95)	**0.022**
Alcohol consumption				
No	1		1	
Yes	1.94 (0.97, 3.88)	**0.059**	3.54 (0.97, 12.87)	0.055
Fasting blood sugar, *mmol/l*	2.01 (1.28, 3.36)	**0.003**	2.07 (1.04, 4.13)	**0.038**
Duration on ART	1.01 (1.00, 1.01)	**0.033**	1.01 (1.00, 1.02)	0.05

ART, antiretroviral therapy; GRZ, government of Zambia

In the JNC 7 criteria WC above 94 cm for men and 80 cm for women and increased sedentary hours remained significantly associated with hypertension in multivariate logistic regression while increased minutes of moderate physical activity were negatively associated with hypertension as shown in Table 11. Sex , age, BMI, employment status, smoking, table salt consumption, alcohol consumption, fasting blood sugar and duration on ART were not siginificantly associated with hypertension (Table 11).

Table 11. Factors associated with hypertension (JNC 7 criteria) in logistic regression

Variable	**Odds Ratio (OR) (95%CI)**	**p-value**	**Adjusted Odds Ratio AOR (95%CI)**	**p-value**
Sex				
Female	1		1	
Male	1.35 (0.64, 2.84)	**0.422**	4.22 (0.35, 50.66)	0.255
Age, *Years*	1.08 (1.04, 1.12)	<0.001	1.05 (0.97, 1.13)	0.216
Body mass index, kg/m^2				
<18.5	1		1	
18.5 – 24.9	2.32 (0.51, 10.65)	**0.277**	1.49 (0.09, 24.87)	0.783
25.0 – 29.9	1.90 (0.34, 10.54)	**0.459**	0.04 (0.00, 5.85)	0.209
≥30	8.86 (1.75, 44.65)	**0.008**	0.23 (0.00, 28.82)	0.551
Waist circumference category, *cm*				
<94 men or < 80 women	1		1	
94-102 men or 80-88 women	1.19 (0.36, 3.92)	**0.774**	15.65 (1.23, 198.92)	**0.034**
>102 men or >88 women	6.38 (2.80, 14.53)	**<0.001**	289.84 (4.57, 18597.15)	**0.008**
Employment Status				
GRZ/Private	1		1	
Self employed	0.57 (0.21, 1.55)	**0.276**	1.38 (0.15, 12.10)	0.771
Retired	3.92 (1.29, 11.96)	**0.016**	6.63 (0.60, 73.89)	0.124
Unemployed	0.59 (0.21, 1.61)	**0.303**	0.50 (0.04, 6.61)	0.602
Minutes of moderate physical activity	0.99 (0.99, 1.00)	**0.063**	0.98 (0.97, 1.00)	**0.048**
Sedentary hours (sitting)	1.00 (1.00, 1.01)	**0.009**	1.00 (1.00, 1.01)	**0.021**
Smoking status				
No	1		1	
Yes	0.60 (0.24, 1.52)	**0.285**	1.97 (0.22, 17.36)	0.541
Table salt consumption				
Never or rarely	1		1	
Sometimes or always	1.62 (0.74, 3.58)	**0.229**	2.33 (0.37, 14.56)	0.365
Alcohol consumption				
No	1		1	
Yes	1.32 (0.51, 3.39)	**0.563**	1.51 (0.25, 8.98)	0.653
Fasting blood sugar	1.446 (0.81, 2.65)	**0.204**	0.83 (0.29, 2.40)	0.731
Duration on ART	1.00 (0.99, 1.01)	**0.399**	1.01 (0.99, 1.02)	0.412

ART, antiretroviral therapy; GRZ, government of Zambia

As shown in Table 12, using the new AHA/ACC criteria shifted 26% normotensive into hypertensive category. Comparison of the JNC7 with the new AHA/ACC criterions was prodded by the fact that lowering the systolic and diastolic values for the diagnosis of hypertension (AHA/ACC) was well established and critical for identification of risk factors. Usage of both criterions could identify more risk factors associated with hypertension and this information used for prevention and interventional purposes.

Table 12. Comparison of Blood pressure categories and status between JNC 7 and new AHA/ACC criteria

Category BP with JNC 7 criteria	**n(%)**	**Category BP with New ACC/AHA criteria**	**n(%)**	**Difference**
BP categories		**BP categories**		
Normal	94 (41.6)	Normal	84 (37.2)	10 (4.4)
Prehypertension	45 (19.9)	Elevated	47 (20.8)	-2 (-0.9)
Stage 1 hypertension	25 (11.1)	Stage 1 hypertension	31 (13.7)	-6 (-2.6)
Stage 2 hypertension	62 (27.4)	Stage 2 hypertension	64 (28.3)	-2 (-0.9)
Hypertension Status		**Hypertension Status**		
Normotensive	191 (84.5)	Normotensive	131 (58)	60 (26.5)
Hypertensive	35 (15.5)	Hypertensive	95 (42)	-60 (-26.5)
Total	226 (100)	Total	226 (100)	

AHA, American heart association; ACC, American college of cardiology; BP, blood pressure; JNC 7, Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure

One BP measurement taken by attending nurses compared to three averaged measurements re-taken were significantly different (Table 13). Systolic, diastolic BPs and pulse measurements taken once by nurses were significantly higher than the standard measurements ($p<0.0001$) with a strong positive correlation. Based on one measurement taken by attending nurses, 4% and 12% of participants would have been misclassified as hypertensive using the JNC 7 and new AHA/ACC criteria respectively (Table 13).

Table 13. Nurse BP measurement versus three averaged standard BP measurements

Variable	Nurses reading Median (IQR)	Standard measurement Median (IQR)	Median of difference 95% CI	rho	p-value
Systolic Blood pressure, *mmHg*	128 (115,144)	124 (113,141)	3 (1,5)	0.8	**<0.0001**
Diastolic blood pressure, *mmHg*	83 (75, 94)	80 (71, 89)	3 (1, 5)	0.7	**<0.0001**
Pulse, *beats per minute*	78 (69, 86)	72 (62, 80)	4 (2, 4)	0.7	**<0.0001**
Misclassified hypertensive using JNC 7 criteria, *n/total NT (%)*	7 /191 (3.7)				
Misclassified hypertensive using new AHA/ACC criteria, *n/total NT (%)*	23/191 (12.0)				

Rho, nonparametric Spearman correlation coefficient; NT, normotensive

4.1.2 Sub cohort 2: Inflammatory surrogate study

CD4 profile using flow cytometry and complete blood counts were performed using hydrodynamic focusing, and SLS haemoglobin method. Absorption photometry for biochemical testing and ion selective electrode for electrolyte profile was also done.

Innate inflammatory surrogate cells (neutrophils, lymphocytes, monocytes) were higher in hypertension compared to the normotensive individuals while CD4% was lower respectively. Hypertensives also had higher red blood cell counts compared to normotensive individuals (Figure 11). However, although the differences were statistically significant ($p<0.05$), the levels of these cells were within the expected range apart from nadir CD4 count, CD4% and erythrocyte sedimentation rate. The statistical, biological and clinical significance of these results are discussed in section 5.1.2.1. Expected values are elaborated in the legend of each figure. Additional supplemental data for the results in the figures are found in appendix C.

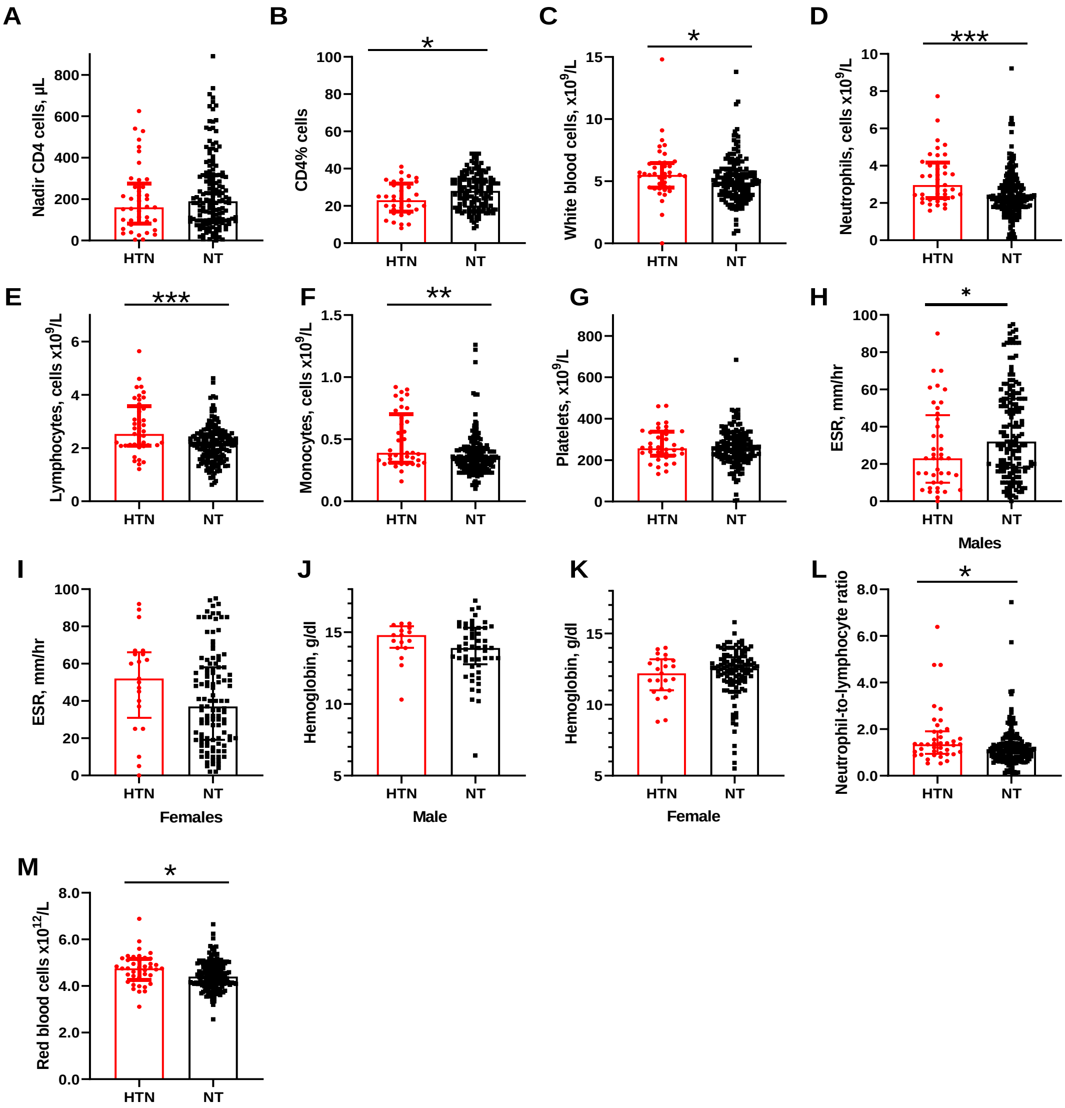


Figure 11. Innate and adaptive immune cells in hypertension and normotensive HIV positive individuals

(A) Nadir CD4 count was similar between hypertensives (HTN) and normotensives (NT) but lower than the expected CD4 count. (B) HTN had lower CD4% than NT below the expected range. HTN had higher (C) white blood cells, (D) neutrophils, (E) lymphocytes, (F) monocytes, (M) red blood cells, and (L) neutrophil-to-lymphocyte ratio compared to the NT. Although the difference was statistically significant, the levels were within the expected reference range. The levels of (G) platelets, (J) hemoglobin in males and (K) hemoglobin in females were not statistically different between HTN and NT and they were within the biological expected range. HTN males had lower (H) erythrocyte sedimentation rate (ESR) compared to the NT but the ESR in females between HTN and NT was not statistically different. Although the ESR levels in both males and females were lower than the biological reference range, this is expected in chronic infections like HIV. See appendix C for detailed supplementary information presented in the graphs. $*p<0.05$, $**P<0.01$, $***P<0.001$, Wilcoxon rank-sum test. 216 HIV positive adults (41 HTN and 175 NT). **Expected values:** HIV positives with a CD4 count over 500 (≈CD4% of 29%) are usually not at high risk of developing opportunistic infections and those who have a CD4 cell count below 200 are at high risk of developing serious illnesses. CD4% 29%-65%; White blood cell count = 4.0-10.0 x 10^9/L; Haemoglobin = 12 -16 g/dl for females and 14-18 g/dl for males; Red blood cell count = 4.5-5.9 x 10^{12}/L; Plateletes = 150-450 x 10^9/L; Neutrophils = 1.8 – 8.8 x 10^9/L; Lymphocytes

= 1.1 – 4. 9 x 10^9/L; Monocytes = 0.90 – 1.10 x 10^9/L; Erythrocyte sedimentation rate = 0 to 22 mm/hr for men and 0 to 29 mm/hr for women; Neutrophil to lymphocyte ratio = 1.0-1.7.

All the lipid levels between hypertensive and normotensive were not statistically different (Figure 12, appendix C).

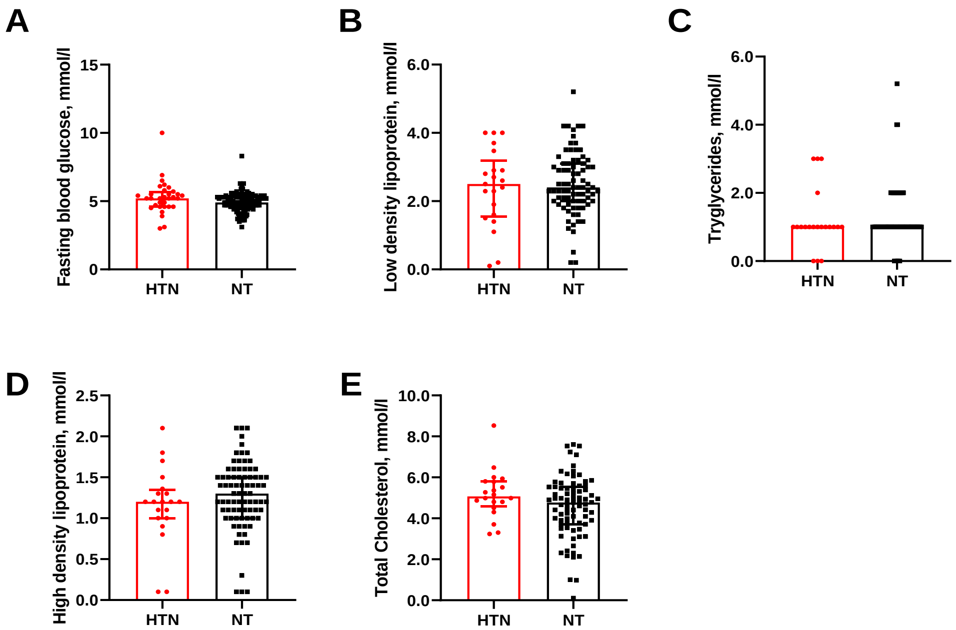


Figure 12. Lipid profile between hypertensive and normotensive HIV positive individualss

Fasting blood sugar (A) and lipid profile (B to E) was comparable between hypertensive (HTN) and normotensive (NT) groups. Wilcoxon rank-sum test. 216 HIV positive adults (41 hypertensives and 175 normotensives). **Expected values:** Fasting blood glucose = <5.6 mmol/L; Low density lipoprotein = <2.59 mmol/L; Tryglyceride = <1.7 mmol/L; Total cholesterol = <5.2 mmol/L ; High density lipoprotein = >1.0 mmol/L.

Plasma creatinine and creatinine clearance were higher and eGFR was lower in hypertensives compared with normotensives ($p<0.05$) (Figure 13).

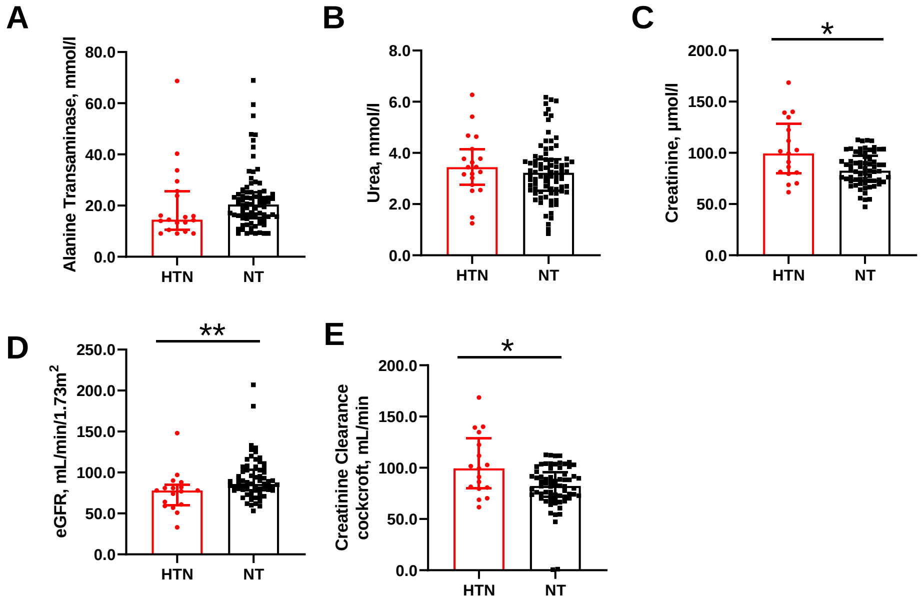


Figure 13. Liver and kidney profile between hypertensive and normotensive HIV positive individuals

(A) Alanine transferase and (B) urea levels were not statistically different between hypertensive (HTN) and normotensive (NT). Although (C) plasma creatinine levels and (E) glomerular filtration rate via creatinine clearance (using the Cockcroft-Gault equation) were higher in hypertensive compared to normotensive, the values were within the expected range. However, the (D) estimated glomerular filtration rate (eGFR) using the modification of diet in renal disease (MDRD) formulae was lower in hypertensive compared to the normotensive and the median levels were lower than the expected range for both groups. *p<0.05, **P<0.01, ***P<0.001, Wilcoxon rank-sum test. 216 HIV positive adults (41 hypertensives and 175 normotensives). **Expected values**: Alanine transaminase <45 mmol/l; Urea 2.5 to 7.1 mmol/L; Creatinine = 45 µmol/L to 110 µmol/L; Creatinine clearance = 88–137 mL/min; eGFR ≥ 90 mL/min/1.73m^2;

The median spot urine potassium, sodium and chloride concentrations were not statistically different between hypertensives and normotensives (Figure 14).

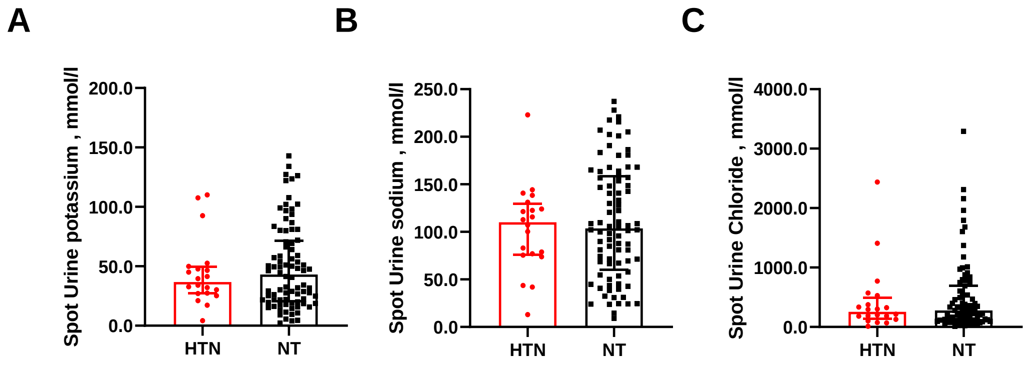


Figure 14. Spot urine concentrations between hypertension and normotensive HIV positive individuals

Spot urine potassium, sodium and chloride concentrations were similar between hypertensive and normotensive groups. Wilcoxon rank-sum test. 216 HIV positive adults (41 hypertensives and 175 normotensives)

Expected values: Spot urine potassium, sodium and chloride are >20 mmol/L and dependent on dietary intake.

Although falling within the expected levels, the possible association between hypertension and neutrophil count, neutrophil-to-lymphocyte ratio, plasma creatinine and red blood cell count remained statistically significant ($p<0.05$) after they were adjusted for age, sex, BMI and duration on ART in logistic regression model (Table 14). The estimated glomerular filtration rate may also remained significantly associated with hypertension and the median values were below the expected levels. The statistical and biological significance of these results are discussed in section 5.1.2.1.

Table 14. Hypertension correlates in multivariate regression adjusted for sex, age, body mass index and duration on antiretroviral therapy in HIV positive individuals.

Variable	Adjusted Odds Ratio AOR (95%CI)	p-value
White blood cell count, $x\ 10^9 cells/L$	1.16 (0.94, 1.44)	0.148
Neutrophil count, $x\ 10^9 cells/L$	1.56 (1.16, 2.11)	**0.003**
Monocyte count, $x\ 10^9 cells/L$	1.12 (0.62, 2.01)	0.687
Lymphocyte count, $x\ 10^9 cells/L$	1.45 (0.94, 2.23)	0.090
CD4 percentage, %	0.96 (0.91, 1.01)	0.190
Neutrophil to lymphocyte ratio	1.57 (1.07, 2.29)	**0.019**
Red blood cell count, $x\ 10^{12} cells/L$	2.42 (1.27, 4.64)	**0.007**
Plasma creatinine, *mmol/l*	1.04 (1.00, 1.09)	**0.032**
Glomerular filtration rate via Creatinine Clearance, *mL/min*	0.98 (0.94, 1.01)	0.319
Estimated glomerular filtration rate using modification of diet in renal disease (MDRD) formulae, $ml/min/1.73m^2$	0.95 (0.91, 0.99)	**0.041**

Owing to the differences in the red blood cell count between the hypertensive and normotensive (Figure 11. M), haemoglobin oxygen saturation was measured to assess the capacity of red blood cells to carry oxygen but there was no difference between groups (Table 15). A plausible explaination for this is discussed in section 5.1.2.1.

Table 15. Oxygen saturation levels in haemoglobin using a pulse oximeter

Clinical characteristics	Hypertension n, 23 (min, max)	Normotensive n, 79(min, max)	p-value
Oxygen saturation, *%*	96.17 (66, 100)	96.09 (86, 100)	0.374
Pulse rate, bpm	75.70 (60, 108)	75.90 (56, 138)	0.891

4.2.0 Inflammation and hypertension studies

4.2.0.1 Specific objective two

To determine the relationship between hypertension and inflammation in HIV

4.2.1 Sub-cohort 3 results

A multiplex flow cytometry kit comprising 13 cytokines was used to quantify plasma supernatant cytokines after a six hour incubation with PMA/Ionomycin for hypertensive and normotensive individuals in both HIV positive and HIV negative participants. The reference range for the cytokine concentration levels produced in the supernatant by controls was used to compare with all groups and is shown under each figure legend (Figure 15 and 16).

4.2.1.1 Study sample characteristics

The subgroup cohort of 85 participants comprising 50.6% (n,43) HIV positive participants and 49.4% (n, 42) HIV negative individuals who served as controls matched for age and sex was studied. The ratio of hypertensive: normotensive and male: female was 1:1 in both HIV positive adults and HIV negative controls. Log transformation (log_2 and log_{10}) for IL-5, IL-9 and IL-13 (Figure 15) was done to only improve visualization. Additional supplemental data for the results in the figures (Figure 15 and 16) are found in appendix C.

4.2.1.2 Inflammatory markers in the study groups between hypertensives and normotensives

Hypertensive PWH had higher plasma levels of IL-6, IL-10 (Figure 15) and IL-17A (16) compared with the normotensive PWH ($p<0.05$). Although the rest of the cytokine concentration levels were not siginificantly different between the HIV positive groups and were within the reference range (obtained from the control group), the hypertensive individuals had slightly higer IL-5 and lower IL-13 compared to the HIV negative control. In the HIV negative group, levels of IL-6, IFN-γ and TNF-α (Figure 15) were higher in the hypertensive when compared with the HIV negative normotensive controls and this difference was statistically significant ($p<0.05$).

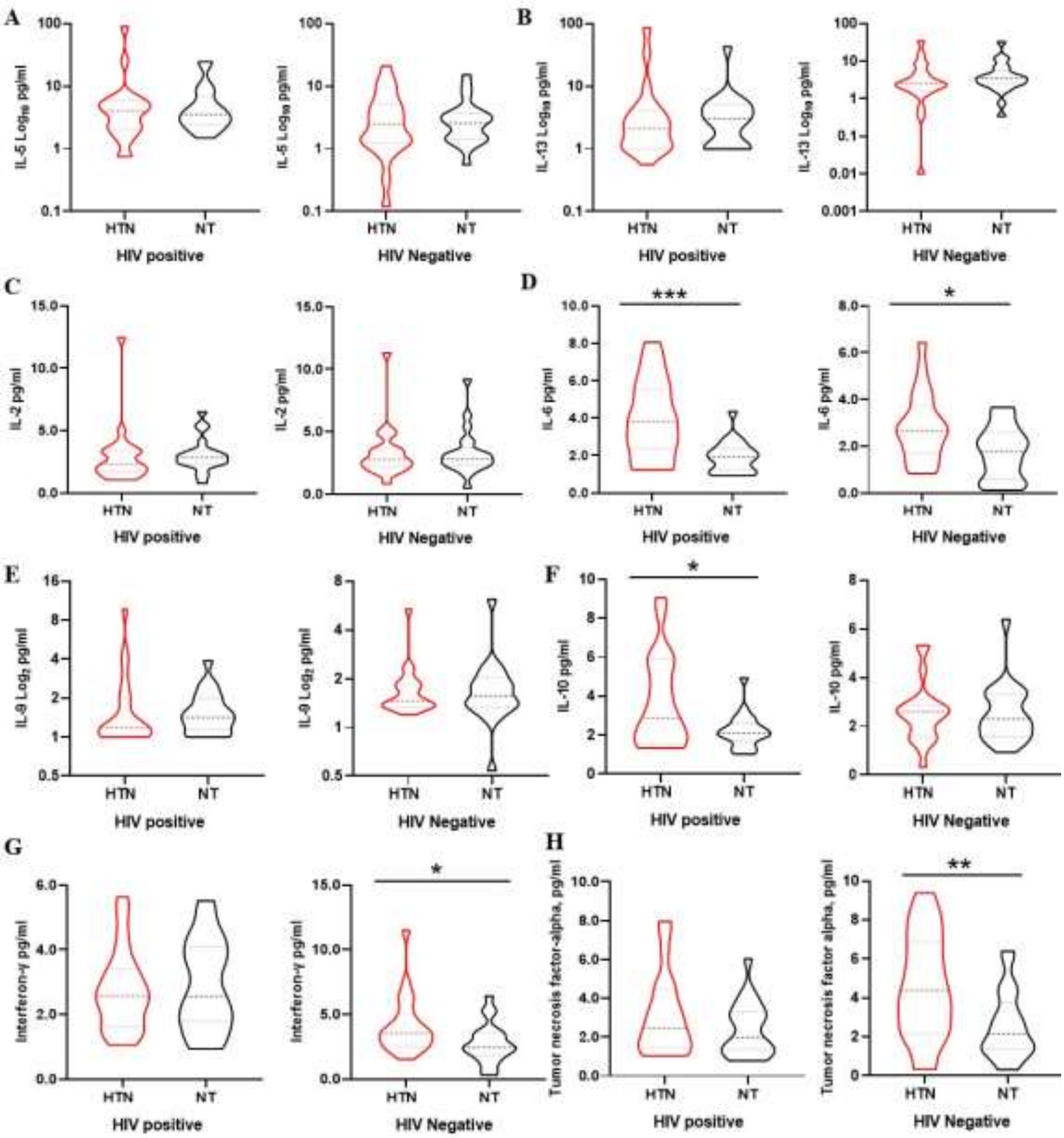


Figure 15. IL-5, IL-13, IL-2, IL-6, IL-9, IL-10, IFN-γ and TNF-α levels in activated whole blood supernatant of HIV positive and HIV negative individuals.
(A) IL-5 was higher in hypertensive (HTN) persons with HIV (PWH) compared to the normotensive (NT) PWH and slightly higher than the reference while the HTN HIV negative had lower levels compared to the reference. However, this difference was not statistically significant. PWH and the HTN HIV inividuals had slightly lower (B) IL-13 levels compared to the reference control but this difference was not statistically significant. (D) HTN had higher IL-6 in both PWH and HIV negative groups and the levels were higher than the reference control. (C) IL-2 and (E) IL-9 levels were not statistically different between HTN and NT and they were within the reference range. (F) IL-10 was higher in HTN compared to NT in PWH but not in the HIV negative, however, the levels of IL-10 were within the reference range. While (G) IFN-γ was higher in HTN HIV negatives compared to the reference. The levels of (H) TNF-α in HTN PWH were not statistically different when compared with NT PWH but HTN had higher TNF-α compared to the control in the HIV negative group. Log transformation was used only to improve visualization.

Biolegend multiplex assay was used for cytokine quantification and GraphPad prism version 8 for graphing. *p<0.05, ***p<0.001, Wilcoxon rank-sum test. For detailed results, see supplimentary data in appendix C. Expected reference levels (in pg/ml) of cytokines secreted in the supernatant from health controls are presented as mean (range): IL-5 = 2.5 (0.5 - 15.2); IL-13 = 3.5 (0.3 – 3.3); IL-2 = 2.8 (0.4 - 9.2); IL-6 = 1.7 (0.1 -3.6); IL-9 = 1.5 (0.5 – 6.2); IL-10 = 2.3 (0.9 – 6.4); IFN-γ = 2.4 (0.3 – 6.4); TNF-α =2.1 (0.3 – 6.4).

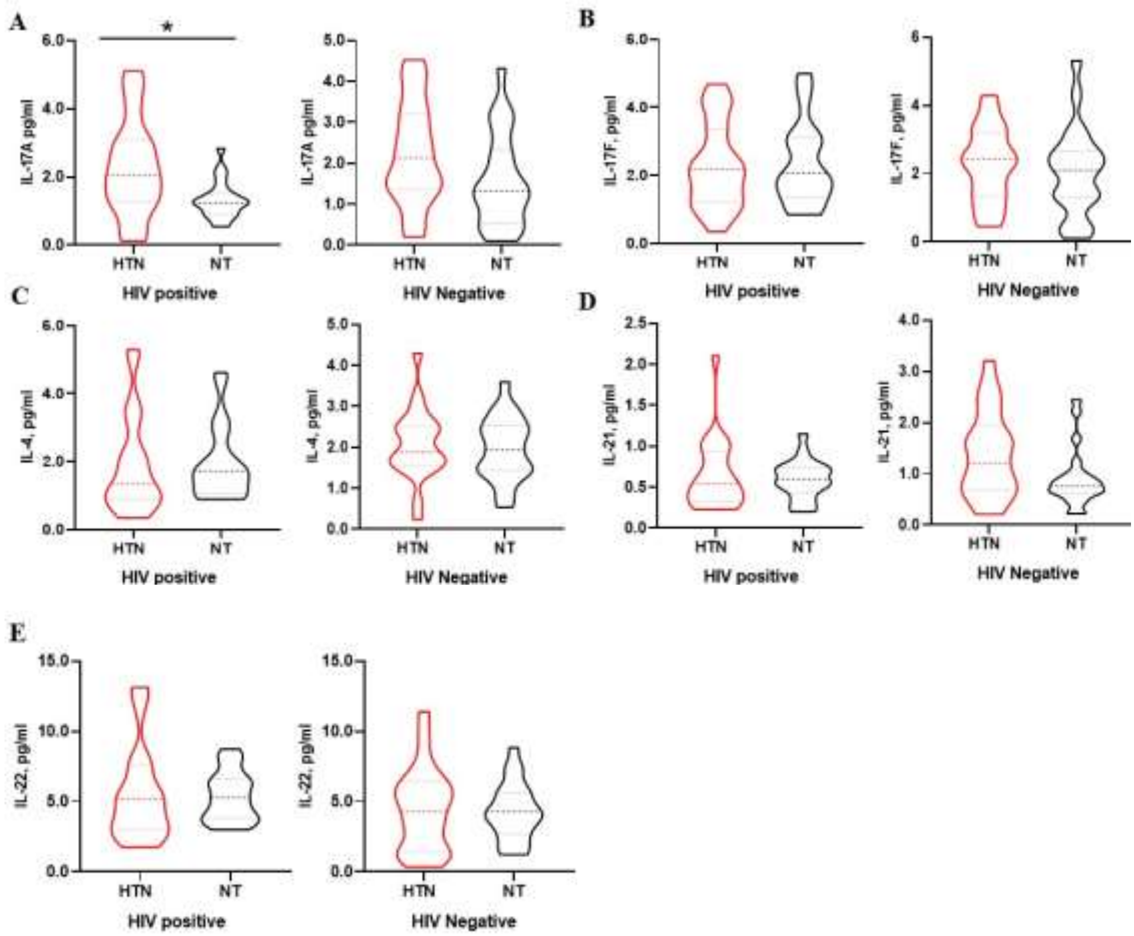


Figure 16. IL-17A, IL-17F, IL-4, IL-21 and IL-22 levels in activated whole blood supernatant of HIV positive and HIV negative individuals.

In PWH, hypertensives (HTN) had high (A) IL-17A compared to normotensives (NT) but not in the HIV negative group. However, the levels were within the expected reference range. Levels of (B) IL-17F, (C) IL-4, (D) IL-21, and (E) IL-22 between HTN and NT in each group were not statistically different, however, HTN had IL-4, and IL-22 levels slightly above the reference control. Biolegend multiplex assay was used for cytokine quantification and GraphPad prism version 8 for graphing *p<0.05, ***p<0.001, Wilcoxon rank-sum test. For detailed results, see supplimentary data in appendix C. Expected reference levels (in pg/ml) of cytokines secreted in the supernatant from health controls are presented as mean (range): IL-17A = 1.3 (0.1 – 4.3); IL-17F = 2.1 (0.1 – 5.3); IL-4 = 1.9 (0.5 – 3.6); IL-21 = 0.7 (0.2 -2.4); IL-22 = 4.3 (1.2 – 8.8).

A comparison of all cytokine levels across the four subgroups found that only IL-6, TNF-α, IL-17A and IL-21 were significantly different (Table 16).

Table 16. Comparison of cytokine levels across all HIV positive and HIV negative groups

	HIV positive group		**HIV negative**		
	Hypertensive (n, 22) Median (IQR)	Normotensive (n, 21) Median (IQR)	Hypertensive (n, 21) Median (IQR)	Normotensive (n, 21) Median (IQR)	P-value
IL-5, *pg/ml*	4.05 (2.05, 5.87)	3.51 (2.42, 6.90)	2.46 (1.25, 5.10)	2.57 (1.45, 3.71)	0.139
IL-13, *pg/ml*	2.11 (0.99, 4.10)	3.04 (1.07, 5.04)	2.54 (1.66, 4.70)	3.54 (2.44, 5.77)	0.428
IL-2, *pg/ml*	2.33 (1.73, 3.40)	2.84 (2.32, 3.40)	2.76 (2.15, 3.63)	2.80 (2.28, 3.71)	0.598
IL-6, *pg/ml*	3.81 (2.37, 5.54)	1.91 (1.20, 2.37)	2.65 (1.70, 3.71)	1.76 (0.59, 2.59)	**<0.001**
IL-9, *pg/ml*	1.19 (1.00, 2.02)	1.42 (1.14, 1.99)	1.45 (1.34, 1.85)	1.56 (1.32, 2.02)	0.102
IL-10, *pg/ml*	2.87 (1.53, 5.89)	2.09 (1.66, 2.61)	2.60 (1.54, 2.86)	2.30 (1.59, 3.33)	0.141
Interferon-gamma, *pg/ml*	2.57 (1.62, 3.39)	2.56 (1.78, 4.09)	3.53 (2.55, 5.03)	2.44 (1.81, 3.59)	0.050
Tumor necrosis factor-alpha, *pg/ml*	2.47 (1.45, 4.45)	2.00 (1.35, 3.30)	4.39 (2.13, 6.86)	2.14 (1.35, 3.76)	**0.014**
IL-17A, *pg/ml*	2.04 (1.27, 3.09)	1.23 (0.91, 1.55)	2.13 (1.37, 3.22)	1.32 (0.54, 2.33)	**0.020**
IL-17F, *pg/ml*	2.18 (1.20, 3.37)	2.06 (1.35, 3.11)	2.42 (1.34, 3.20)	2.10 (1.31, 2.66)	0.914
IL-4, *pg/ml*	1.34 (0.89, 2.61)	1.71 (1.05, 2.56)	1.88 (1.56, 2.52)	1.94 (1.43, 2.53)	0.433
IL-21, *pg/ml*	0.54 (0.33, 0.93)	0.60 (0.43, 0.74)	1.21 (0.68, 1.94)	0.76 (0.62, 0.99)	**<0.001**
IL-22, *pg/ml*	5.19 (3.00, 7.61)	5.29 (3.82, 6.60)	4.30 (1.39, 6.41)	4.30 (2.70, 5.66)	0.349

Kruskal-Wallis test used. Reference levels (in pg/ml) of cytokines secreted in the supernatant from health controls presented as mean (range): IL-5 = 2.5 (0.5 - 15.2); IL-13 = 3.5 (0.3 – 3.3); IL-2 = 2.8 (0.4 - 9.2); IL-6 = 1.7 (0.1 -3.6); IL-9 = 1.5 (0.5 – 6.2); IL-10 = 2.3 (0.9 – 6.4); IFN-γ = 2.4 (0.3 – 6.4); TNF-α =2.1 (0.3 – 6.4); IL-17A = 1.3 (0.1 – 4.3); IL-17F = 2.1 (0.1 – 5.3); IL-4 = 1.9 (0.5 – 3.6); IL-21 = 0.7 (0.2 -2.4); IL-22 = 4.3 (1.2 – 8.8).

A follow-up posthoc test was performed for cytokine levels that were statistically significant across subgroups on the Kruskal-Wallis test (Table 16) in order to assess the subgroup that was significantly different when compared to the control. As shown in Table 17, IL-6 was higher in HIV positive hypertensive compared to the controls. The HIV positive normotensive and hypertensive subgroups had IL-6 levels that did not differ when compared to the controls ($p>0.05$). Although the hypertensive HIV negative subgroup had higher levels of TNF-α compared to all groups including the controls (Table 16), this was not statistically significant. IL-17A was slightly higher among hypertensive in both HIV and HIV negative group (Table 16), however, only the hypertensive HIV positive group had statistically significant higher levels when compared with controls (Table 17). While IL-21 levels were different when compared across all subgroups (Table 16), this was abrogated by multiple comparison using the controls as a comparator (Table 17).

Table 17. PostHoc test for HIV negative normotensive controls compared with all HIV positive subgroups

		HIV positive group		**HIV negative**
		Hypertensive p value	Normotensive p value	Hypertensive p value
Controls	IL-6	**<0.001**	>0.999	0.069
	Tumor necrosis factor-alpha	0.908	0.179	>0.99
	IL-17A	**0.048**	>0.999	0.211
	IL-21	>0.999	0.345	>0.999

Dunns' multiple comparison test

Figure 17 shows gating strategy for all specific cell populations analyzed for HIV positive and HIV negative individuals. Flowjo version 10 was used for this purpose. Refer to section 3.4.1.7.3 for details.

4.2.1.3 Gating strategy for immune cell markers by flow cytometry

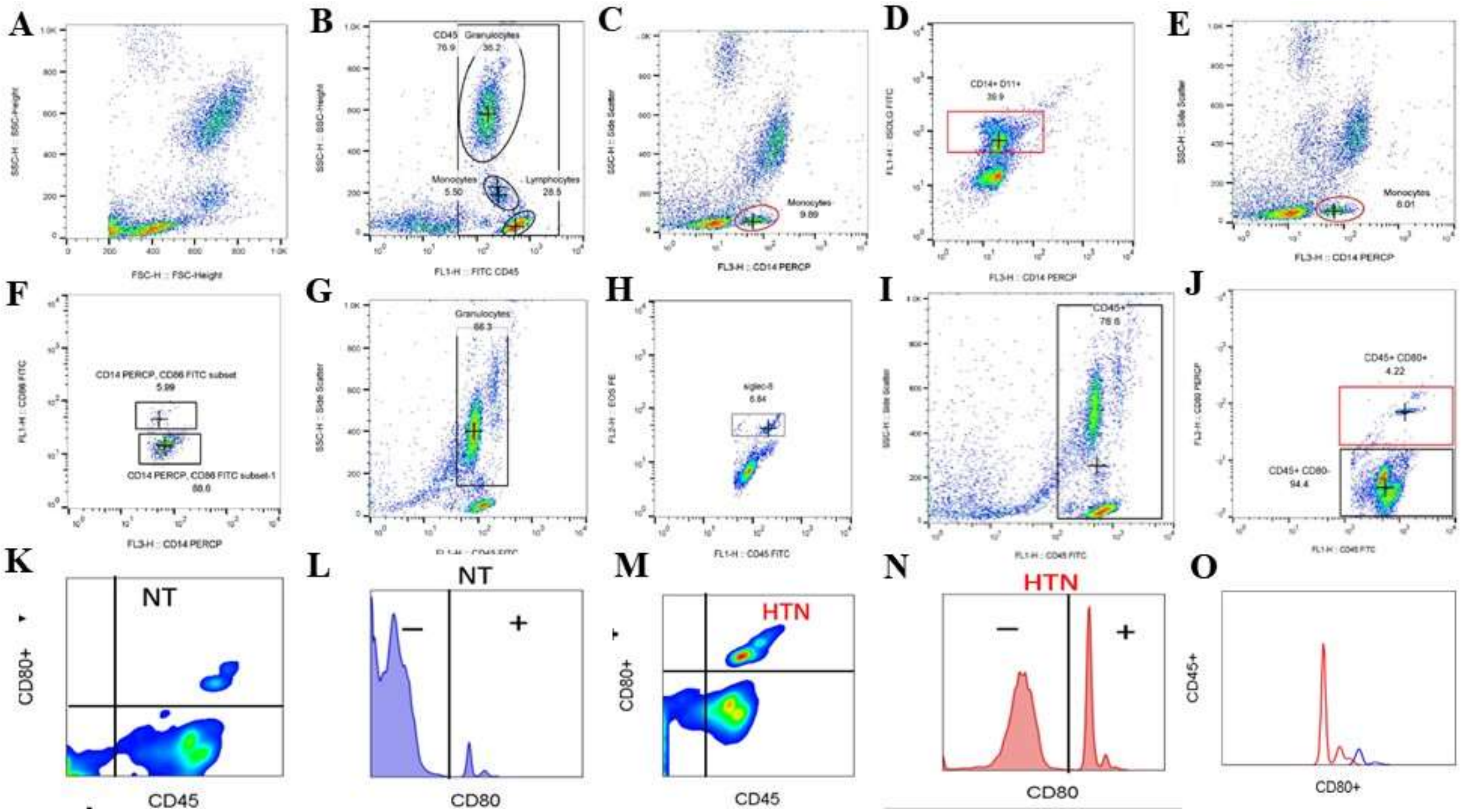


Figure 17. Gating strategy to identify cell populations and immune markers of activation by flow cytometry

(A) side-scatter versus forward scatter was employed to visualize cells and (B) Monocytes, lymphocytes and granulocytes were visualized using CD45 versus side-scatter. Gating on (C) monocytes expressing (D) Isolevuglandin (D11) were identified and quantified. The (E) monocyte gate was also used to identify and quantify activated monocytes expressing (F) CD86. Using side-scatter versus CD45 (G) a gate on granulocytes was created and (H) eosinophils were identified and quantified using siglec-8. To identify activated leucocytes expressing CD80, a gate was set on (I) total leucocytes and using a CD80 marker, (J) activated leucocytes were identified and quantified. A comparative strategy to compare flouriscence intensities of total activated leucocytes (CD80+) between (K) normotensive (NT) and (M) hypertensive (HTN) is shown for the HIV positive. Their histograms of flouriscence intensities between (L) NT and (N) HTN is also shown. Flouriscence intensities were higher in HTN compared with NT (O). The red wave represents HTN while the blue represent NT (O). Flowjo was used for analysis.

4.2.1.4 Immune cell markers between hypertensive and normotensive individuals

Hypertension was associated with immune activation in the HIV positive group and increased expression of Isolevuglandins (D11) in monocytes of both HIV positive and HIV negative groups. There was high expression of (A) CD80+ on unstimulated leucocytes (CD45) in hypertensive HIV positive group, $p<0.05$ (Figure 18).

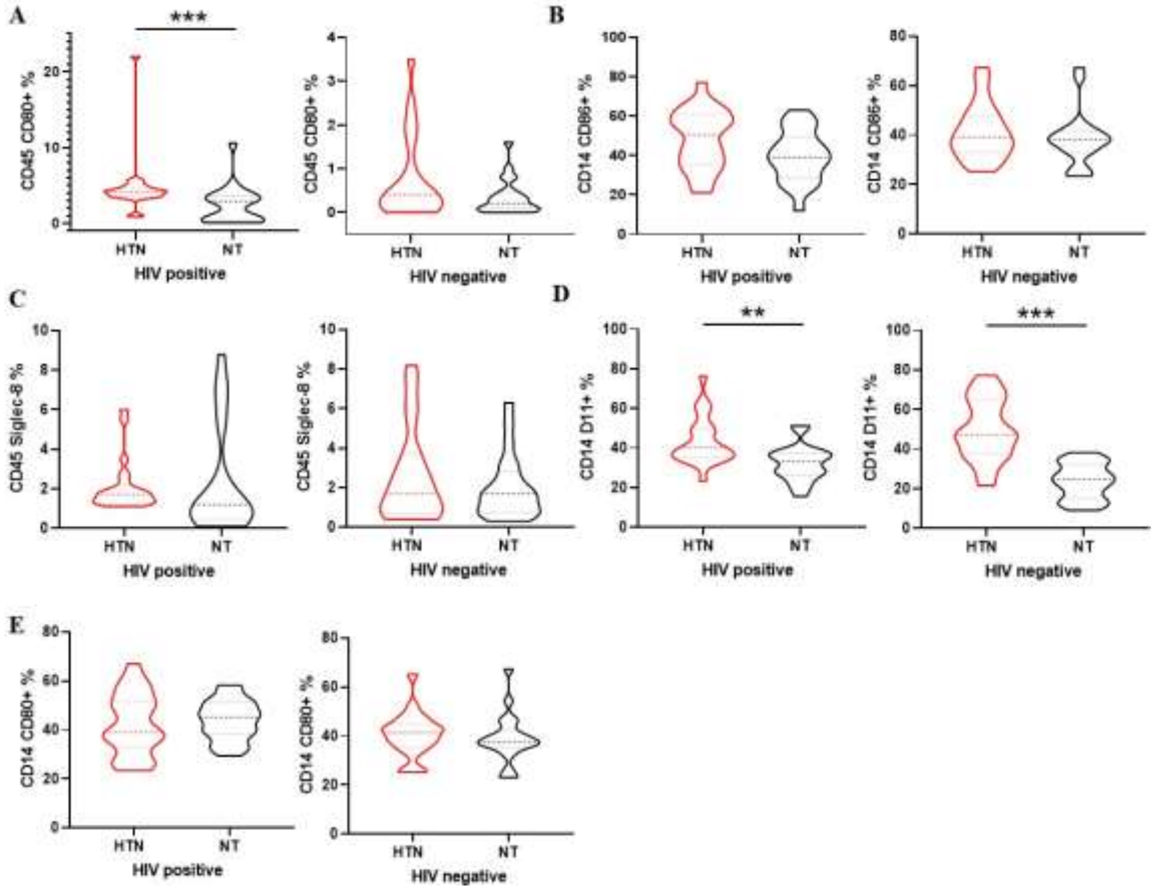


Figure 18. Expression of CD80+ and Siglec-8 on leucocytes, CD80+, CD86+ and isoLGs on monocytes in HIV positive and HIV negative groups

(A) CD45 CD80+% was higher (above expected range) in the hypertensive (HTN) compared with the normotensive (NT) in the HIV positive group but not in the HIV negative group. (B) CD14 CD86+% and (E) CD14 CD80% was not significantly different between HTN and NT in both groups and they were within the expected range. Although (C) CD45 Siglec-8% was not significantly different between HTN and NT in both groups, NT HIV positive and HTN HIV negative had levels slightly above the reference group (NT HIV negative). (D) CD14 D11+% was higher in HTN (above expected range) compared with the NT in both groups. Siglec-8 is a marker for eosinophils. Expected levels derived from the control: CD45 CD80% = 0 – 1.6%; CD14 CD86% = 23 – 67%; CD45 Siglec-8% = 0.3 – 6.3%; CD14 D11% = 9 – 38%; CD14 CD80% = 23 – 67%. Flow cytometry was used and Flowjo version 10 for analysis and graphing. Dotted lines denote median with upper and lower quartiles. $^{**}p<0.01$, $^{***}p<0.001$, Wilcoxon rank-sum test.

In PWH, the possible association between IL-6, IL-10, percentage of CD80+ expression on CD45 cells, Isolevuglandin adducts in monocytes and hypertension remained statistically significant after adjustment in logistic regression model (Table 18).

Table 18. Association between hypertension and covariates in multivariate logistic regression among PWH adjusted for sex, age, body mass index, duration on ART, viral load and CD4 count

Variable	**Adjusted Odds Ratio AOR (95%CI)**	**p-value**
IL-6, pg/ml	3.14 (1.26, 7.82)	**0.014**
IL-17A, pg/ml	1.96 (0.92, 4.15)	0.08
IL-10, pg/ml	2.81 (1.14, 6.93)	**0.025**
CD45 CD80+, %	1.91 (1.12, 3.26)	**0.017**
CD14 D11 (isoLevuglandin), %	1.18 (1.05, 1.32)	**0.004**

In HIV negative group, IL-6, tumor necrosis factor-alpha and Isolevuglandin adducts in monocytes remained significantly associated with hypertension after adjustment in logistic regression model (Table 19).

Table 19. Association between hypertension and covariates in multivariate logistic regression among HIV negative group adjusted for sex, age and body mass index

Variable	**Adjusted Odds Ratio AOR (95%CI)**	**p-value**
IL-6, pg/ml	5.08 (1.52, 16.92)	**0.008**
Tumor necrosis factor-alpha, pg/ml	2.12 (1.12, 4.02)	**0.020**
Interferon gamma, pg/ml	0.91 (0.59, 1.40)	0.671
CD14 D11 (isoLevuglandin), %	1.22 (1.08, 1.38)	**0.001**

As depicted in Figure 19, CD45 80+ cells were associated with hypertension across all 4 subgroup categories and immune activation was higher in hypertensive HIV positive group in comparison to each subgroup. While D11 expression in monocytes was higher in hypertension across all 4 subgroup categories, there was higher D11 expression in the hypertension HIV positive group in comparison to the HIV positive and HIV negative normotensive group but not the HIV negative hypertensive group.

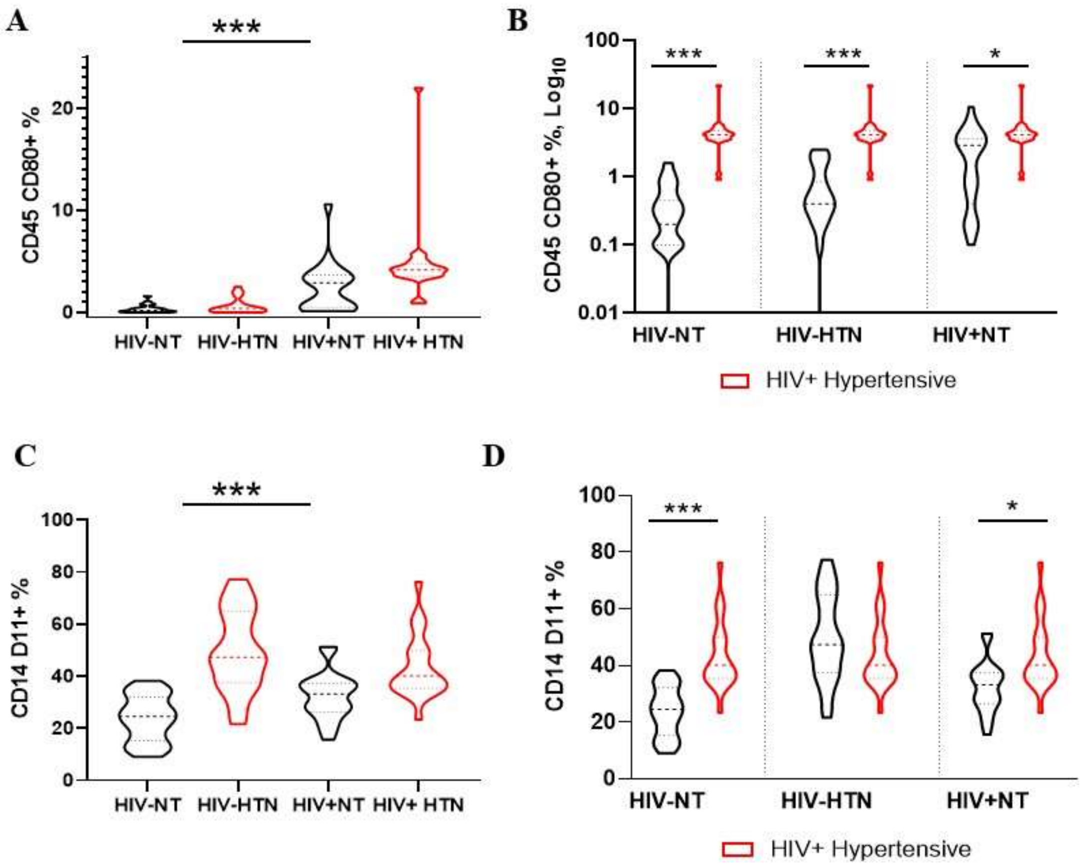
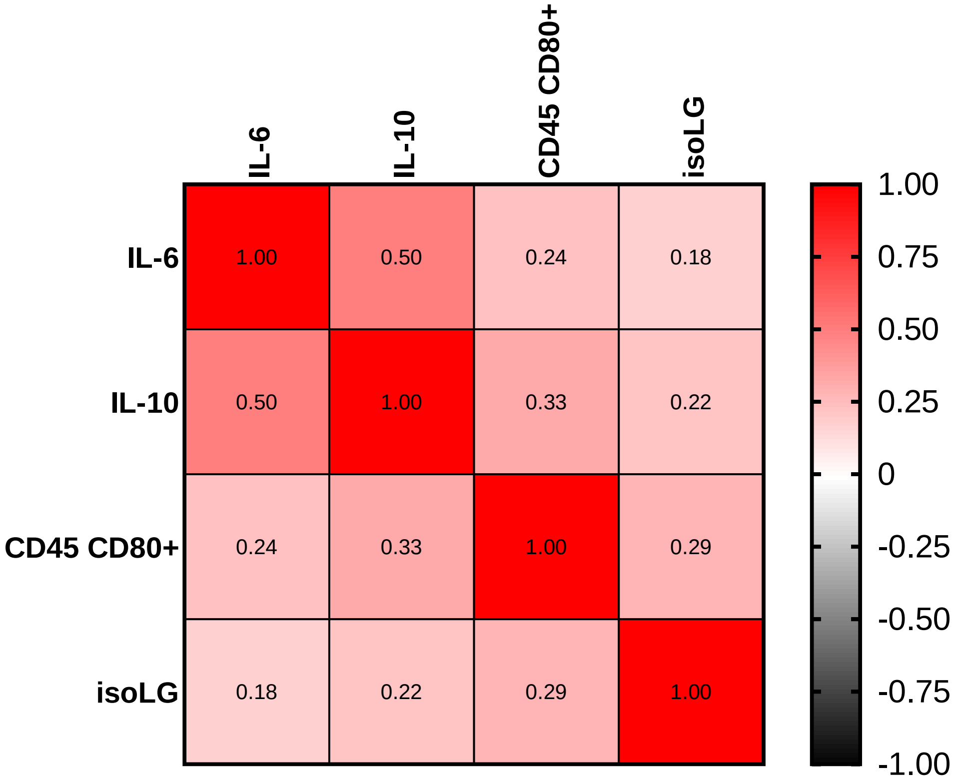


Figure 19. Subgroup multiple comparisons of CD80+ and IsoLGs in HIV positive and HIV negative groups

(A) CD45 CD80+% and (C) CD14 D11+% were significantly different across all subgroups (Kruskal-Wallis test). The HIV positive (HIV+) hypertensive (HTN) group had higher expression of (B) CD80+% on CD45 cells compared to each subgroup. On multiple comparison, the HIV+ HTN had higher (D) CD14 D11+% compared to the HIV negative (HIV-) and HIV positive (HIV+) normotensive (NT) but not the HIV negative hypertensive (Dunn's multiple comparison test used). Expected levels derived from the control: CD45 CD80% = 0 – 1.6%; CD14 D11% = 9 – 38%. *p<0.05, ***p<0.001. Log transformation (B) was employed to improve visualization. GraphPad prism version 8 was used for graphing. Horizontal Dotted lines denote median with upper and lower quartiles.

A correlation compared between the markers that remained significantly associated with hypertension in PWH in logistic regression (Table 18) shows, IL-6 positively correlated with IL-10 levels (rho= 0.50, p=0.001) and IL-10 positively correlated with activated (CD80+) leucocytes (CD45) (rho= 0.33, p=0.029) (Table 20).

Table 20. IL-6, IL-10, CD45 CD80+ and IsoLGs Correlation matrix



A spearman correlation matrix which shows spearman correlation coefficients

4.2.2 Cohort 4: Adiposity and Immune Activation Cohort (AIAC) results

This cohort is from the USA. A paper has been published on this (Masenga et al., 2020).

4.2.2.1 Gating strategy for immune cell markers

Gating was perfomed on peripheral blood mononuclear cells and T cell populations were identified as shown in Figure 20.

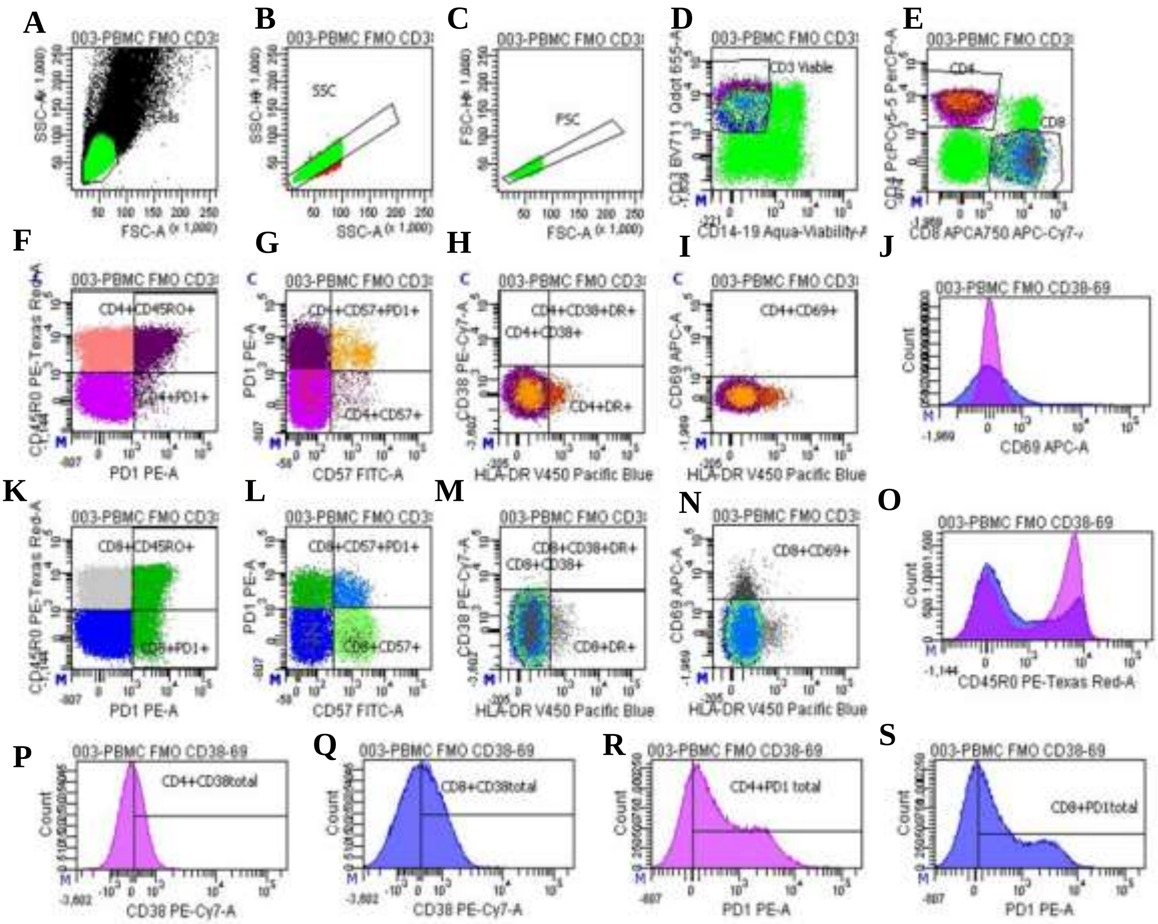


Figure 20 Gating strategy to identify T cell subtypes.
(A) Forward-scatter area (FSC-A) versus side-scatter area (SSC-A) was used to exclude debris and gate on lymphocytes. Doublets were then identified using (B) side-scatter area versus side scatter height (SSC-H) from (C) singlets which were identified by forward scatter height (FSC-H) versus side forward scatter area (SSC-A). (D) Viable lymphocytes were then seperated using a viability dye (CD3 BV711 versus CD14-19 aqua viability) and a gate on live cells was created to seperate (E) T cell populations using CD4+ and CD8+ markers. Activated T cells for both CD4 (F-J) and CD8 (K-O) were quantified and differentiated by the expression of PD1 (F and K) and additional expression of CD57 (G and L). CD4 and CD8 T cell populations expressing CD38+HLA-DR+ (H and M respectively), CD69+ HLA-DR+ (I and N respectively) were quantified. (J) CD4 CD 69+, activated T cells (O), CD38+ on CD4+ (P) and CD8+ (Q), PD1 on CD4+(R) and CD8+ (S) were quantified.

4.2.2.2 Participant characteristics

Clinical characteristics of the HIV-positive study AIAC cohort are shown in Table 21. About 44 % were hypertensive. These were also older and had higher BMI, FMI, average WC, mid-upper arm circumference, visceral adipose tissue, amyloid A, leptin and were obese as compared with the normotensive ($p<0.05$). The rest of the characteristics were comparable between hypertensives and normotensives.

Table 21. HIV positive participant characteristics

Variables	**N=70**	**Normotensive n=39**	**Hypertensive n=31**	p-value
Age *median years (IQR)*	42 (35, 47)	43 (35,47)	49 (43,52)	**0.002**
Female n (%)	30 (43)	16 (41)	14 (45)	0.810
Duration on treatment median *years (IQR)*	6.2 (4.3, 10.1)	6.1 (4.3, 11.1)	6.4 (4.3, 8.2)	0.804
BMI *median (IQR) kg/m2*	32.3 (26.3, 37.1)	26.5 (22.8, 32.8)	33.9 (28.2, 40.0)	**0.001**
FMI x 10^3 *median (IQR)*	12.8 (8.8, 16.3)	8.8 (5.7, 13.2)	13.3 (9.9, 17.5)	**0.003**
CD4+ count *median (IQR)* cells/µl	701 (540, 953)	700 (523, 924)	690 (581, 969)	0.554
Nadir CD4+ count *median (IQR)* cells/µl	257 (140, 378)	276 (183, 393)	240 (100, 371)	0.301
CD8+ count *median (IQR)* cells/µl	752 (600, 1004)	774 (630, 949)	675 (585, 1062)	0.582
Smokers *n (%)*	25(36)	14 (36)	11 (35)	1.000
Cigarettes per day (N=69)	0.0(0.0,4.0)	0.0(0.0,4.0)	0.0(0.0,4.0)	0.787
Non-white n (%)	38 (54)	23 (59)	15 (48)	1.000
Hepatitis C infection *n (%)*	8 (11%)	2 (5%)	6 (19)	0.127
Fasting total cholesterol *median (IQR)* mg/dl	176 (154, 202)	176 (160, 203)	171 (142, 200)	0.460
Fasting LDL *median (IQR)* mg/dl	105 (88, 123)	111(89, 123)	101 (85, 124)	0.435
Fasting HDL *median (IQR)* mg/dl	45 (36, 52)	47 (35, 55)	43 (36, 50)	0.619
Fasting TG *median (IQR)* mg/dl	101 (73, 139)	98 (80, 147)	102 (73, 138)	0.953
obese *n (%)*	35 (50)	14 (35.9)	21 (67.7)	**0.008**
Average waist circumference *median (IQR)* cm	104 (88, 122)	96 (82, 110)	114 (101, 128)	**0.001**
Average mid-upper arm circumference *median (IQR)* cm,	33 (30, 36)	31 (28, 35)	35 (31, 38)	**0.006**
Visceral adipose tissue *(IQR)* cm	1554 (630, 2219)	906 (381, 2061)	1950 (1316, 3014)	**0.002**
Taking NSAIDS *n (%)*	10 (14.3)	5 (12.8)	5 (16.1)	0.694
Taking Daily Aspirin	6 (8.6)	0 (0.0)	6 (19.4)	**0.006**
Highly sensitive CRP *(IQR) mg/L*	2.4 (1.1, 6.2)	2.1 (0.8, 3.4)	3.2 (1.5, 6.6)	0.05
Amyloid A *(IQR) pg/ml* $x10^6$	1.8 (0.9, 5.0)	1.4 (0.7, 2.2)	4.2 (1.8, 9.7)	**<0.001**
Leptin *median (IQR) ng/ml*	17.3 (8.5, 32.1)	11.5 (5.9, 30.2)	23.6 (12.4, 37.2)	**0.02**

For the quantitative variables age, duration on treatment, BMI, FMI, CD4 and CD8 counts, the Wilcoxon rank-sum test was used. The chi-square test was used for the remaining binary categorical variables. BMI, body mass index; FMI, fat mass index. P values less than 0.05 are shown in bold.

4.2.2.3 Immune cell markers between hypertensive and normotensive individuals

In unadjusted comparisons, no significant differences were observed among CD4$^+$ and CD8$^+$ naïve and memory T cell subtypes between hypertensive and normotensives. The study also found no significant differences among CD4$^+$ and CD8$^+$T cells expressing CD57 and PD1. However, hypertension was associated with lower CD4$^+$ T cells expressing the CD38 activation marker, but not CD8$^+$CD38$^+$ T cells and there were no significant differences in dual expression of CD38$^+$ and HLA-DR$^+$ cells (Figure 21).

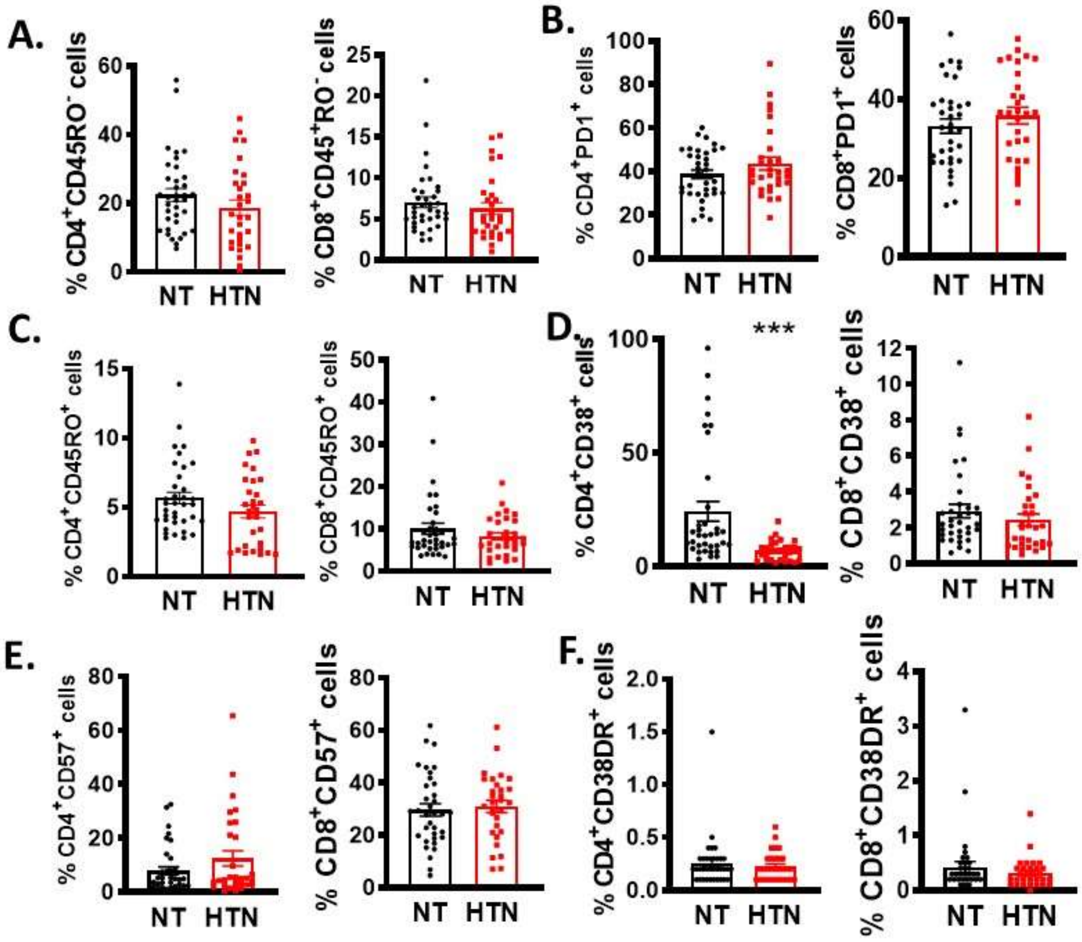


Figure 21. CD4+ and CD8+ activation markers in HIV positive individuals
Increased CD4 T cell activation in people living with HIV is negatively associated with hypertension. Flow cytometry analysis of T cell subsets in people living with HIV showed pairs of (A) naïve and (B) memory CD4$^+$ and CD8$^+$T cell subtypes, (C) T cells expressing CD57, (D) PD1, (E) CD38 and (F) the CD38 receptor in normotensives (NT) compared to hypertensives (HTN). Expected ranges are not well established hence the data is interpreted on comparison of the two groups (HTN and NT). ***p<0.001. Mann-Whitney test

Increased macrophage activation in PWH maybe associated with prevalent hypertension. The study observed no significant difference in levels of soluble CD14 (sCD14) in the normotensive compared to the hypertensive PWH participants (Figure 22A). However, it was found that hypertension was associated with a marked increase in macrophage activation markers and chemokines including soluble CD163 (sCD163) (Figure 22B) and macrophage inflammatory protein-1 alpha (MIP-1α) (Figure 22C). In addition, it was found that hypertension was likely associated with a significant increase in monocyte chemoattractant protein 1 (MCP-1) (Figure 22D), and both VCAM-1 (Figure 22E) and ICAM-1 (Figure 22F). See supplementary data to figure 22 in appendix C for details. However, the values of sCD163 were within the normal expected range in health individuals whereas MIP-1α, MCP-1, VCAM-1 and ICAM-1 were above the expected range in healthy individuals. This was probably due to the HIV infection. These results suggest that increased macrophage activation and expression of vascular adhesion molecules are a feature of inflammation and there may be a possible association with hypertension among PWH on ART.

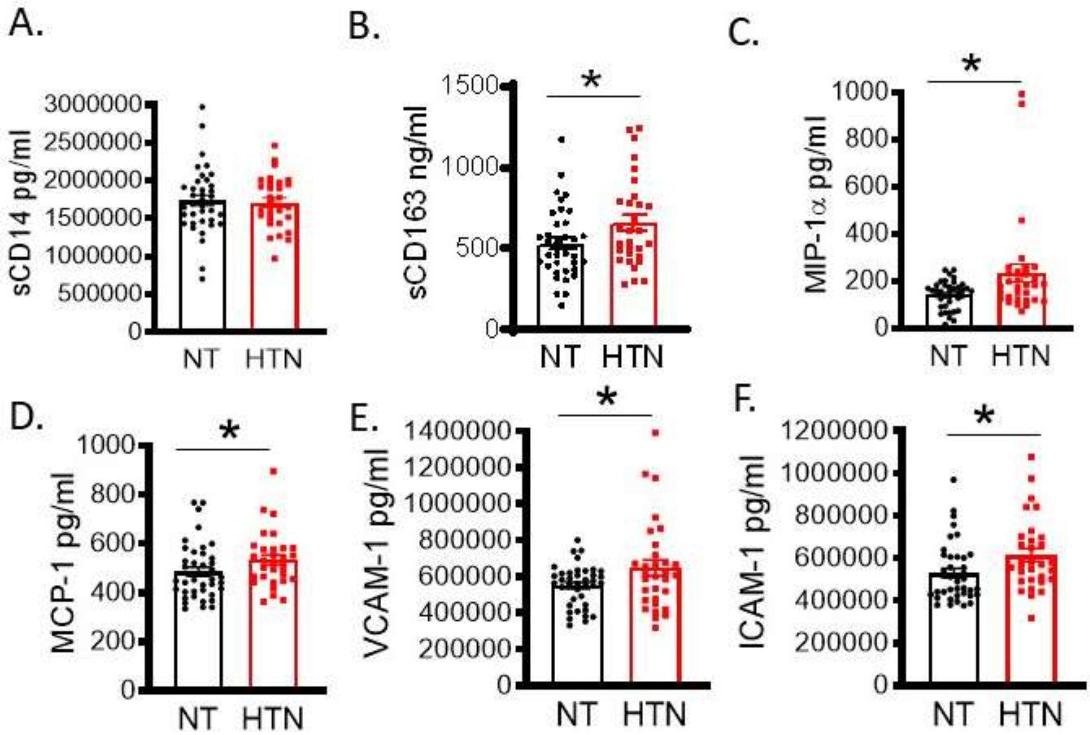


Figure 22. Plasma levels of macrophage and chemokine markers between hypertension and normotensive HIV positive participants

Plasma levels of (A) soluble CD14 (sCD14) were not statistically different between the hypertensive (HTN) and normotensive (NT) groups and they were within the expected reference range. (B) soluble CD163 and (C) macrophage inflammatory protein 1 alpha (MIP-1α) were higher in HTN compared to NT but within the normal expected range, although MIP-1α had some values above the reference. (D) Monocyte chemoattractant protein 1 (MCP-1) and adhesion molecules including (E) vascular cell adhesion molecule 1 (VCAM-1) and (F) intracellular adhesion molecule 1 (ICAM-1) were higher in HTN compared to NT and were above the expected range in healthy individuals. See supplementary data in appendix C for details. Cytokine concentrations were analyzed in plasma using enzyme-linked immunosorbent assay. *$p<0.05$ using the Mann-Whitney U test. Expected levels: sCD14 = 800, 000 – 2,500, 000 pg/mL; sCD163= 387 - 1785 ng/mL ; MIP-1α = 0-208 pg/ml; MCP-1 = 69.5–175.2 pg/mL ; VCAM-1 = 122, 000 -529 000 pg/mL; ICAM-1 = 64,000 -303, 000 pg/mL

Previous studies have found that inflammatory cytokines, including IL-17, contribute to the development of hypertension (Chiasson et al., 2011; Madhur et al., 2010; Nguyen et al., 2013). The current study found that hypertensive PWH had significantly higher levels of IL-17 (Figure 23A). It also found that hypertension was associated with increased circulating IL-6 (Figure 23B) but the levels were within the expected reference. In addition, the anti-inflammatory cytokine IL-10 (Figure 23C), and pro-inflammatory levels of TNF-α (Figure 23D) and soluble tumor necrosis factor receptor 2 (TNFR2) (Figure 23E) were not significantly different between hypertensives and normotensives, but a significant elevation of TNFR1 (Figure 23F) was found. While the levels of IL-10 and TNF-α were within the expected range, TNFR2 and TNFR1 were above the expected range. Plausible explainations are discussed in section 5.2.2.1. Thus, hypertension in PWH is possibly associated with higher circulating levels of IL-17 and sTNFR1.

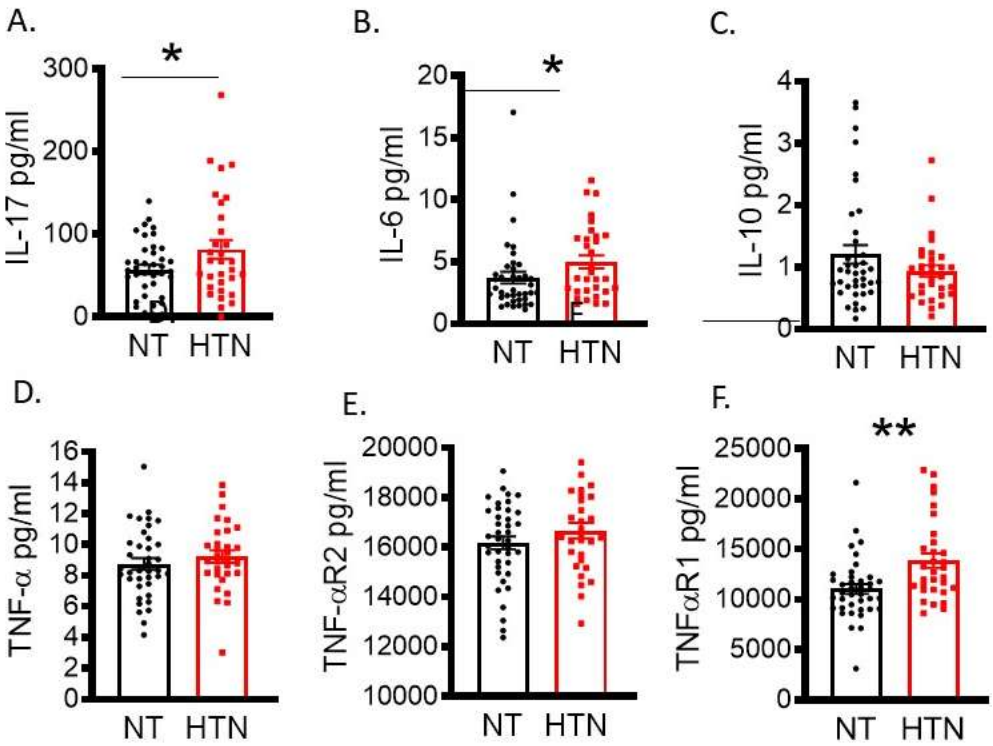


Figure 23. Cytokine production in HIV positive participants.
Increased cytokine production in virally suppressed HIV positive participants on ART is possibly associated with hypertension (HTN). (A) interleukin (IL)-17 and (E) TNF receptor 1 were higher in hypertension above the normal expected range while (B) IL-6 was higher in HTN compared to normotensives (NT) but within the reference range. (C) IL-10 and (D) Tumor Necrosis Factor (TNF)-α were not significantly different between HTN and NT and were within the normal expected range. The levels of (F) TNF receptor 2 were above that expected in healthy individuals but did not differ significantly between HTN and NT. See supplementary data in appendix C for details. Cytokine concentrations were analyzed in plasma using enzyme-linked immunosorbent assay. *p<0.05, **p<0.01 using the Mann-Whitney U test. Expected levels: IL-17 = 0.0–13.4 pg/mL; IL-6 = 0.0–12.7pg/mL; IL-10 = 13.68 pg/mL; TNF-α = 0=50 pg/mL; TNF-α R1 = 0 - 900 pg/mL; TNF-α R2 = 1000 – 2900 pg/mL

Although the eosinophil count (Figure 24B) and percentage (Figure 24A) were within the expected range, this study found higher circulating eosinophils in hypertension compared to normotensive in virally suppressed PWH. Prior studies have indicated that eosinophils play a role in a number of immune mediated diseases (Jacobsen et al., 2012). Additionally, IL-5 was also similarly elevated in hypertensive PWH when compared with normotensives (Figure 24C).

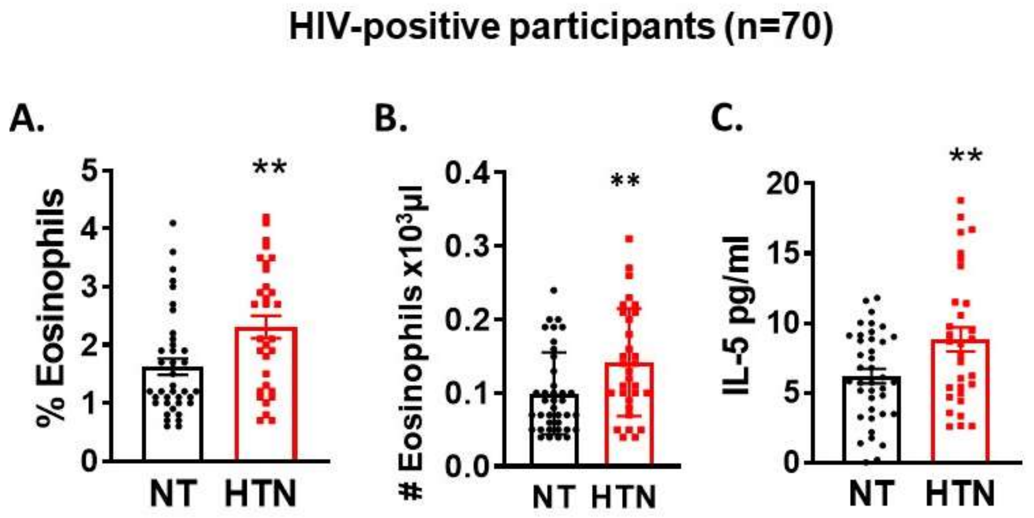


Figure 24. Eosinophil and IL-5 in HIV positive participants.
(A) Eosinophil percentage and (B) eosinophil absolute count were higher in hypertension (HTN) compared with normotensives (NT) but the levels were within the reference range. (C) IL-5 was higher in HTN than in NT and the levels were both above the expected levels. Analysis of eosinophils was performed using automated differential count of white blood cells and expressed as percentage (A) and absolute counts (B). Interleukin-5 was analyzed using enzyme-linked immunosorbent assay in plasma. **$p<0.01$ using the Mann-Whitney U test. Expected values: Eosinophil percentage = 0-5; Eosinophil absolute count = 0 – 500µl; IL-5 = 0 – 7 pg/mL

The positive correlation between eosinophils and hypertension in virally suppressed PWH remained significant after adjusting for age, sex and FMI in a multivariate analysis (Table 22). IL-17, IL-6, TNFaR1, MIP1-a, MCP1, VCAM-1, ICAM-1, sCD163, $CD4^{+}CD3^{+}$ T cells and IL-5 did not remain significantly associated with hypertension (Table 22).

Table 22. Association between hypertension and inflammatory cell subset/biomarkers in HIV using logistic regression adjusted for fat mass index, age, and sex.

					Adjusted OR (75% to 25%)	**Adjusted OR (75% to 25%, 95% CI)**		
Variable	25% Quantile	75% Quantile	Value Diff (75%-25%)	p-value	Odds Ratio (Diff: 75%-25%)	OR (Diff, Lower 95%)	OR (Diff, Upper 95%)	p-value
IL-17	36.25	87	50.75	0.056	1.4	0.708	2.766	0.333
IL-6	2.282	5.743	3.46	0.09	1.064	0.492	2.301	0.874
TNFaR1	9927.125	13650.625	3723.5	**0.005**	1.616	0.817	3.196	0.168
MIP1-a	121.75	199.75	78	**0.025**	1.905	0.961	3.775	0.065
MCP1	421.75	574.5	152.75	0.094	1.458	0.7	3.036	0.313
ICAM1	445708	646911.125	201203.125	**0.033**	1.814	0.821	4.008	0.141
VCAM1	471585.5	729.025	312.05	**0.04**	1.733	0.787	3.815	0.172
sCD163	416.975	5.743	3.46	0.09	1.064	0.492	2.301	0.874
$CD4^+CD3^+$ T cells	5.3	10.3	5.6	**0.006**	0.394	0.158	0.985	0.063
IL-5	4.645	9.448	4.803	**0.013**	1.988	0.975	4.055	0.059
Eosinophils	1.1	2.7	1.6	**0.007**	2.797	1.106	7.078	**0.03**

Notably, it was found that the eosinophil maturation and differentiation factor IL-5 was also associated with hypertension in virally suppressed PWH in a univariate analysis. This association was robust. The finding of both high circulating eosinophils and increased plasma levels of a key maturation factor (IL-5) strongly suggests that expansion of the eosinophil population are more likely to be a feature of hypertension in HIV.
Given the small sample size, a goodness of fit analysis using the p-values and the Nagelkerke R-squared as well as the C-index analysis was peformed. Both these tests indicated goodness of fit (Appendix D and E).

Comprehensive fat mass measurements were also performed and compared between hypertensive and normotensive and the results are presented in Table 23. The total fat mass, FMI, fat mass in the left arm, left trunk, right arm, right trunk and in the android was higher in the hypertensive participants compared with the normotensive ($p<0.05$). Fat mass in the left and right legs and in the gynoid were not statistically different between the hypertensive and normotensive participants.

Table 23. Fat Mass measurements between hypertensives and normotensive

	HIV Positive n=69		p-value
	Normotensive n=39 *median (IQR)*	**Hypertensive n=30** *median (IQR)*	
Left Arm FM, kg	1.18 (0.80, 1.53)	1.56 (1.21, 2.15)	**0.003**
Left Leg FM, kg	4.82 (2.90, 6.10)	4.71 (3.26, 7.05)	0.321
Left trunk FM, kg	7.18 (3.60, 10.83)	11.62 (8.88, 15.64)	**<0.001**
Left total FM, kg	13.23 (7.29, 19.33)	17.85 (13.86, 25.58)	**0.003**
Right arm FM, kg	1.25 (0.81, 1.60)	1.59 (1.32, 2.15)	**0.002**
Right leg FM, kg	4.88 (2.96, 6.07)	5.59 (3.55, 7.64)	0.160
Right trunk FM, kg	7.40 (3.49, 11.07)	11.76 (8.73, 15.91)	**0.001**
Right total FM, kg	13.80 (7.89, 19.32)	18.31 (14.24, 25.40)	**0.003**
Arms FM, kg	2.43 (1.60, 3.19)	3.13 (2.53, 4.31)	**0.003**
Legs FM, kg	9.77 (5.85, 12.17)	9.88 (6.82, 14.40)	0.204
Trunk FM, kg	14.09 (7.09, 21.84)	22.81 (17.51, 31.61)	**<0.001**
Android FM, kg	2.58 (1.03, 3.90)	4.14 (3.11, 6.18)	**0.001**
Gynoid FM, kg	4.31 (3.10, 6.49)	5.72 (3.92,7.46)	0.056
Total FM, kg	27.03 (15.04, 38.47)	36.04 (28.10, 51.22)	**0.003**
FMI, kg/m^2	8.8 (5.7, 13.2)	13.3 (9.9, 17.5)	**0.003**

Wilcoxon rank sum test used. FM, fat mass, FMI, fat mass index. P values less than 0.05 are shown in bold.

4.2.3 Control Cohort 5 and 6: Vanderbilt University Medical Center and Vanderbilt Synthetic Derivative control cohort results

To determine whether hypertension was also associated with elevated eosinophil counts in HIV negative individuals, prodded by the novel findings from cohort 4 (AIAC), an additional Vanderbilt University Medical Center (VUMC) cohort of 50 HIV-negative participants including 25 normotensives and 25 hypertensives and another cohort comprising of 81, 039 from the Vanderbilt synthetic derivative data base (VSDD) cohort was recruited. Characteristics of the VUMC cohort of participants are shown in Table 24. Hypertensives had higher systolic and diastolic blood pressure, higher MAP and BMI compared with the normotensive participants.

Table 24. Demographics and Clinical Data For HIV-negative Subjects

	Normotensive controls (n=25)	**Hypertensive** patients (n=25)	**p-value**
Age median years (IQR)	40.4 (27.5, 51.2)	45.5 (38.3, 54.9)	*<0.06*
Female n (%)	20(80%)	18(72%)	1.00
Male, n (%)	5 (20%)	7 (28)	
Whites n (%)	19(76%)	17(68%)	1.00
Non whites, n (%)	6 (24%)	8 (32%)	
Systolic BP (mmHg, median (IQR)	112.0 (105.7, 121.0)	137.0 (121.0, 147.0)	**<0.0002**
Diastolic BP (mmHg, median (IQR)	68.00 (62.67, 74.00)	80.00 (74.00, 87.00)	**<0.0001**
MAP (mmHg, median (IQR)	82.67 (76.11, 88.78)	97.00 (90.44, 106.00)	**<0.0001**
BMI median (IQR) kg/m2	23.80 (21.12, 27.05)	32.20 (28.10, 36.00)	**<0.0001**

n = number of subjects in each group; x±SEM = mean ± standard error of the mean; F = female; M= male; W= white; BP = blood pressure; MAP = mean arterial pressure; BMI = body mass index. Non whites included African American blacks, Asians and native Americans. P values for the unpaired t-test and chi-square comparisons between groups are in the rightmost column.

It was found that eosinophil counts were higher in hypertensive when compared to normotensive people without HIV (Figure 25) in both the Vanderbilt University Medical Center cohort (A and B) and the Vanderbilt synthetic derivative database cohort (C). However, the levels were within the expected range. This suggests that there is a possible biological interaction occurring that may be related to hypertension. Although a plausible explaination is beyond the scope of this study, more is discussed in section 5.2.2.1.

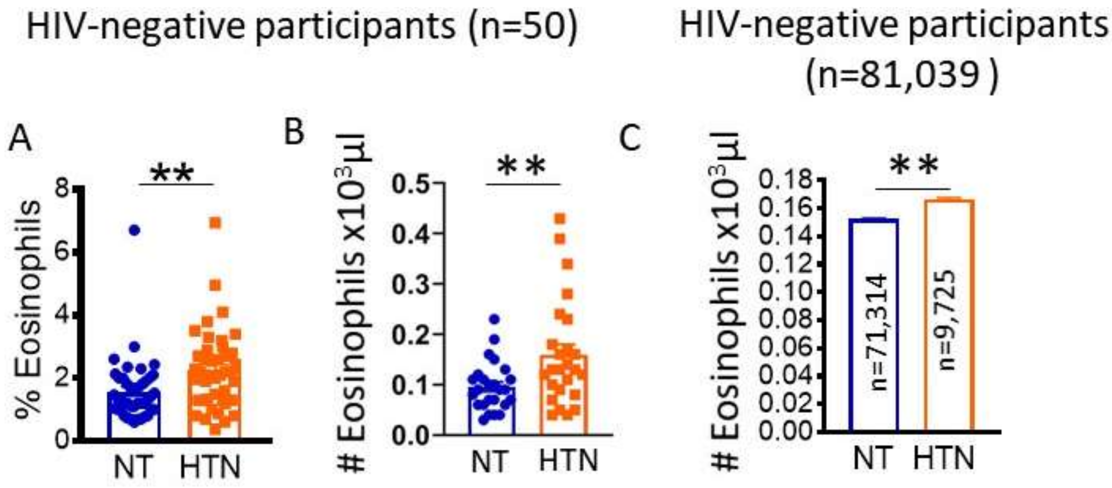


Figure 25. Eosinophil in the VUMC and Vanderbilt synthetic database of HIV negative participants

Elevated percentage and absolute counts of eosinophils in blood is associated with increased hypertension in human immunodeficiency virus-negative normotensive and hypertensive participants. Analysis of eosinophils was performed using automated differential count of white blood cells and expressed as percentage and absolute counts in HIV negative participants. **$p<0.01$ using the Mann-Whitney U test. A is the Vanderbilt university medical center cohort and B is the Vanderbilt synthetic derivative database cohort.

However, in a multivariate analysis after adjusting for sex, age and BMI, eosinophil counts were not associated with hypertension, but they were significantly associated with increased BMI in the VUMC cohort (See Table 25 for details).

In addition, a regression analysis was performed on the combined cohort comprising of both the 70 HIV-positive and the 50 HIV-negative participants. The clinical characteristics for the combined group are shown in Table 26. After multivariate analysis of this combined cohort controlling for age, sex and BMI, the association between eosinophils and hypertension was robust but did not reach statistical significance again (Table 25 and 27). The prediction results with one unit increase of eosinophilia in HIV positive and negative cohorts is shown in appendix E. These results suggest that unlike in HIV-negative participants where higher BMI explains the elevated eosinophil counts, HIV infection is accompanied by adipose tissue dysfunction that could mimic obesity, and this may increase eosinophil counts in HIV even without increase in BMI.

Table 25. Association between hypertension and demographic and clinical characteristics in the HIV-negative cohort using logistic regression adjusted for body mass Index, age, and gender.

				OR (75% to 25%)	**OR (75% to 25%, 95% CI)**			**Adjusted OR (75% to 25%)**	**Adjusted OR (75% to 25%, 95% CI)**		
Variable	25% Quantile	75% Quantile	Value Diff (75%-25%)	OR (Diff: 75%-25%)	OR (Diff, Lower 95%)	OR (Diff, Upper 95%)	p-value	Odds Ratio (Diff: 75%-25%)	OR (Diff, Lower 95%)	OR (Diff, Upper 95%)	p-value
Eosinophil	68.95	153.7	84.75	2.543	1.11	5.826	**0.027**	2.646	0.8318	8.416	0.0993
Age	36.39	54.23	17.84	1.978	0.848	4.616	0.115	1.955	0.6237	6.13	0.25
BMI	23.8	32.7	8.9	11.577	2.799	47.877	**<0.001**	12.43	2.684	57.58	**0.0013**
Gender, M:F				1.556	0.419	5.779	0.509	1.58	0.2535	9.845	0.6243

BMI, body mass index; M, male; F, female

When the HIV positive and HIV negative cohorts were combined, the distribution of sex and HIV status was different but eosinophil count, age, BMI and hypertension status were not significantly different (Table 26).

Table 26. Demographics and clinical data for both the 50 HIV-negative and 70 HIV-positive cohorts combined

	HIV$^-$ ***n=50***	**HIV$^+$** ***n=70***	**Combined** ***n=120***	**p-value**
Eos median (IQR)	1.68 (1.22, 2.20)	1.70 (1.10, 2.70)	1.70 (1.10, 2.43)	0.793
Age median years (IQR)	44.34 (36.39, 54.23)	45.00 (39.00, 50.00)	45.00 (37.24, 51.00)	0.868
Female n (%)	38(76)	30(43)	68(57)	**<0.001**
Male n (%)	12 (24)	40 (57)	52 (43)	
BMI median (IQR) kg/m2	27.60 (23.80, 32.70)	30.29 (23.98, 35.61)	28.30 (23.84, 34.60)	0.192
Hypertensive	25(50)	31(44)	56(47)	0.536
HIV	0(0)	70(100)	70(58)	**<0.001**

n is the number of subjects in each group. BMI, body mass index; Eos, eosinophils; IQR, interquartile range

In multivariate logistic regression as shown in Table 27, eosinophil count, sex and HIV status were not significantly associated with hypertension when the 50 HIV negative were combined with the 70 HIV positive participants. However, age and BMI were significantly associated with hypertension.

Table 27. Association between hypertension and demographic/clinical characteristics in the both the 50 HIV-negative and 70 HIV-positive cohorts' logistic regression

Variable	OR	OR (Lower 95%)	OR (Upper 95%)	p-value
Eosinophil	1.518	8.004706e-06	0.007796146	0.0521
Age	1.063	1.015124e+00	1.113708391	**0.0095**
BMI	1.177	1.093814e+00	1.266014678	<**0.0001**
Gender, M:F	2.011	9.961915e-01	2.314035505	0.1561
HIV	0.399	1.523392e-01	1.047409729	0.0621

BMI, body mass index; M, male; F, female

An additional study using the Synthetic Derivative, (a Vanderbilt University Medical Center database of ~2.5x10^6 de-identified electronic medical records) to identify additional HIV-negative normotensive and hypertensive participants was done. This was done to increase power and further justify the novel finding that eosinophils were higher in hypertensive when compared to normotensive individuals but within the expected range. The characteristics of this cohort are shown in Table 28. Hypertensive participants were older, had more males, more whites, higher eosinophil count and higher BMI compared to the normotensive participants (p<0.001). However, the eosinophil count was within the expected range.

Table 28. Demographics and clinical data for the confirmatory HIV-negative cohort from the synthetic derivative

	Normotensive controls (n= 71314)	**Hypertensive** patients (n= 9725)	p-value
Age median years (IQR)	43.77 (36.02, 53.37)	51.75 (43.34, 56.95)	***<0.001***
Female n (%)	39912 (56)	3911 (40)	***<0.001***
Male n (%)	31402 (44)	5814 (60)	
Whites n (%)	63538 (89)	8010 (82)	***<0.001***
Non whites (%)	7776 (11)	1715 (18)	
Eos median (IQR)	0.120 (0.065, 0.200)	0.135 (0.080, 0.220)	***<0.001***
BMI median (IQR) kg/m2	27.02 (23.43, 31.73)	30.98 (26.58, 36.38)	***<0.001***

n = number of subjects in each group. Non whites included African American blacks, Asians and native Americans. BMI = body mass index. P values for the unpaired t-test and chi-square comparisons between groups are in the rightmost column.

In the VSDD cohort of 81, 039, it was found that hypertensive HIV-negative subjects had a higher eosinophil count when compared to the normotensives (165.81±1.29, n=9,725 hypertensive versus 151.97±0.49, n=71,314 normotensive, p<0.0001). However, like the smaller cohort (VUMC) of 50 HIV-negative participants, higher eosinophil count was significantly correlated with the higher BMI in the hypertensive participants (p<0.000001). It is possible, that there is a link between hypertension and eoniphil count given that eosinophil count remained significantly associated with hypertension following a multivariate analysis (Table 29) in the VSDD cohort. Although a biological explaination for this was beyond the scope of this study, possible explainations are suggested and discussed in detail in section 5.2.2.1.

Eosinophil count, age, BMI and gender remained significantly associated with hypertension in multivariate logistic regression (Table 29).

Table 29. Association between hypertension and demographic and clinical characteristics in the HIV-negative cohort from the synthetic derivative using logistic regression

Variable	25% Quantile	75% Quantile	Value Diff (75%-25%)	OR (Diff: 75%-25%)	OR (Diff, Lower 95%)	OR (Diff, Upper 95%)	p-value
Eosinophils	0.069	0.2	0.131	1.034	1.012	1.056	**0.0021**
Age	36.435	54.204	17.769	1.981	1.902	2.064	**<0.0001**
BMI	23.687	32.365	8.678	1.738	1.697	1.779	**<0.0001**
Gender, M:F				1.893	1.810	1.979	**<0.0001**

BMI, body mass index; M, male; F, female

4.3.0 HIV, Immune-activation Salt-sensitive hypertension study results

4.3.0.1 Specific objective three

The objective of this study was to quantify and compare the effect of high dietary salt on immune cell activation and blood pressure

4.3.1 Sub-cohort 3

Hypertensives were 43 and normotensives were 42 in the combined group (Table 30). The distribution of age, sex and HIV status was equal in the whole study group between the hypertensive and normotensive groups (Table 30). Median age was 40. Among hypertensive participants, 84% (36) were salt sensitive and 16% (7) were salt resistant. Among the normotensive participants, 19% (8) were salt sensitive and 81% (34) were salt resistant. Salt sensitivity was associated with hypertension ($p<0.001$)

Table 30. Combined HIV positive and HIV negative Study cohort demographics

	Hypertensive, 43	**Normotensive n, 42**	**p-value**
Age	40 (38, 42)	40 (38, 41)	0.389
Sex, *n(%)*			
Male	20 (46.5)	18 (42.9)	0.735
Female	23 (53.5)	24 (57.1)	
HIV Status, *n(%)*			
Positive	22 (51.2)	21 (50.0)	0.915
Negative	21 (48.8)	21 (50.0)	
Salt sensitivity, *n(%)*			
Salt sensitive	36 (83.7)	8 (19.0)	**<0.001**
Salt resistant	7 (16.3)	34 (81.0)	

Column percentage shown

When segregated by salt sensitivity status (Table 31), salt sensitivity was associated with hypertension and dipping status on low and high salt diet in the HIV positive and HIV negative group (Table 31). The prevalence of salt sensitivity among hypertensive and normotensive was 91% and 9% in the HIV positive group and 71% and 29% in the HIV negative group respectively. Non-dipping increased by about 26% when shifting from low to high salt diet among salt-sensitive non-dippers in the HIV positive group while non-dipping increased by about 28% when shifting from low to high salt among salt-sensitive non-dippers in the HIV negative group.

Table 31. General characteristics of the HIV positive and HIV negative group

	HIV positive group			**HIV negative group**		
	Salt-sensitive n, 23	**Salt-resistant n, 20**	**p-value**	**Salt-sensitive n, 21**	**Salt-resistant n, 21**	**p-value**
Age, *median (interquartile range)*	40 (39, 42)	41 (38, 42)	0.883	39 (37, 41)	40 (37, 43)	0.527
Sex, *n(%)*						
Male	12 (60.0)	8 (40.0)	0.425	9 (42.9)	9 (42.9)	1.00
Female	11 (47.8)	12 (52.2)		12 (57.1)	12 (57.1)	
Body mass index, *kg/m²*	24 (20, 27)	22 (19, 25)	0.268	25 (23, 29)	23 (21, 25)	0.087
Hypertension status, *n(%)*						
Hypertensive	21 (95.5%)	1 (4.5%)	**<0.001**	15 (71.4)	6 (28.6)	**0.005**
Normotensive	2 (9.5)	19 (90.5)		6 (28.6)	15 (71.4)	
Dipping status on low salt, *n (%)*						
Dipper, ≥10%	8 (33.3)	16 (66.7)	**0.003**	8 (33.3)	16 (66.7)	**0.013**
Non-dipper, <10%	15 (78.9)	4 (21.1)		13 (72.2)	5 (27.8)	
Dipping status on high salt, *n (%)*						
Dipper, ≥10%	2 (10.5)	17 (89.5)	**<0.001**	2 (11.8)	15 (88.2)	**<0.001**
Non-dipper, <10%	21 (87.5)	3 (12.5)		19 (76.0)	6 (28.6)	

Row percentage shown

Dipping status was conducted using ambulatory blood pressure monitor. The ambulatory BP automated report showing dipping results is shown in Figure 26.

Examine Result

All BP Averages:	109.8/69.8mmHg		
Day BP Averages:	112.3/71.9mmHg	**BP threshold:**	120/85mmHg
Night BP Averages:	98.5/60.3mmHg	**BP threshold:**	110/70mmHg

Day BP Load Value:Nomal<40%	**Night BP Load Value:Nomal<50%**
SYS(>120mmHg) 17.2%	SYS(>110mmHg) 7.7%
DIA(>85mmHg) 1.7%	DIA(>70mmHg) 7.7%

Maximum SYS	143mmHg	**Time**	2019/6/24 09:30	**Minimum SYS**	83mmHg	**Time**	2019/6/24 02:30
Maximum DIA	88mmHg	**Time**	2019/6/24 12:45	**Minimum DIA**	46mmHg	**Time**	2019/6/24 02:30

Circadian rhythm of BP:SYS Night Dec. 12.3% **DIA Night Dec.** 16.2% **Normal:10%-20%**

BP CV:	**All:SYS**	10.3%	**DIA**	10.9%
	Day:SYS	9.2%	**DIA**	8.3%
	Night:SYS	8.2%	**DIA**	11.2%

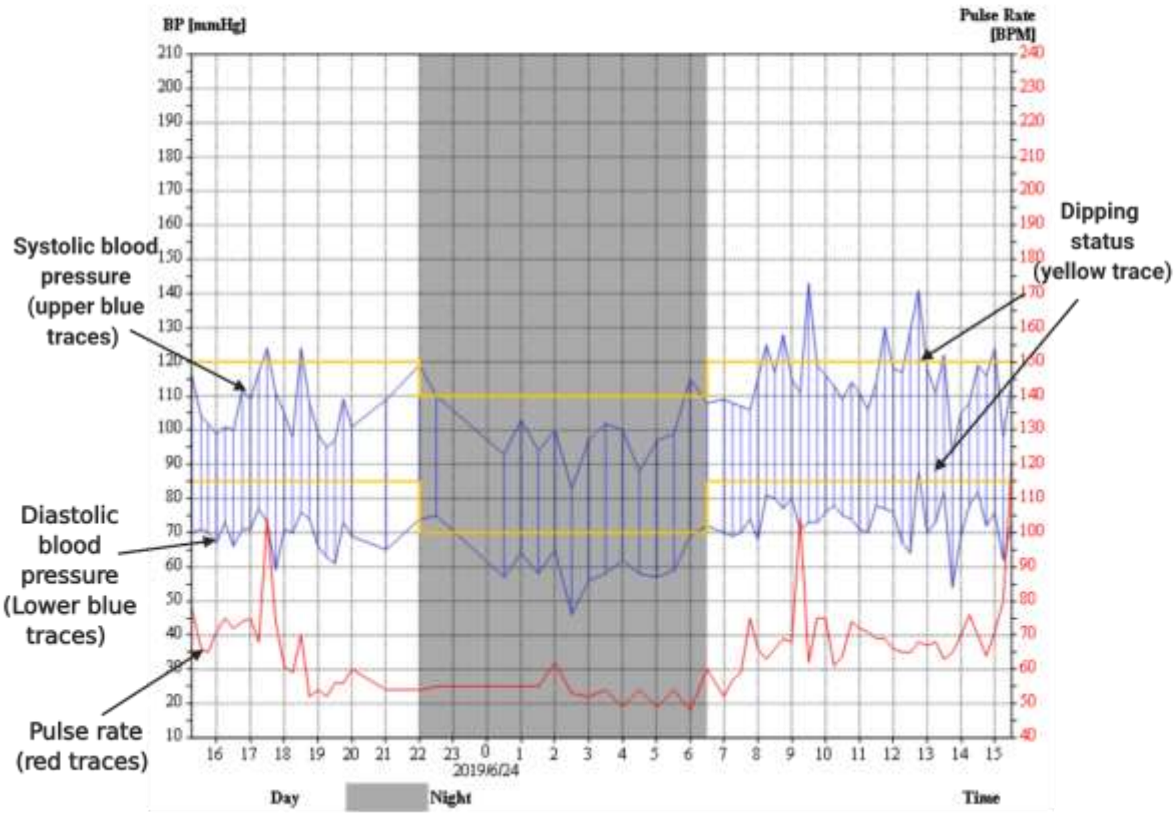


Figure 26. Dipper report for JNC 7 normotensive HIV positive participant
Showing an excerpt report from an ambulatory blood pressure monitor PM50. The dipping status yellow trace follows the blood pressure of an HIV positive paticipant. In this case, a symmetrical valley between 22:00hrs when the paticipant was sleeping and 06:30 hrs when the paticipant woke up, represents the dipping phase. Both systolic and diastolic blood pressure dropped by more than 10%. This participant was a normotensive by JNC 7 criteria.

The ambulatory BP automated report showing dipping results is shown in figure 27.

Examine Result

All BP Averages:	132.9/89.0mmHg		
Day BP Averages:	131.8/89.0mmHg	**BP threshold:**	120/70mmHg
Night BP Averages:	136.0/88.9mmHg	**BP threshold:**	110/60mmHg

Day BP Load Value:Nomal<40%	**Night BP Load Value:Nomal<50%**
SYS(>120mmHg) 77.4%	SYS(>110mmHg) 100.0%
DIA(>70mmHg) 98.1%	DIA(>60mmHg) 100.0%

Maximum SYS	175mmHg	**Time**	2019/9/20 21:30	**Minimum SYS**	107mmHg	**Time**	2019/9/20 15:30
Maximum DIA	112mmHg	**Time**	2019/9/20 21:00	**Minimum DIA**	70mmHg	**Time**	2019/9/20 11:45

Circadian rhythm of BP:SYS Night Dec. -3.1% **DIA Night Dec.** 0.1% **Normal:10%-20%**

BP CV:	**All:SYS**	10.1%	**DIA**	9.7%
	Day:SYS	10.3%	**DIA**	10.5%
	Night:SYS	9.2%	**DIA**	6.8%

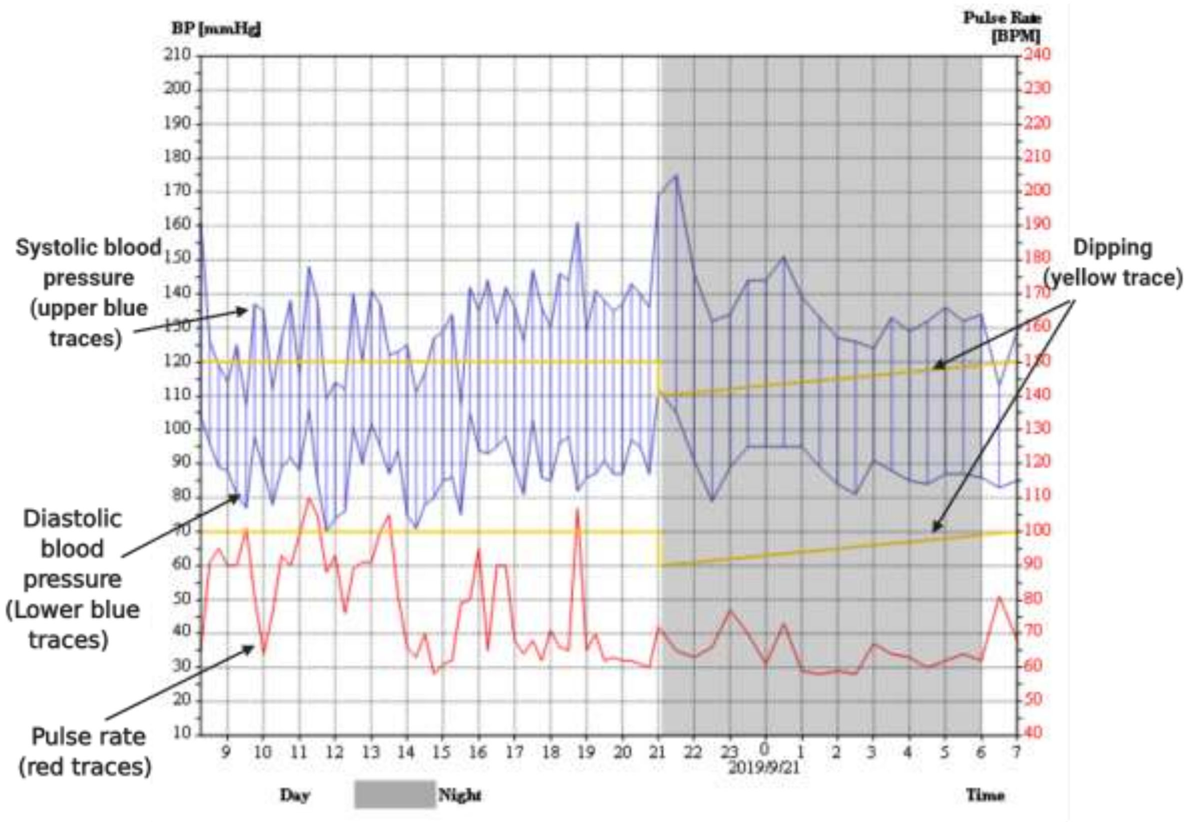


Figure 27. Non-dipper report for JNC 7 hypertensive HIV positive participant

Showing an excerpt report from an ambulatory blood pressure monitor PM50. The dipping status yellow trace follows the blood pressure of an HIV positive paticipant. In this case, no symmetrical valley is created between 21:00hrs when the paticipant was sleeping and 06:00 hrs when the paticipant woke up, representing no dipping phase. Both systolic and diastolic blood pressure remained approximately constant similar to day blood pressure. This participant was a hypertensive by JNC 7 criteria.

Salt sensitivity was associated with IL-6, monocyte count, CD45 CD80+ and IsoLG expression in monocytes in HIV positive participants but non of the markers were associated with salt sensitivity in the HIV negative group prior to the low- and high-salt diets (Table 32). However, IL-6 levels and monocyte count were within the expected range.

Table 32. Clinical outcomes and salt-sensitivity in the HIV positive and HIV negative group

	HIV Positive			HIV Negative		
Clinical characteristics	**Salt-sensitive n, 23 (IQR)**	**Salt-resistant n, 20 (IQR)**	**p-value**	**Salt-sensitive n, 21 (IQR)**	**Salt-resistant n, 21 (IQR)**	**p-value**
IL-5, *pg/ml*	3.8 (2.2, 5.8)	3.6 (2.2, 7.5)	0.761	2.4 (1.3, 5.1)	2.5 (1.3, 3.7)	0.821
IL-13, *pg/ml*	1.9 (0.9, 3.9)	3.1 (1.2, 5.1)	0.286	2.5 (1.5, 3.9)	3.5 (2.3, 7.0)	0.092
IL-2, *pg/ml*	2.4 (1.7, 3.4)	2.8 (2.2, 3.3)	0.635	2.7 (2.0, 3.5)	3.1 (2.3, 3.8)	0.473
IL-6, *pg/ml*	3.4 (2.1, 5.4)	1.8 (1.1, 2.5)	**0.002**	2.5 (1.4, 3.0)	2.1 (1.0, 3.1)	0.589
IL-9, *pg/ml*	1.1 (1.0, 1.6)	1.4 (1.1, 2.0)	0.202	1.4 (1.3, 1.8)	1.7 (1.3, 2.1)	0.687
IL-10, *pg/ml*	2.6 (1.5, 5.8)	2.2 (1.5, 2.7)	0.066	2.6 (1.8, 2.8)	2.1 (1.5, 3.3)	0.669
Interferon-gamma, *pg/ml*	2.4 (1.6, 3.5)	2.7 (1.9, 4.1)	0.543	3.1 (2.4, 3.6)	3.2 (2.1, 5.2)	0.782
Tumor necrosis factor-alpha, *pg/ml*	2.4 (1.3, 4.4)	2.2 (1.3, 3.3)	0.414	4.3 (2.1, 6.3)	2.5 (1.4, 3.4)	0.053
IL-17A, *pg/ml*	1.9 (1.1, 2.8)	1.2 (0.9, 1.7)	0.147	1.6 (1.1, 3.0)	1.4 (0.8, 2.8)	0.597
IL-17F, *pg/ml*	1.7 (1.2, 3.3)	2.0 (1.6, 3.1)	0.450	2.5 (1.3, 3.2)	2.1 (1.1, 2.5)	0.199
IL-4, *pg/ml*	1.3 (0.8, 2.4)	1.6 (1.0, 2.7)	0.494	1.7 (1.4, 2.4)	1.9 (1.5, 2.8)	0.159
IL-21, *pg/ml*	0.56 (0.38, 0.91)	0.58 (0.37, 0.74)	0.903	0.8 (0.6, 1.6)	0.8 (0.6, 1.7)	0.753
IL-22, *pg/ml*	5.0 (3.1, 5.7)	5.6 (3.8, 7.1)	0.252	3.7 (1.2, 6.5)	4.3 (3.1, 5.6)	0.465
Neutrophils, $x10^9$cell/L	2.6 (2.1, 3.5)	1.9 (1.5, 3.7)	0.105	0.35 (0.17, 0.46)	0.27 (0.15, 0.45)	0.521
Lymphocytes, $x10^9$cell/L	2.1 (1.5, 2.6)	1.9 (1.2, 2.2)	0.361	0.36 (0.10, 1.00)	0.25 (0.05, 0.40)	0.255
Monocytes, $x10^9$cell/L	0.58 (0.37, 0.75)	0.34 (0.25, 0.44)	**0.002**	37.3 (32.2, 47.8)	38.4 (32.8, 42.3)	0.763
CD45 CD80+, *%*	4.1 (3.5, 4.6)	2.9 (0.5, 3.6)	**0.004**	1.5 (0.7, 3.2)	1.7 (0.7, 3.4)	0.920
CD14 CD86+, %	45 (34, 57)	41 (28, 54)	0.289	38.4 (29.1, 48.2)	26.3 (19.7, 36.8)	0.064
Siglec-8 on CD45+, %	1.8 (1.3, 2.5)	1.1 (0.4, 2.2)	0.10	39.4 (33.6, 44.8)	38.3 (36.0, 43.9)	0.870
D11 on CD14+, %	39 (35, 51)	31 (25, 37)	**<0.001**	2.4 (1.3, 5.1)	2.5 (1.3, 3.7)	0.821
CD14 CD80+, %	40 (34, 50)	45 (37, 51)	0.551	2.5 (1.5, 3.9)	3.5 (2.3, 7.0)	0.092

Row percentage used; Wilcoxon rank-sum test used. Expected values are indicated in appendix A under the 'biological samples' section. Medians are presented with interquartile range (IQR). Participants grouped into Salt sensitive and salt resistance regardless of hypertension status.

Salt-sensitive HIV positive individuals secreted less sodium in a random 24-hour sample, more sodium in a nocturnal sample on high salt diet and first morning urine as shown in Table 33 in the HIV positive group. Random 24-hour potassium and chloride excretion, nocturnal potassium and chloride excretion on low and high salt diets were not significantly different between the salt sensitive and salt resistant groups. The 24-hour sodium, potassium and chloride excretion on low and high salt diets were also not significantly different between the salt sensitive and salt resistant groups (Table 33).

Table 33. Electrolytes excretion and salt-sensitivity in the HIV positive group

Clinical characteristics	Salt-sensitive n, 23	Salt-resistant n, 20	p-value
Random 24-hr urine potassium excretion, *mmol/day*	70.8 (53.4, 92.8)	73.5(56.5, 92.0)	0.75
Random 24-hr urine sodium excretion, *mmol/day*	205.4 (169.7, 248.2)	279.5 (245.0, 333.6)	**0.007**
Random 24-hr urine Chloride excretion, *mmol/l*	84.9 (64.7, 152.6)	95.4 (81.9, 166.9)	0.40
Nocturnal potassium excretion on low-salt diets, mmol/l	14.2 (11.3, 16.2)	13.4 (11.8, 17.2)	0.94
Nocturnal sodium excretion on low-salt diets, mmol/l	40.2 (33.2, 55.6)	33.1 (29.4, 48.9)	0.05
Nocturnal chloride excretion on low-salt diets, mmol/l	57.3 (45.6, 71.1)	49.2, 38.6, 68.0)	0.09
Nocturnal potassium excretion on high-salt diets, mmol/l	27.0 (23.4, 33.5)	36.2 (29.3, 42.6)	0.05
Nocturnal sodium excretion on high-salt diets, mmol/l	77.6 (65.3, 90.0)	65.2 (56.5, 74.5)	**0.009**
Nocturnal chloride excretion on high-salt diets, mmol/l	89.4 (79.3, 101.6)	78.4 (68.5, 90.0)	0.05
24-hr potassium excretion on low-salt diets, *mmol/l*	147.6 (101.8, 176.9)	145.1 (114.8, 186.0)	0.94
24-hr sodium excretion on low-salt diets, *mmol/day*	78.65 (75.0, 84.2)	78.65 (73.0, 80.8)	0.40
24-hr chloride excretion on low-salt diets, *mmol/l*	281.0 (226.5, 326.8)	305.8 (222.9, 439.8)	0.18
24-hr potassium excretion on high-salt diets, *mmol/l*	293.3 (234.2, 344.2)	282.8 (205.8, 315.6)	0.36
24-hr sodium excretion on high-salt diets, *mmol/day*	225.8 (189.7, 279.5)	207.9 (156.1, 259.8)	0.40
24-hr chloride excretion on high-salt diets, *mmol/l*	1415.0 (1134.2, 1690.3)	1611.9 (1120.6, 1788.9)	0.42
First morning urine potassium excretion, mmol/l	34.1 (20.4, 63.0)	35.0 (15.5, 47.1)	0.46
First morning urine sodium excretion, mmol/l	118.4, 85.1, 186.9)	85.5 (49.7, 119.0)	**0.03**
First morning urine chloride excretion, mmol/l	239.0 (156.7, 477.0)	279.9 (90.3, 587.2)	0.71

Participants are grouped into Salt sensitive and salt resistance regardless of hypertension status. Row percentage shown; Wilcoxon rank sum test used. P-value less than 0.05 are in bold. Medians are presented with interquartile range (IQR).

In the HIV negative group, the salt-sensitive group secreted more sodium and chloride in random 24-hour urine excretion and nocturnal sodium excretion on low salt as shown in Table 34. Random 24-hour potassium excretion, nocturnal potassium and chloride excretion on low and high salt diets were not statistically significant between the salt sensitive and salt resistant groups. In addition, nocturnal sodium excretion on high salt diet, 24-hour potassium, sodium, chloride excretion and first morning urine excretion of potassium, sodium and chloride did not differ significantly between groups (Table 34).

Table 34. Electrolytes excretion and salt-sensitivity in the HIV negative group

Clinical characteristics	Salt-sensitive n, 21	Salt-resistant n, 21	p-value
Random 24-hr urine potassium excretion, *mmol/day*	71.3 (54.6, 84.6)	61.5 (51.1, 71.9)	0.06
Random 24-hr urine sodium excretion, *mmol/day*	255.2 (216.5, 295.8)	204.8 (162.6, 240.7)	**0.006**
Random 24-hr urine Chloride excretion, *mmol/day*	111.6 (86.9, 140.8)	95.4.(60.7, 105.3)	**0.029**
Nocturnal potassium excretion on low-salt diets, mmol/l	12.3 (11.2, 15.3)	12.5 (11.1, 13.4)	0.50
Nocturnal sodium excretion on low-salt diets, mmol/l	51.4 (34.1, 60.3)	37.5 (31.2, 45.3)	**0.027**
Nocturnal chloride excretion on low-salt diets, mmol/l	64.2 (42.1, 77.4)	47.4 (41.8, 55.4)	0.07
Nocturnal potassium excretion on high-salt diets, mmol/l	48.3 (37.2, 60.9)	51.3 (42.2, 54.2)	0.94
Nocturnal sodium excretion on high-salt diets, mmol/l	84.3 (77.3, 89.3)	74.5 (72.2, 83.1)	0.06
Nocturnal chloride excretion on high-salt diets, mmol/l	96.4 (91.2, 111.0)	93.4 (85.3, 109.3)	0.41
24-hr potassium excretion on low-salt diets, *mmol/l*	123.2 (106.4, 149.4)	130.8 (111.8, 176.1)	0.45
24-hr sodium excretion on low-salt diets, *mmol/day*	78.6 (75.0, 84.1)	78.1(72.6, 81.1)	0.49
24-hr chloride excretion on low-salt diets, *mmol/l*	255.6 (232.7, 301.1)	264.6 (216.1, 313.6)	0.77
24-hr potassium excretion on high-salt diets, *mmol/l*	268.5 (239.8, 308.0)	303.2 (247.9, 330.4)	0.20
24-hr sodium excretion on high-salt diets, *mmol/day*	209.1 (186.3, 248.1)	228.9 (187.3, 256.8)	0.38
24-hr chloride excretion on high-salt diets, *mmol/l*	1368.6 (1116.3, 1604.6)	1180.5 (1019.5, 1609.4)	0.67
First morning urine potassium excretion, mmol/l	26.1 (11.4, 54.5)	25.2 (14.9, 54.9)	0.83
First morning urine sodium excretion, mmol/l	63.5 (32.9, 89.0)	66.9 (48.8, 114.3)	0.57
First morning urine chloride excretion, mmol/l	258.4 (75.1, 380.9)	131.2 (58.0, 285.6)	0.26

Participants are grouped into salt sensitive and salt resistance regardless of hypertension status. Row percentage shown; Wilcoxon rank sum test used. P-value less than 0.05 are in bold. Medians are presented with interquartile range (IQR).

In the HIV positive group, salt sensitivity remained significantly associated with IL-6, monocyte count, IsoLGs in monocytes, non-dipping blood pressure on both low and high salt diets, random 24-hour sodium and chloride excretion, 24-hr sodium excretion on high salt diet as shown in Table 35.

Table 35. Association between Salt-sensitivity and each clinical-pathological characteristic in HIV positive adults adjusted for sex, age, body mass index, duration on ART

Variable	**Adjusted Odds Ratio AOR (95%CI)**	**p-value**
IL-6, *pg/ml*	2.17 (1.18, 3.98)	**0.013**
Monocyte count, *x10^{9}cell/L*	2.4 (0.44, 13.4)	**0.007**
CD45 CD80, *%*	1.03 (0.85, 1.24)	0.759
CD14 D11 (isoLevuglandin), *%*	1.21 (1.05, 1.40)	**0.008**
Non-dipping blood pressure on low salt	11.86 (2.20, 63.86)	**0.004**
Non-dipping blood pressure on high salt	8.9 (0.9, 86.30	**0.004**
Random 24-hr sodium excretion, *mmol/day*	1.03 (1.00, 1.06)	**0.016**
Random 24-hr Chloride excretion, *mmol/day*	1.04 (1.01, 1.08)	**0.007**
Nocturnal sodium excretion on high salt diet	1.04 (0.98, 1.09)	0.125
24-hr sodium excretion on high salt diet, *mmol/day*	1.01 (1.0, 1.02)	**0.013**
First morning urine sodium	1.11 (0.99, 1.020	0.076

P-value less than 0.05 are in bold

Nocturnal sodium excretion and random 24-hr urine chloride excretion did not remain significantly associated with salt sensitivity in the HIV negative group while random 24-hr sodium excretion remained significantly associated with salt sensitivity (Table 36).

Table 36. Association between Salt sensitivity and each clinical-pathological characteristic in HIV negative adults adjusted for sex, age, body mass index

Variable	Adjusted Odds Ratio AOR (95%CI)	p-value
Nocturnal sodium excretion on low-salt diet	1.03 (0.98, 1.08)	0.134
Random 24-hr sodium excretion, *mmol/day*	1.01 (1.00, 1.03)	**0.026**
Random 24-hr urine Chloride excretion, *mmol/day*	1.01 (0.99, 1.03)	0.091

P-value less than 0.05 are in bold

To confirm if spot urine electrolyte excretion and plasma electrolyte concentrations was different between hypertensive and normotensive PWH, spot urines and plasma were measured with additional participants and all the electrolyte concentrations were similar between groups except plasma chloride, which was lower in the hypertensive compared to the normotensive (Table 37). The implication of this is unknown.

Table 37. Urine and plasma electrolytes between hypertensive and normotensive HIV positive adults

Clinical characteristics	Hypertension n, 25 (IQR)	Normotensive n, 131 (IQR)	p-value
Spot urine potassium excretion, *mmol/l*	33.0 (16.7, 64.3)	31.6 (17.5, 53.7)	0.789
Spot urine sodium excretion, *mmol/l*	85.6 (49.8, 125.0)	95.1 (62.7, 135.7)	0.398
Spot urine Chloride excretion, *mmol/l*	274.7 (117.3, 557.5)	209.2 (89.0, 804.3)	0.133
*Plasma potassium, *mmol/l*	4.19 (3.39, 4.69)	4.22 (3.96, 4.48)	0.660
*Plasma sodium, *mmol/l*	133.5 (132.4, 134.1)	132.4 (131.6, 133.8)	0.376
*Plasma Chloride, *mmol/l*	97.2 (96.0, 99.1)	100.4 (99.1, 101.7)	**0.007**

***5 hypertensive Vs 45 normotensive.** Medians are presented with interquartile range (IQR)

The urine electrolyte analysis was repeated using hypertension status as the outcome. Hypertensive HIV positive participants excreted more random 24-hour urine sodium and chloride, nocturnal urine chloride and sodium on low salt diet, nocturnal urine sodium and chloride on high salt diet, 24-hour urine sodium on low salt and high salt diets and 24-hour urine chloride on high salt diet ($p<0.05$, Table 38). In the HIV negative group (Table 39), hypertensives secreted more random 24-hour urine sodium, nocturnal sodium and chloride on low salt diet, nocturnal sodium on high salt diet, and chloride in a first morning urine sample (p<0.05).

Table 38. Electrolytes excretion and Hypertension in the HIV positive group

Clinical characteristics	Hypertension n,	Normotensive n,	p-value
Random 24-hr urine potassium excretion, *mmol/l*	55.5 (45.4, 64.7)	50.3 (45.0, 58.7)	0.274
Random 24-hr urine sodium excretion, *mmol/l*	186.3 (157.1, 211.5)	156.4 (136.4, 168.2)	**0.004**
Random 24-hr urine Chloride excretion, *mmol/l*	225.9 (178.6, 264.7)	166.8 (152.4, 185.1)	**<0.001**
Nocturnal potassium excretion on low salt diets, *mmol/l*	15.0 (11.3, 16.8)	13.5 (11.9, 15.9)	0.610
Nocturnal sodium excretion on low salt diets, *mmol/l*	39.7 (33.2, 56.0)	33.2 (29.7, 43.1)	0.068
Nocturnal chloride excretion on low salt diets, *mmol/l*	60.1 (45.3, 74.8)	52.1 (39.0, 64.90	**0.029**
Nocturnal potassium excretion on high salt diets, *mmol/l*	27.0 (23.4, 34.4)	36.2 (26.2, 43.2)	0.080
Nocturnal sodium excretion on high salt diets, *mmol/l*	78.0 (69.4, 90.0)	65.3 (57.3, 72.2)	**0.009**
Nocturnal chloride excretion on high salt diets, *mmol/l*	89.4 (79.3, 101.6)	78.4 (72.3, 89.3)	**0.046**
24-hr potassium excretion on low salt diets, *mmol/l*	93.0 (79.4, 103.0)	90.4 (78.4, 112.3)	0.437
24-hr sodium excretion on low salt diets, *mmol/l*	159.4 (153.1, 187.0)	156.3 (145.3, 159.4)	**0.029**
24-hr chloride excretion on low salt diets, *mmol/l*	180.8 (169.4, 204.0)	174.2 (164.2, 187.3)	0.094
24-hr potassium excretion on high salt diets, *mmol/l*	184.6 (177.3, 202.0)	188.0 (174.0, 199.0)	0.688
24-hr sodium excretion on high salt diets, *mmol/l*	597.1 (545.2, 645.0)	543.0 (511.3, 578.0)	**0.011**
24-hr chloride excretion on high salt diets, *mmol/l*	987.5 (884.3, 1213.4)	883.4 (785.0, 994.3)	**0.037**
First morning urine potassium excretion, *mmol/l*	35.5 (21.2, 54.5)	33.0 (14.4, 48.1)	0.451
First morning urine sodium excretion, *mmol/l*	115.9 (85.1, 186.9)	85.6 (50.1, 121.5)	0.068
First morning urine chloride excretion, *mmol/l*	242.2 (156.7, 486.1)	258.4 (103.2, 398.1)	0.846

Row percentage used; Wilcoxon rank sum test used. Medians are presented with interquartile range (IQR). Significant p values below 0.05 are indicated in bold

Table 39. Electrolytes excretion and Hypertension in the HIV negative group

Clinical characteristics	Hypertension n,	Normotensive n,	p-value
Random 24-hr urine potassium excretion, *mmol/l*	44.4 (37.5, 54.0)	44.0 (36.4, 54.4)	0.840
Random 24-hr urine sodium excretion, *mmol/l*	166.4 (150.8, 176.4)	145.3 (137.1, 159.8)	**0.014**
Random 24-hr urine Chloride excretion, *mmol/l*	179.5 (161.8, 197.2)	172.2 (156.8, 183.1)	0.222
Nocturnal potassium excretion on low salt diets, *mmol/l*	13.2 (11.5, 15.3)	12.3 (11.0, 13.4)	0.232
Nocturnal sodium excretion on low salt diets, *mmol/l*	51.4 (39.3, 63.8)	33.3 (22.3, 46.4)	**<0.001**
Nocturnal chloride excretion on low salt diets, *mmol/l*	64.2 (47.6, 79.4)	44.9 (32.9, 55.3)	**<0.001**
Nocturnal potassium excretion on high salt diets, *mmol/l*	51.4 (38.7, 59.3)	49.2 (35.5, 56.8)	0.352
Nocturnal sodium excretion on high salt diets, *mmol/l*	84.3 (75.3, 93.8)	76.3 (70.2, 81.2)	**0.006**
Nocturnal chloride excretion on high salt diets, *mmol/l*	96.3 (87.1, 118.3)	95.4 (88.9, 104.8)	0.435
24-hr potassium excretion on low salt diets, *mmol/l*	88.0 (77.7, 94.7)	85.3 (75.4, 94.7)	0.450
24-hr sodium excretion on low salt diets, *mmol/l*	154.6 (145.8, 158.6)	153.4 (144.1, 156.6)	0.268
24-hr chloride excretion on low salt diets, *mmol/l*	175.0 (164.6, 179.7)	167.4 (157.2, 175.3)	0.186
24-hr potassium excretion on high salt diets, *mmol/l*	188.4 (178.9, 195.3)	188.5 (174.4, 193.5)	0.624
24-hr sodium excretion on high salt diets, *mmol/l*	587.4 (536.8, 601.2)	588.4 (568.4, 599.8)	0.466
24-hr chloride excretion on high salt diets, *mmol/l*	905.3 (824.8, 1003.7)	893.2 (641.4, 9864.7)	0.274
First morning urine potassium excretion, *mmol/l*	33.0 (16.7, 64.3)	18.6 (8.7, 47.7)	0.128
First morning urine sodium excretion, *mmol/l*	73.4 (49.8, 116.5)	57.9 939.6, 85.0)	0.242
First morning urine chloride excretion, *mmol/l*	301.5 (127.1, 557.5)	77.9 (46.9, 147.7)	**0.001**

Row percentage used; Wilcoxon rank sum test used. Medians are presented with interquartile range (IQR). Significant p values below 0.05 are indicated in bold.

Comparison of inflammatory cytokines in the supernatant produced by PBMCs on low and high salt in both HIV negative and HIV positive participants was done and results indicate that only IL-2 in HIV positive and IL-21 in HIV negative groups were significantly different between the low and high salt diets (Table 40 and 41 respectively). However, both cytokines are non-specific, so their relevance is unknown in this case. Among the HIV positive participants, the normotensive but not the hypertensive had higher IL-2 in the high salt diet compared to the low salt diet (Table 40). The rest of the cytokines were not significantly different between the low and high salt diets for both the normotensive and hypertensive groups.

Table 40. Change in Inflammatory markers on low and high salt diet in a selected HIV positive adult group

Clinical characteristics	**Normotensive, n(10)**			**Hypertensive, n (11)**		
	Low salt diet	**High salt diet**	**p-value**	**Low salt diet**	**High salt diet**	**p-value**
IL-5, *pg/ml*	3.2 (2.4, 7.8)	2.9 (2.0, 4.4)	0.322	4.3 (1.4, 5.8)	3.5 (2.3, 4.8)	0.5195
IL-13, *pg/ml*	2.4 (0.9, 5.0)	3.3 (2.3, 4.2)	0.845	2.4 (0.9, 5.1)	3.4 (1.3, 5.3)	0.7646
IL-2, *pg/ml*	2.4 (1.9, 3.2)	2.9 (2.7, 5.2)	**0.0137**	2.4 (1.3, 3.7)	2.3 (1.8, 3.6)	0.7002
IL-6, *pg/ml*	1.8 (1.4, 2.1)	2.1 (1.2, 3.2)	0.5078	3.4 (1.5, 4.1)	3.8 (2.9, 5.2)	0.0674
IL-9, *pg/ml*	1.4 (1.1, 1.7)	1.4 (1.1, 2.6)	0.5566	1.1 (1.0, 3.6)	2.4 (1.3, 3.2)	0.8984
IL-10, *pg/ml*	2.0 (1.3, 2.4)	1.5 (0.4, 2.4)	0.2324	3.7 (2.5, 4.7)	2.5 (1.5, 4.3)	0.4648
Interferon-gamma, *pg/ml*	2.4 (1.5, 3.8)	1.3 (0.3, 3.4)	0.3223	2.4 (1.5, 3.3)	2.4 (1.1, 3.2)	0.3652
Tumor necrosis factor-alpha, *pg/ml*	1.7 (1.3, 3.1)	1.8 (1.4, 2.4)	0.5078	2.2 (1.3, 3.6)	2.4 (1.4, 3.5)	0.4648
IL-17A, *pg/ml*	1.1 (0.8, 1.2)	1.3 (0.9, 2.4)	0.1523	1.7 (1.2, 2.4)	2.1 (1.3, 2.4)	0.7646
IL-17F, *pg/ml*	1.7 (1.4, 2.9)	1.4 (1.0, 2.2)	0.4316	2.0 (1.2, 2.7)	1.7 (1.2, 2.5)	0.7002
IL-4, *pg/ml*	1.4 (1.0, 2.1)	1.4 (1.3, 2.3)	0.6953	1. (0.8, 2.4)	1.7 (1.2, 2.4)	0.3096
IL-21, *pg/ml*	0.5 (0.4, 0.7)	0.4 (0.2, 0.6)	0.1699	0.4 (0.3, 1.0)	0.3 (0.2, 0.4)	0.3770
IL-22, *pg/ml*	5.6 (3.7, 6.4)	3.8 (2.9, 4.7)	0.1309	5.5 (3.1, 7.8)	4.2 (3.5, 6.3)	0.3652

Medians are presented with interquartile range (IQR)

Among the HIV negative participants, all the cytokines were not significantly different between the low and high salt diet for both normotensive and hypertensive groups except for the hypertensive who had higher levels of IL-21 in the low salt diet compared with the high salt diet (Table 41).

Table 41. Change in Inflammatory markers on low and high salt diet in HIV negative adults

Clinical characteristics	**Normotensive, n(8)**			**Hypertensive, n(10)**		
	Low salt diet	**High salt diet**	**p-value**	**Low salt diet**	**High salt diet**	**p-value**
IL-5, *pg/ml*	3.2 (1.8, 6.3)	2.6 (1.5, 4.9)	0.2500	4.0 (1.5, 6.0)	2.9 (1.1, 3.9)	0.4473
IL-13, *pg/ml*	4.9 (2.4, 10.1)	3.4 (3.2, 5.8)	0.3828	2.5 (1.7, 4.6)	2.7 (1.4, 4.8)	0.8457
IL-2, *pg/ml*	2.3 (1.5, 2.5)	2.4 (2.0, 3.2)	0.5469	2.3 (1.1, 2.7)	2.4 (1.8, 3.3)	0.1660
IL-6, *pg/ml*	1.4 (0.5, 2.10	1.5 (0.6, 2.5)	0.8438	1.9 (1.4, 3.0)	1.5 (1.0, 2.4)	0.1016
IL-9, *pg/ml*	1.3 (1.2, 1.8)	1.6 (1.4, 1.8)	0.5469	1.4 (1.3, 1.7)	1.6 (1.1, 1.7)	0.5566
IL-10, *pg/ml*	2.8 (2.1, 3.4)	2.4 (1.3, 2.6)	0.1484	2.6 (1.5, 2.9)	2.5 (1.4, 4.2)	0.8457
Interferon-gamma, *pg/ml*	2.3 (0.8, 3.6)	1.9 (0.7, 3.0)	0.4375	2.0 (1.0, 3.6)	3.6 (3.1, 5.1)	0.0840
Tumor necrosis factor-alpha, *pg/ml*	2.4 (1.3, 4.3)	2.7 (0.6, 3.3)	0.2656	4.4 92.1, 7.5)	4.0 (1.9, 5.7)	0.6250
IL-17A, *pg/ml*	1.2 (0.6, 2.2)	1.7 (1.2, 3.0)	0.5781	2.2 (1.0, 2.5)	2.5 (1.3, 2.7)	0.4316
IL-17F, *pg/ml*	1.4 (0.5, 2.2)	1.8 (1.4, 3.0)	0.3828	2.4 (1.5, 2.6)	2.4 (1.2, 2.8)	0.9219
IL-4, *pg/ml*	1.7 (1.4, 2.5)	1.5 (1.2, 2.1)	0.3125	1.8 (1.3, 2.5)	2.1 (1.2, 2.7)	0.4922
IL-21, *pg/ml*	0.6 (0.2, 0.6)	0.5 (0.3, 0.5)	0.9453	0.9 (0.6, 1.8)	0.5 (0.4, 0.7)	**0.0313**
IL-22, *pg/ml*	3.5 (1.6, 5.7)	4.6 (2.5, 5.6)	0.4609	3.8 (1.2, 5.8)	2.9 (2.5, 4.5)	0.9219

Medians are presented with interquartile range (IQR)

4.3.2 Hypertensive individuals exhibit higher blood pressure changes than normotensive on a high salt diet.

HIV positive group and HIV negative group matched for age and sex showed similar trends of blood pressure change from low to high salt diet. Hypertensive groups had a significant rise in both systolic and diastolic blood pressure ($p<0.001$) while normotensives showed no significant change except for the HIV negative group ($p=0.0466$) (Figure 28).

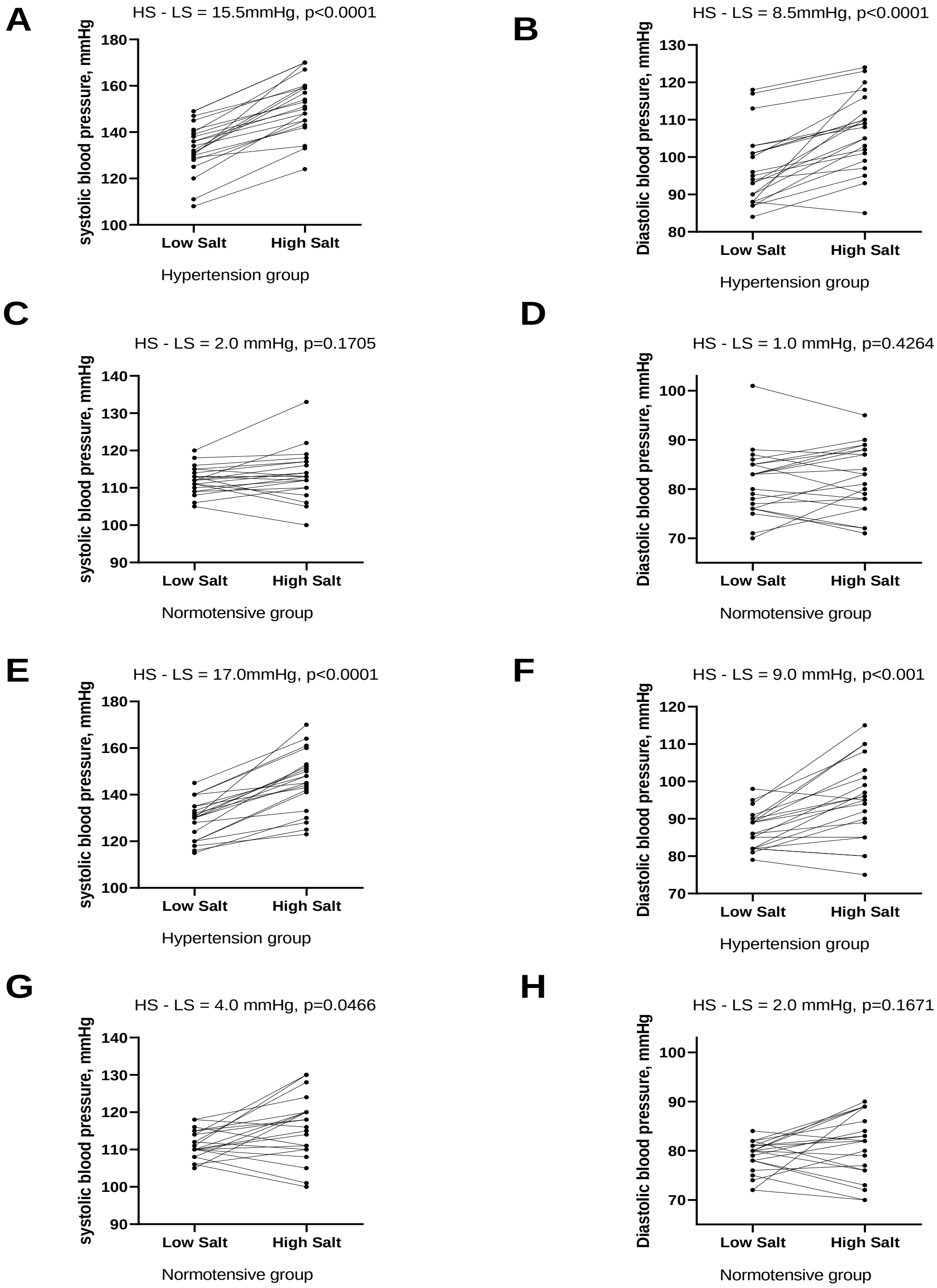


Figure 28. Blood pressure change in HIV positive (A-D) and HIV negative (E-H) groups between low and high salt diets

In hypertension, median systolic blood pressure (SBP) and diastolic blood pressure (DBP) change was (A) 15.5 mmHg and (B) 8.5 mmHg (p<0.0001) in the HIV positive group while SBP and DBP change in HIV negative was (E) 17.0 mmHg and (F) 9.0 mmHg, (p<0.001). Normotensive groups exhibited the least SBP and DBP changes of (C) 2.0 mmHg (p=0.1705) and (D) 1.0 mmHg (p=0.4264) in HIV positive group and (G) 4.0 mmHg (p=0.0466) and (H) 2.0 mmHg (p=0.1671) in the HIV negative. Wilcoxon matched-pairs signed-rank test used. HS, high salt; LS, low salt. The JNC 7 criteria was used to classify participants as hypertensive in all cases. History of antihypertensive medication was used to diagnose hypertension.

5.0.0 CHAPTER FIVE: DISCUSSION

5.1.0 Risk factors and inflammatory surrogate study

5.1.1 Specific Aim one: Cohort 1

5.1.1.1 Prevalence of hypertension in HIV positive participants

While using the JNC 7 criteria puts the prevalence of hypertension in this study at 16%, the new AHA/ACC criteria shift the prevalence to 42% representing a 26% shift of those previously considered normotensive into the hypertensive category (Table 4 and 5 respectively). The reported prevalence of hypertension in PWH in our study is confirmed by the prevalence in low- and middle-income countries ranging from 4 to 54% (Korem et al., 2018; Martin-Iguacel et al., 2016; Nguyen et al., 2015). In ART-treated PWH, hypertension prevalence ranging from 17 to 38% have been reported (Brennan et al., 2018; Dimala et al., 2016; Kagaruki et al., 2014; Korem et al., 2018; Muhammad et al., 2013; Peck et al., 2014) based on the JNC 7 criteria. However, with the new AHA/ACC criteria the results from this study indicate an urgent need for intervention.

5.1.1.2 Routine and non-routinely collected factors associated with hypertension in PWH

Among the social-demographic data, age, employment, marital status and education were positively associated with hypertension ($p<0.05$) (Table 4 and 5). Age is routinely collected while the rest are not at LCH. Neither the JNC 7 nor the new AHA/ACC recommend consideration for employment, marital status or education when assessing risk for hypertension or treatment, however, the majority who were hypertensive in this study were married (46% hypertensive versus 43% normotensive; Table 4), in GRZ/private employment (31% hypertensive versus 21% normotensive; Table 4) and attained tertiary education (40% hypertensive versus 17% normotensive; Table 5). While the JNC 7 and AHA/ACC does not discuss the influence of these factors on blood pressure, further studies are needed to determine how they relate to hypertension in low-income countries. Using the new AHA/ACC criteria in multivariate logistic regression (Table 10), being older [1.0 (1.03, 1.15); AOR 95%CI] was positively associated with hypertension in HIV positive participants. Briefly, increasing age especially above 50 years old correlates positively with high blood pressure in Sub-Saharan Africa HIV positive individuals (Albrecht et al., 2019; Peltzer and Pengpid, 2018). Being unemployed had reduced effect on hypertension [0.19 (0.04, 0.95); AOR 95% CI] even when adjusted in multivariate logistic regression (Table 10). Employment, marital status and education could positively contribute to hypertension due to stress and psychosocial burden faced by HIV positive individuals (Fahme et al., 2018; Temu et al., 2017). Contrary to this study, unemployment correlated positively with hypertension in HIV positive individuals from South Africa (Gómez-Olivé et al., 2018). The difference in findings could be attributed to age disparities as the cohort from South Africa were much older than in this current study (mean age of 61 versus 43 years respectively).

Among dietary and lifestyle non-routinely collected variables, factors such as minutes of weekly moderate activity and daily physical activity and were associated with hypertension ($p<0.05$) (Table 6 and 7) confiming what has already been reported (Whelton et al., 2017). Moreover, sedentary life and physical inactivity was associated with hypertension in PWH (Duong et al., 2003; Princewel et al., 2019) while consistent moderate physical activity was associated with reduced odds for developing hypertension (Peltzer and Pengpid, 2018). These findings suggest that increased physical activity and spending less time sitting modulates cardiovascular health which is linked to lower risk for developing hypertension.

While addition of salt while cooking and intake of processed foods high in salt were associated with hypertension, addition of salt at the table was negatively associated with hypertension in this study (Table 6 and 10). It was not expected to find that those who added salt sometimes or always on the table would have reduced odds [0.17 (0.04, 0.95; AOR, 95%)] for developing hypertension compared to those who rarely or never added salt on the table. These results were contrary to a study conducted in neighboring Zimbabwe were adding salt to food at the table was an independent risk factor for uncontrolled hypertension (Magande et al., 2017). However, the study population was not PWH. Several studies consistently report that higher salt intake is positively associated with high BP and hypertension (Azinge et al., 2011; Oelke et al., 2016; Ware et al., 2017), albeit not in PWH. A previous study from Zambia reported higher salt intake more than twice that recommended by WHO in the general population (Oelke et al., 2016). Studies that address salt intake and how it relates to BP and hypertension in PWH are scarce. There is also the issue of salt sensitivity which varies among individuals (Choi et al., 2015; Kirabo, 2017). However, the current study cohort was beyond the scope of assessing actual salt quantities and sensitivity. In multivariate logistic regression (Table 11), individuals with longer sedentary lifestyles, which is the amount of time spent seated, were at higher risk of developing hypertension [1.0 (1.00, 1.01); AOR, 95%CI, p=0.021] while minutes of moderate physical activity had reduced effects [0.98 (0.97, 1.00) 95%CI, p=0.048] on risk of being hypertensive. These results are consistent with the AHA/ACC report on hypertension (Whelton et al., 2017).

The clinical factors associated with hypertension included BMI, WC, duration on ART, PP, MAP, mid-BP, FBS, diabetes risk scores and risk category (Table 8 and 9). Among these, only BMI is routinely collected at LCH. In multivariate logistic regression (Table 10), those who were overweight (BMI =25-29.9) and obese (BMI equal to or greater than 30) were eight (p=0.043) and twenty-three (p=0.027) times more likely to be hypertensive respectively. Compared to those with normal WC (<94 cm men; <88 cm women), individuals with a WC between 94-102 for men and 80 to 88 for females and those with a WC above 102 for men and above 88 for females were sixteen times (1.2, 198.9; 95% CI) and 290 times respectively, more likely to be hypertensive (p<0.05) (Table 11). These findings are consistent with another study conducted locally in Zambia (Rush et al., 2018) as well as what has been reported by (Whelton et al., 2017). Obese individuals with high WC are predisposed to hypertension owing to adiposity effects on the vessel and tendance to elicit an inflammatory milieu (Koethe, 2017). Effects of HIV-infection and ART treatment on adipose maturation, cytokine signaling and expression of adipocyte regulatory genes such as PPAR-γ (Koethe, 2017) alter adipose tissue morphology, distribution and metabolism, resulting in the lipodystrophy phenotype in HIV positive individuals that contributes to hypertension (Zicari et al., 2019). ART treatment for longer than 2 years has been reported to drive hypertension (Martin-Iguacel et al., 2016). In terms of effects of ART on the metabolic profile of HIV positive individuals, there has been some controversy regarding differential effects of different drugs. However, it is well established that the protease inhibitors (PIs), particularly lopinavir/ritonavir and indinavir but less so, for some newer agents, exert profound effects on triacylglycerols and lipid transport (Martin-Iguacel et al., 2016). This leads to dyslipidemias, lipodystrophy, and insulin resistance, and promotes adipokine secretion, with consequent vascular inflammation and endothelial dysfunction contributing to hypertension (Martin-Iguacel et al., 2016; Zicari et al., 2019). Combination therapy with NRTIs and non-nucleoside reverse transcriptase inhibitors (NNRTIs) was associated with more severe stiffening of large elastic arteries in those with hypertension or those not receiving ART (Msoka et al., 2018), suggesting that regardless of the combination's contribution to hypertension, it definitely exaggerates arterial remodeling that contributes to hypertension.

BP components, PP, MAP and mid-BP were only included in the analysis to indirectly assess the effect of antihypertensive drugs. It is expected that hypertensive patients taking antihypertensives will generally have comparable or lower BPs when compared with normotensive individuals except when resistant hypertension is present or lack of adherence to medications. This study found that PP, MAP and mid-BPs were higher in hypertensive patients. This is owing to the fact that even though 89% of hypertensives (Table 9) were on antihypertensive drugs, almost all (95%) had uncontrolled high BPs. Unfortunately, no data was collected on adherence to antihypertensive drugs was not done and therefore we cannot ascertain the cause for this. The implication of pulse pressure to hypertension is that it is a surrogate for arterial stiffness in older adults associated with high BP and fatty deposits on the walls of the arteries (atherosclerosis) (Strandberg and Pitkala, 2003). Pulse pressure can therefore be used to help create the need for arterial stiffness (Bakris and Laffin, 2019) testing to evaluate if hypertension is adversely affecting the vessels. However additional testing is required to confirm this. A high MAP (>100 mmHg) may indicate increased pressure in the arteries eventually leading to blood clots or damage to the heart muscle and reflects the inadequacy of tissue perfusion (Safar, 2018; Yeo et al., 2019). Mid-BP (calculated as [SBP+DBP]/2), is an important marker of coronary heart disease (CHD) (Safar, 2018; Yeo et al., 2019). However, further additional testing are usually needed to rule out CHD and arterial stiffness using pulse wave velocity, aortic augmentation index and brachial blood pressure (Ngatchou et al., 2013).

Hypertensive patients had high diabetes risk scores and FBS (Table 10 and 11). Often the factors associated with hypertension are likely related to diabetes risk too, hence, the need to have employed a diabetes risk assessment tool to assess if hypertensive patients were at risk of developing diabetes mellitus type 2. The value for diabetes risk assessment scores was described from the recent previous study conducted in HIV positive individuals (Masenga et al., 2019c). Conducting diabetes risk assessment would be cardinal among the HIV positive individuals with hypertension. More especially that fasting blood sugar was positively associated with hypertension (p=0.038) as those with higher fasting blood sugars were twice more likely to be hypertensive (1.0, 4.1,95% CI) (Table 10). Similar to this study, higher FBS correlating with hypertension were reported in HIV positive individuals with high risk for cardiovascular disease (Wu et al., 2019). Additionally, weight gain, longer duration on ART and hypertension were independent predictors of dysglycaemia in another study (Duncan et al., 2018). A study in South Africa found that HIV infection, ART, and use of antihypertensive had a poor modulating effect on FBS (Khoza et al., 2018). Contrally to what our study found, a study in Cameroon found that FBS was not affected with ART duration and hypertension (Ekali et al., 2013).

In general, absence of alcohol consumption and smoking, and a tendency to have moderate and vigorous physical exercises, inclusion of fresh fruit and vegetable in diets are helpful to prevent high FBS and high BP (Chikowore et al., 2017; Kiama et al., 2018; Loonam and Mullen, 2012) and these should be highly recommended in HIV positive individuals.

The results provide evidence that several non-routinely collected variables need to be incorporated in routine ART services and care to prevent hypertension and its attendant adverse outcomes. A summary of the risk factors of hypertension in HIV infected adults in Zambia is given in figure 30 below.

Figure 29. Summary concept on risk factors of hypertension in HIV infected adults at Livingstone Central Hospital
Age, BMI, employment status, fasting blood sugar and table salt consumption were associated with hypertension using the new AHA/ACC diagnostic criteria while waist circumference and sedentary lifestyle were positively associated with hypertension using the JNC 7 criteria (P<0.05). Moderate physical activity was negatively associated with hypertension (P<0.05).

5.1.1.3 Implications of the new AHA/ACC guidelines

The treatment and care of PWH previously overlooked the burden of NCD comorbidity such as hypertension. Recently (2018), the Zambian government through the Ministry of Health integrated hypertension management in PWH (see www.hivst.org). However, the guidelines are not detailed and are lacking in most critical areas of hypertension diagnosis, treatment and care emphasized in the new AHA/ACC criteria.

It was found in this study, that the current routine practice for the measurement of BP in PWH at Livingstone Central Hospital was not very accurate. As shown in Table 13, routine measurements that did not take into consideration the preparation of the patient such as resting, sitting position and taking more than one BP measurement resulted in higher BPs than the standard measurement with median SBP and SDP differences of 3mmHg. As a result, about 4% (7/191) and 12% (23/191) of normotensives would have been misclassified as hypertensive using the JNC 7 and new AHA/ACC diagnostic criteria respectively. All measurement guidelines stipulated in the new AHA/ACC guidelines can be implemented in low-cost settings. Self-monitoring of BP is also encouraged to enhance accuracy in BP measurements and to avoid white coat hypertension.

The advantage of using the new AHA/ACC criteria is that most of the patients can prevent hypertension-related health complication through lifestyle changes alone such as reducing salt intake, increased physical activity, reducing sedentary time and eating more vegetables and fruits (He et al., 2013; He and MacGregor, 2010; Whelton et al., 2017). These changes are feasible in low-cost settings and can potentially reduce the burden and complications of hypertension.

Another important but routinely unpracticed assessment at the institution where the study was conducted is the 10-year risk for heart disease and stroke using the atherosclerotic cardiovascular disease (ASCVD) risk calculator elaborated in the new AHA/ACC guidelines. The components of ASCVD risk include age, sex, race, SBP, DBP, total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, history of diabetes, smoking status, antihypertensive drug history, history of statin use and aspirin use. Screening for ASCVD in low-cost settings is feasible although LDL, HDL testing is not yet routine at Livingstone Central Hospital.

It is evident from this study that the new AHA/ACC guidelines are needed. Therefore, integrating the guidelines into routine care for PWH are warranted.

5.1.2 Sub cohort 2: Inflammatory surrogate cells associated with inflammation

Higher innate inflammatory surrogate cells (neutrophils, monocytes), neutrophil-to-lymphocyte ratio, low adaptive immune cells (CD4 T cells), higher red blood cell count and lower kidney functional capacity (high plasma creatinine and lower estimated glomerular filtration rate (eGFR) using the modification of diet in renal disease (MDRD) formulae) in HIV positive individuals was found to be possibly associated with hypertension (Figure 11 and Table 14).

5.1.2.1 Statistical, biological and clinical significance of the results

These results should be interpreted in-context with reference to the clinical implication. Although, the association between hypertension and levels of neutrophils, lymphocytes, monocytes, white blood cell count, red blood cell count, neutrophil-to-lymphocyte ratio, creatinine and creatinine clearance were statistically significant ($p<0.05$), these levels were within the expected normal range in healthy individuals. Statistical significance just indicates that the relation or association is likely not by chance. There could be some other underlying interactions which are beyond the scope of this study. Statistical significance does not always indicate clinical significance especially in this case as some of the results may not impact on clinical practice (Ranganathan et al., 2015). A longitudinal study to assess these levels with respect to longer time periods is warranted.

Although within the normal reference, some plausible pathophysiological explainations for the involvement of these innate inflammatory surrogates in hypertension has been reported. Neutrophils, neutrophil-to-lymphocyte ratio and monocytes are implicated in the progression of hypertension in both human and animal studies (Jaworowski et al., 2019; Schweitzer et al., 2019; Sinha and Feinstein, 2019). Increased monocyte activation and formation of neutrophil extracellular traps in HIV contribute to hypertension and cardiovascular disease (Sinha and Feinstein, 2019). However, the levels of monocytes, lymphocytes and neutrophils were within the expected clinical range for most patients except for the neutrophil-to-lymphocyte ratio which was slightly high for some hypertensive and normotensive HIV positive individuals. Neutrophil-to-lymphocyte ratio has been proved to be a useful indicator of inflammation and cardiovascular risk as well as an effective indicator for arterial stiffness using brachial-ankle pulse wave velocity in both HIV positive and HIV negative (Wang et al., 2017). Neutrophil-to-lymphocyte ratio has also been positively correlated with systemic endothelial dysfunction (Martínez-Urbistondo et al., 2016), and a predictor of hypertension (Liu et al., 2015). However, this current study could not confirm this as brachial-ankle pulse wave velocity was not conducted. Studies on the link between neutrophils and hypertension are likely unique to this study. The mechanisms underlying this connection is unknown and beyond the scope of this study.

The link between erythrocytes (red blood cells) being higher in HIV positive hypertensive patients compared with their normotensive counterparts is unique in the context of being within the normal range as there seems to be no literature available at the time of this write-up. High blood pressure has been correlated with lower hemoglobin and erythrocytes in some studies (Marketou et al., 2010) while in other studies, higher hemoglobin correlated positively with BP (Chen et al., 2018). Several studies have reported hematological derangements in essential hypertension including erythrocyte counts and sedimentation rates (Merad-Boudia et al., 2019). In HIV negative cohorts (Guedes et al., 2019), hypertensive patients exhibit changes in erythrocyte elasticity, morphology and stiffness that lead to changes in systemic blood flow thereby contributing to blood flow perturbations in hypertension. These changes have potential effects in reducing oxygen-carrying capacity of hemoglobin in erythrocytes and hence more production of erythrocytes is necessitated (Revin et al., 2016).

An oxillometric oxygen saturation was conducted in a sample of hypertensive and normotensive individuals to test the hypothesis that erythrocytes' hemoglobin to transport oxygen to the tissues was altered. It was found that there was no significant difference between oxygen hemoglobin saturation levels in the hypertensive and normotensive. However, more robust methods used in other studies such as laser interference microscopy (LIM) and Raman spectroscopy are required to confirm if oxygen functionality is altered other than the method used in the current study. We could therefore neither refute nor confirm the functionality of erythrocytes in the research paticipants from this study or confirm findings from (Revin et al., 2016). Red blood cells are normally increased when the need for oxygen by tissues is not supplied adequately (Guedes et al., 2019; Revin et al., 2016).

Of clinical relevance, CD4%, eGFR and ESR (in males) were lower in hypertensive PWH and outside the reference levels (less than 29%, 90 mL/min/1.73 m^2 and 23 mm/hr respectively). This association was statistically significant and may have clinical implications.

Several cross-sectional and clinical trial studies (Althoff et al., 2019; Chepchirchir et al., 2018; Hatleberg et al., 2018) from Sub-Saharan Africa and developed countries have reported similar results, that low CD4 cells and renal impairment (low eGFR) were associated with hypertension (Fiseha et al., 2019). The association between low CD4 count and hypertension could arise from the fact that inflammation and subclinical atherosclerosis contribute to the development of hypertension with synergistic effects from other factors such as age, duration on ART and renal impairment (Fiseha et al., 2019; Siwamogsatham et al., 2019). Increased viral replication injures the kidney vasculature that may impair renal function (Winston, 2010). Low eGFR are prevalent among PWH and has been associated as a determinant of low creatinine clearance (Longo et al., 2012). In the current study, both the Cockcroft-Gault and the MDRD equation was used to assess renal function. While the estimated GFR using the Cockcroft-Gault method was not significantly associated with hypertension in multivariate logistic regression, the eGFR using the MDRD was lower in hypertension even after adjusting for multiple clinical variables (Table 14). Though uncertainties and controversies exist (Chudek et al., 2018; Palacio-Lacambra et al., 2018) on the best equation to use, there is evidence that the MDRD has the smallest bias and highest accuracy (Schwandt et al., 2017). Hypertension and treated HIV-infection are all risk factors for kidney impairment though the mechanisms are unknown (George et al., 2017, 2019). Given than the median levels of eGFR were mildly decreased (slightly lower than 90 mL/min/1.73 m^2), it was beyond the scope of this study to ascertain the clinical implication.

A summary mechanism of the possible linkage between HIV, inflammatory surrogates and hypertension is shown in Figure 30.

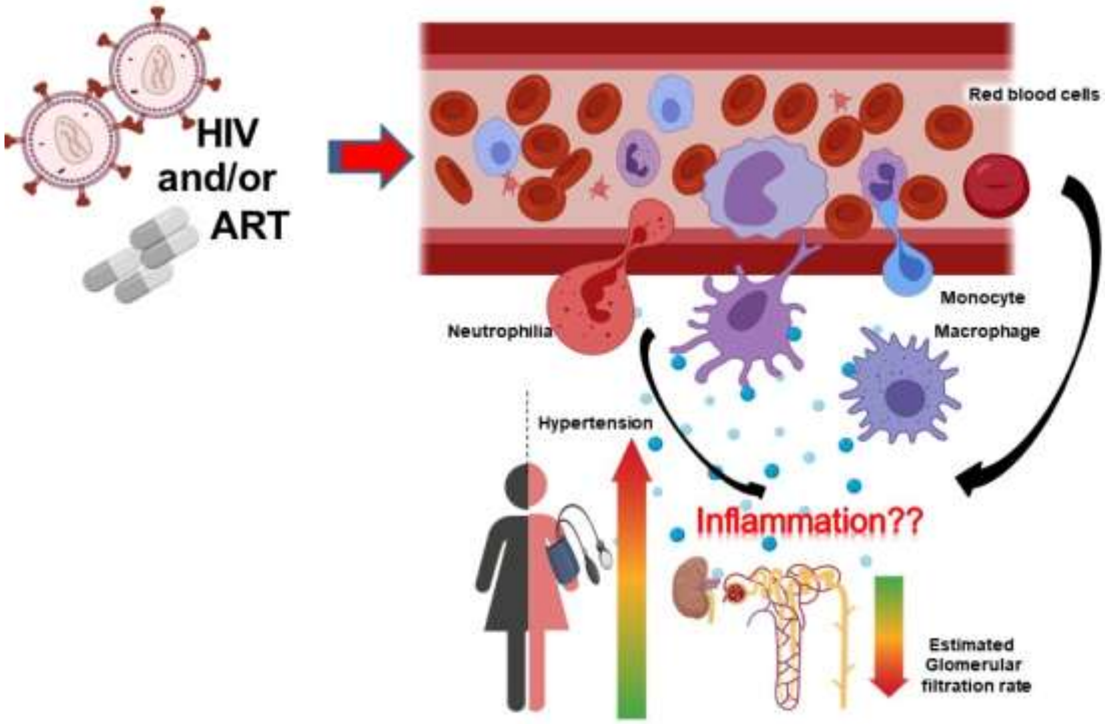


Figure 30. Inflammatory surrogate cells in treated HIV-infected adults with hypertension

It is hypothesized that neutrophils, monocyte-macrophages are increased in hypertension owing to an inflammatory stimulus from HIV-infection and/ or antiretroviral therapy (ART) that exists in hypertension through unknown mechanisms. Red blood cell functionality may be impaired or due to low kidney perfusion or lowered filtration function, the bone marrow increases red blood cell production in hypertension to meet oxygen demand. However, these mechanisms need to be investigated in order to be validated. The black arrows denote possible inducement of inflammation by neutrophils and monocytes.

5.2.0 Inflammation and hypertension study

5.2.1 Aim two: Sub cohort 3

In a cohort of HIV positive, it was found that inflammation was possibly associated with increased secretion of IL-6, IL-17A and IL-10 (Figure 15 and 16) while in the HIV negative control group cells of hypertensive secreted higher IL-6, IFN-γ (Interferon-gamma) and TNF-α (Tumor necrosis factor-alpha) (Figure 15 and 16) compared to normotensive participants.

5.2.1.1 Statistical and Biological significance of the results

5.2.1.1.1 Experimental setting

As opposed to measuring serum or plasma cytokine levels, this study measured cytokine levels in the supernatant of in vitro-stimulated whole blood with PMA/ionomycin. This type of experiment measures the overall ability of cells to produce cytokines. While reference values for serum or plasma cytokines are available with variability between laboratories and methods used, reference levels in supernatant samples are not well established. Cytokines in the supernatant do not reflect the reference range for plasma or serum cytokines and the levels can be much lower in the supernatant (Jason et al., 2001). Levels of cytokines in plasma only weakly reflect peripheral blood cell cytokine production and balances (Jason et al., 2001). To be able to interpret the results of these assays, a comparison group of healthy individuals is required to establish normal ranges of cytokine production (Whiteside, 2002). Therefore, levels of cytokines in the supernatant of HIV negative normotensives are used to interpret the results.

5.2.1.1.2 Biological and clinical relevance

The levels of IL-10 and IL-17A in the supernatant of hypertensive HIV positive participants were higher compared to the normotensive. However, they were within the normal reference. IL-6 was higher in hypertensive compared to the normotensive participants in both the HIV positive and HIV negative groups ($p<0.05$) and the levels of IL-6 were above the reference. TNF-α and IFN-γ were higher in hypertensives compared to normotensives in both groups and the levels were above the reference. However, the association between hypertension and higher levels of these cytokines was only statistically significant in the HIV negative group.

These cytokines are important in pro-inflammatory and anti-inflammatory processes. IL-10, IL-17A, IL-6, TNF-α and IFN-γ have been found to be high in hypertensive participants (Tanase et al., 2019). IL-17A, IL-6, TNF-α and IFN-γ possibly play a role in the pathogensis of hypertension. However, their role in this study is unknown. IL-10 is increased in hypertension as a protective and inhibitory mechanism against inflammation (IL-17A), artherosclerosis and viral replication (Jiang et al., 2018; Mikolajczyk and Guzik, 2019; Tanase et al., 2019; Wilson and Brooks, 2011).

IL-6 is a proinflammatory cytokine and is also considered a myokine (produced by muscle cells during contraction) with extensive anti-inflammatory functions in its role as a myokine (Petersen and Pedersen, 2005). IL-6 is produced by several cells such as monocytes, macrophages, dendritic cells, muscle and endothelial cells (De Pablo-Bernal et al., 2016; Shirakura et al., 2018). There is evidence that increased production of both IL-6 and IL-10 is due to a dysfunction of inactivated classical monocytes during HIV infection (De Pablo-Bernal et al., 2016). Whether this was occurring in our study participants remains unknown. Elevated IL-6 levels have been mostly implicated in a lot of pathological conditions including incident hypertension (Batavia et al., 2018; Lacerda et al., 2014) although the association with hypertension has not be reported in some studies (Ghazi et al., 2020). However, the association between IL-6 and hypertension has been confirmed (Lacerda et al., 2014).

It was expected that the levels of IL-10 would be reduced in hypertension as reported from another study (Kumari et al., 2018). However, IL-6 was positively correlated with IL-10 levels and an explanation for its increase is evidently plausible (Table 20). As a myokine, IL-6 can act as an anti-inflammatory cytokine by inducing the production of IL-10 while inhibiting the production of the proinflammatory cytokine TNF-alpha and in this way protective against chronic diseases associated with low-grade inflammation such as cardiovascular diseases (Petersen and Pedersen, 2005). Higher anti-inflammatory markers such as IL-10 are reported in a study of PWH as a protective mechanism for atherosclerosis (Desvarieux et al., 2013). Up-regulation of IL-10 has been reported to suppress viral replication in increased viremia (Brockman et al., 2009; Ng and Oldstone, 2014).

Expression of Immune-activation marker CD80+ on unstimulated leucocytes (CD45) was evidently higher in cells of hypertensive compared with the normotensive in PWH and above the reference levels (Figure 18). A similar study reported increased expression of CD80 on monocytes and T-cells in PWH due to the effects of HIV viral particles and an inflammatory process following infection with HIV (Boasso et al., 2008). However, there was no study found that reported total activated (CD80+) leucocytes (CD45) being increased in hypertension. In the HIV negative, there was no difference between the percentage of CD45 CD80+ cells in hypertensive and normotensive controls (Figure 18).

In logistic regression, the possible association between hypertension and IL-6, IL-10, CD80+ expression on total leucocytes and isoLevuglandin but not IL-17A, remained statistically significant after adjusting for sex, age, body mass index, duration on ART, HIV viral load and CD4 count in the HIV positive group (Table 18). In the HIV negative group, the possible association between hypertension and IL-6, TNF-α and isoLevuglandin but not IFN-γ, remained statistically significant (Table 19). A multiple comparison test to compare inflammatory cytokines that were significant on Wilcoxon rank-sum test in the HIV positive hypertensive group compared with the rest of the subgroups was performed (Table 17). IL-6 and IL-17A remained high in the HIV positive hypertensive group.

5.2.1.1 Isolevuglandins production is higher in hypertensive when compared with normotensive individuals.

Monocyte cells of hypertensive individuals expressed higher isolevuglandin (IsoLG) adducts compared to the normotensive in both HIV positive and HIV negative groups (Figure 18). This is likely the first study to report an association between IsoLG and hypertension in PWH. Known studies to report IsoLG as it relates to hypertension were conducted in murine models (Barbaro et al., 2017; Kirabo et al., 2014; Norlander et al., 2018, 2017).

In murine model studies, IsoLG accumulate in DCs and activate them. DCs then produce and secrete high levels of IL-6, IL-1β, and IL-23 and express an increase in costimulatory proteins CD80 and CD86 (Barbaro et al., 2017; Kirabo et al., 2014). Activated DCs promote T-cell proliferation and production of IFN-γ and IL-17A contributing to the development of hypertension (Barbaro et al., 2017; Kirabo et al., 2014). This concept is possibly similar to that in humans (Barbaro et al., 2017; Kirabo et al., 2014).

Figure 31 summarizes the the study cohort findings representing the cell and possible inflammatory cytokine linkage with hypertension in PWH on ART.

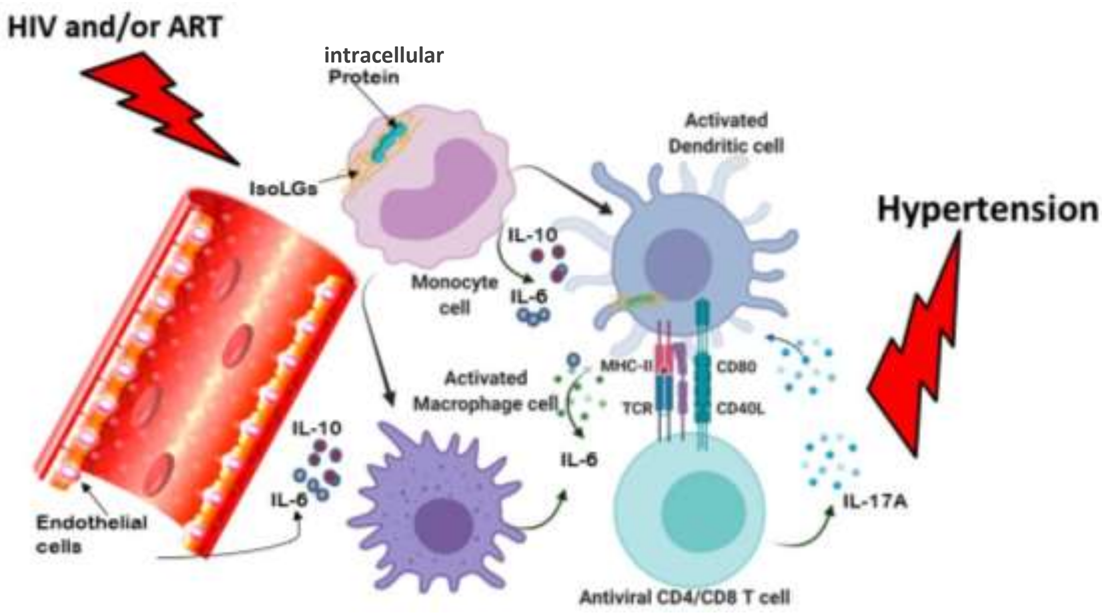


Figure 31. Inflammation, immune activation and IsoLG are elevated in hypertensive PWH

IsoLG-laden monocytes/dendritic cells and endothelial cells likely produce inflammatory cytokines IL-6 and IL-10 that modulate vascular and adaptive immune function. Additionally, dendritic cells with IsoLG-protein adducts (IsoLG adducted to intracellular proteins) stimulate T-cells via expression of co-stimulatory molecule CD80+. T-cells are activated and produce IL-17A and may induce or propagate hypertension through unknown mechanisms. Biorender.com was used to create this figure.

5.2.2 Adiposity and Immune Activation Cohort (AIAC) and HIV-negative control cohorts

5.2.2.1 Statistical and clinical significance of the results

It is important to interprete the findings relating to inflammatory cytokines from this study in context to avoid confusing statistical significance with biological or clinical impact. For example, while levels of sCD163, IL-6, eosinophils and MIP-1α were higher in hypertension compared to the normotensives and this finding being statistically significant ($p<0.05$), this may have no clinical implications as these values were within the normal expected range except for MIP-1α which had some values exceeding the reference. Additionally, MCP-1, VCAM-1, ICAM -1, IL-17, TNFR1 and IL-5 were higher in the hypertensive compared to the normotensives. This association was statistically significant and the levels were above the expected range. These finding may have clinical significance in relation to hypertension and HIV. As most cytokines are related, it is also important to interprete interactions between cytokines that may be within normal range and those that are elevated by referring to what others have previously reported.

5.2.2.2 Biological significance and how the findings relate to literature

An interesting finding of the present study is that hypertension in PWH compared with the normotensive was associated with low levels of CD38 expression on $CD4^+$ T cells (Figure 21D). The reference range for CD38 expression and other immune activation markers on various cells including CD4 cells is not established due to the variable expression of these markers. Many immune cell types such as bone marrow progenitors, natural killer cells, monocytes, and activated T- and B- lymphocytes express CD38 (Malavasi et al., 2008). CD38 is a transmembrane glycoprotein indicating T cell activation. There are few studies that report the clinical implications of a low CD4+CD38+ in PWH. CD38+ in HIV may be related to the viral clearance of HIV as increased expression of CD38+ on both CD4+ and CD8+ T cells reflect decreased HIV-1 replication (Almeida et al., 2007). Whether the reverse (lower expression of CD38+ on CD4+) is true as reported in this current study is unknown. This study found no significant difference in expression of CD38 on $CD8^+$ T cells in hypertensives versus normotensives (Figure 21D), which has been found to be a marker of residual virus replication in chronically HIV-infected patients receiving cART (Benito et al., 2004). Indeed, all participants in the cohort had been virally suppressed (VL < 50 copies/ml limit of detection) for a minimum the 12 months prior to the study. Further research is needed to determine the role of $CD4^+$ T cells expressing CD38 and how they may interact with other immune cells in the HIV-positive hypertensive individuals.

This study also found that monocyte/macrophage activation was possibly associated with hypertension in PWH (Figure 22A-D). This was indicated by a higher level in the shedding of the hemoglobin-haptoglobin scavenger receptor CD163 into the circulation as sCD163 by the activated macrophages and monocytes in hypertensive PWH compared with the normotensive. Although this difference was statistically significant ($p<0.05$), this finding was not clinically relevant as the levels of sCD163 in both hypertensive and normotensive were within the reference range. In addition, the study found increased MIP-1α and MCP-1 in hypertensive when compared to normotensive PWH (Figure 22). The levels of MIP-1α (for some patients) and MCP-1 were above the normal range. This was possibly due to HIV infection. However, it was beyond the scope of this study to ascertain the reason. The current findings from this study are in keeping with increasing evidence indicating that cells of the innate immune system including monocytes and macrophages may potentially play a role in the pathogenesis of hypertension. Previous studies have found that activated macrophages contribute to hypertension and the deletion of monocytes markedly reduced experimental hypertension (Higaki et al., 2019; Wenzel et al., 2011; Zhang et al., 2015). Macrophages accumulate in the kidney and the vasculature in experimental models of hypertension and promote hypertension (Ko et al., 2007; Tian et al., 2007). Moreover, hypertension in PWH was associated with increased expression of adhesion molecules including VCAM-1 and ICAM-1, which govern migration and infiltration of immune cells into tissues. These have been implicated in the pathogenesis of hypertension in experimental animal models (Rudemiller and Crowley, 2017).

A notable finding of this current study is that hypertension in PWH was possibly associated with increased cytokine production and signaling including IL-6 and IL-17 (Figure 23). However, IL-6 was within the normal expected reference while IL-17 was above that expected in healthy controls. Previous studies have found that T cells and their cytokines possibly contribute to hypertension (Crowley et al., 2010, 2008). The importance of IL-17 in possibly contributing to hypertension is reported in murine models where mice lacking T cells (RAG-1$^{-/-}$ mice) and mice lacking the T cell cytokine IL-17A (that contributes to hypertension when present) result in blunted hypertension (Guzik et al., 2007; Madhur et al., 2010). Hypertension augments the capacity of DCs to produce IL-6 and these polarized T cells to produce IL-17 and TNF-α (Barbaro et al., 2017; Kirabo et al., 2014). Although the contribution of IL-17 and other inflammatory cytokines in human hypertension is not clearly known, hypertension in PWH may be associated with some inflammatory components in the immune system.

It is important to note that plasma reference ranges of many cytokines is variable depending on disease conditions and other factors that may not be accounted for such as laboratory techniques (Aziz et al., 1999). Larger variations in plasma do occur for the same cytokine between and within the same patient (Aziz et al., 1998). This accounts for the reason some cytokines are not yet well established and used in the clinical practice. Several studies assessing cytokines compare between those with disease and those without the disease, other than just using the reference levels from other studies or laboratories. However, it is important to use both reported reference levels and compare disease conditions between those with and those without disease to ascertain the clinical relevance. As an example, IL-17 levels in one study assessing anti-neutrophil cytoplasm antibody-associated vasculitis were significantly elevated in the patients (0–72,940 pg/ml) compared to healthy controls (range 0–252 pg/ml) ($p < 0.01$)(Nogueira et al., 2010). However, the reference for health controls (range 0 - 252 pg/ml) in this study was higher than in a study assessing patients with ankylosing spondylitis were patients had higher levels (range 50 – 90 pg/ml) compared with controls (range 22 – 60 pg/ml) (Chen et al., 2012). Similarly, this current study found IL-17 levels in hypertensive (range 0 – 140 pg/ml) to be higher when compared with normotensive (range 0 – 268 pg/ml).

In this cohort, it was found that virally suppressed hypertensive PWH and on antiretroviral therapy (ART) exhibited higher levels of circulating eosinophils and their maturation marker IL-5 compared to the normotensive (Figure 24). Although eosinophils were within expected levels, after adjustment for multiple clinical and demographic factors, the possible association between circulating eosinophil counts and hypertension remained statistically significant in this population while TNF-α1, IL-6, IL-17, intercellular adhesion molecule 1 (ICAM-1) and macrophage inflammatory protein 1-alpha (MIP-1α) did not remain significantly associated with hypertension (Table 22). However, eosinophil counts were not significantly associated with hypertension in a smaller cohort of HIV-negative participants (control cohort 5) after adjusting for multiple clinical and demographic factors (Table 25) while the possible association remained statistically significant with hypertension in a larger cohort control (Table 27). Although these results suggest a link between eosinophils and hypertension based on statistical inferences, this may or may not have any clinical implication as the eosinophil count was within the expected range. More research is therefore required to ascertain the possible link between hypertension and eosinophils.

IL-5 and eosinophils have been implicated in several inflammatory conditions including asthma, drug hypersensitivity, neoplastic disorders and helminths infections (Kouro and Takatsu, 2009). IL-5 is produced by Th2 cells, mast cells, γδT cells, NK and NKT cells and other non-hematopoietic cells (Desreumaux et al., 1992; Moon et al., 2004; Sakuishi et al., 2007; Takatsu, 2011; Takatsu et al., 1994). It was originally identified as a T cell-derived cytokine involved in antibody production from B cells (Moon et al., 2004). Recently, IL-5 has been implicated in mediating maturation and differentiation of eosinophils in mice and humans (Moon et al., 2004). In addition, eosinophils play a controversial role in obesity-mediated inflammation (Bolus et al., 2018; Wu et al., 2011). In the LifeLines Cohort Study of over 13,000 members of the general population in Europe, higher eosinophil counts were associated with higher triglycerides, total cholesterol, and hemoglobin A1c, but lower high-density lipoprotein, in addition to higher odds of obesity and metabolic syndrome (Amini et al., 2016). Despite this association between eosinophils and cardio-metabolic disease, no prior studies have found any association between eosinophils and hypertension.

In summary, further research is needed to determine the specific contribution of the HIV-positive status versus ART on inflammation and how they may contribute to hypertension and cardiovascular disease. It should be acknowledged that this is a cross-sectional study in a limited cohort of 70 HIV-positive participants and therefore cannot address causality. While these findings obviously warrant further validation, it is thought that there is a clinical utility for the treatment of hypertension in or outside the context of HIV. In addition, most of the inflammatory markers fall within the expected reference range, contextual interpretation is therefore warranted.

5.2.2.3 Eosinophils in the HIV negative population

5.2.2.3.1 Control cohort 5 and 6

Two control cohorts were recruited to examine if the possible association between eosinophil count and hypertension was the same in the HIV negative participants who served as controls for the AIAC cohort of PWH. As shown in Figure 25 (where A and B is the VUMC cohort and C is the VSDD cohort), eosinophil count was higher in HIV negative hypertensive patients when compared with the normotensive ($p<0.05$). However, the eosinophil count was still within the normal expected range. In the VUMC cohort consisting of 50 HIV negative participants (25 hypertensive and 25 normotensive), this possible association was abrogated by BMI when adjusted (Table 25). An additional (VSDD) cohort with a larger sample size was recruited. This cohort was comprised of 9, 725 hypertensive and 71, 314 normotensive. The eosinophil count remained higher in hypertensive when compared to the normotensive (Table 28) even after adjustment in multivariate analysis (Table 29). These results indicate that while eosinophil counts are high in hypertensive PWH independent of BMI, they are dependent on BMI in HIV-negative persons (Table 25 and 27 respectively). The sample size for the VSDD cohort was was very large, increasing statistical power especially that the exclusion criteria was rigourous to exclude all participants with conditions that are known to raise eosinophils such as asthma, rhinitis, parasitic disease etc. However, the disadvantage from the control cohorts 5 and 6 is that additional data on other markers of inflammation, immune activation and T cell subsets to compare comprehensively with the HIV positive participants was lacking.

Comparing the association in HIV positive and the HIV negative populations, these findings suggest that in HIV negative, higher BMI may play a role in inducing eosinophil count where as in HIV positive, HIV infection and ART usage may possibly synergize with adiposity dysfunction (Koethe, 2017) to induce eosinophil increase. Recently, eosinophils are reported to be increased in adipose tissue were they possibly contribute to adipose homeostasis and drive energy expenditure, however, their role remains unknown to date (Knights et al., 2018). Some studies have suggested that in chronic inflammation elicited by adipose tissue, eosinophils with other cells play a protective role to mitigate inflammation responsible for insulin resistance (Chang et al., 2017; McLaughlin et al., 2017).

Previously, eosinophils were thought to be only associated with Th2 inflammatory disorders including parasitic infections and allergic reactions (Furuta et al., 2014). However, recent studies have found that eosinophils can infiltrate adipose tissue and regulate its function (Wu et al., 2011). Wither *et al* 2017 found that eosinophils play a role in obesity-related hypertension where they regulate perivascular adipose tissue and vascular function (Withers et al., 2017). Mice lacking eosinophils do not elicit an anti-contractile effect resulting from adiponectin and adipocyte-derived nitric oxide (Withers et al., 2011). This effect mimics the obese phenotype and is restored by the reconstitution of eosinophils. The role of eosinophils in hypertension in the setting of HIV infection is not known. Acquired lipodystrophy occurs in 60 to 80% of patients with HIV on cART (Garg, 2004), and is associated with endothelial dysfunction leading to cardiovascular disease (Behrens et al., 2003; Masiá et al., 2011; Stein et al., 2001). Thus, it is likely that although elevated eosinophil counts were independent of increased BMI in the hypertensive PWH, they may be associated with acquired lipodystrophy, which may create a metabolic derangement similar to increased BMI. Whether this was true in this study is unknown. Understanding how eosinophils impact cardiovascular function and hypertension may have important implications for the treatment of metabolic disorders associated with obesity and HIV.

Eosinophils have been found to regulate macrophage activation in adipose tissue. Wu *et al* 2011 found that in mice, eosinophils are major producers of IL-4 in white adipose tissues and increase infiltration of alternatively activated macrophages. Mice fed on a high-fat diet develop increased body fat, impaired glucose tolerance, and insulin resistance in the absence of eosinophils (Wu et al., 2011). These studies suggest that eosinophils play a role in metabolic homeostasis through the maintenance of adipose alternatively activated macrophages. However, unlike in the HIV-negative participants, eosinophil counts are increased in HIV independent of BMI. It is not clear what role these cells play in hypertension associated with HIV and whether they contribute to macrophage activation. Moreover, peripheral blood eosinophilia is often observed in patients with chronic kidney disease, acute kidney injury, or patients on renal replacement therapy and this may be associated with bio-incompatibility of the dialysis material, acute allograft rejection, or *Strongyloides* hyperinfection. Further scientific effort is required to determine if eosinophilia is associated with kidney disease in hypertensive PWH (Gauckler et al., 2018).

There is evidence that CD8$^+$ T cells activated in the presence of IL-4 can exhibit a Th2-like phenotype and produce cytokines IL-4, IL-5, IL-6, and IL-10 (Gonzalez et al., 2017). The role of CD8+ T cells in HIV-1 infection is not fully understood, however, it is possible that they are responsible for the production of high levels of IL-5 (Gonzalez et al., 2017; Le Gros and Erard, 1994), which may drive the eosinophilia. While any significant differences in CD8$^+$ T cells between normotensive and hypertensive PWH were not observed, previous studies have found that adipose tissue from PWH is enriched for CD8$^+$ T cells compared to HIV-negative controls, and similar changes seen have been observed in obesity (Couturier et al., 2015; Koethe et al., 2018). This role for CD8$^+$ T cells in IL-5 production does raise the possibility that the elevated eosinophils observed in hypertensive PWH may not represent a causal mechanism, but rather be a reflection of a link between the CD8$^+$ T cell anti-viral immune response and cardiovascular function. In keeping with this, the absence of eosinophils has been associated with worsening of cardiometabolic health and restoration of eosinophils leads to increased vascular relaxation and improved glucose homeostasis in murine models (Wu et al., 2011). However, other studies have failed to demonstrate an effect of these cells in rescuing metabolic impairment(Bolus et al., 2018), and Amini *et al* 2016 found that higher eosinophil counts were associated with risk factors of metabolic syndrome including higher triglycerides, total cholesterol, and hemoglobin A1c. Studies have found increased levels of other cells known to be anti-inflammatory such as T regulatory cells in disease. For example, T regulatory cells are increased in the circulation of patients with idiopathic pulmonary arterial hypertension (Austin et al., 2010; Ulrich et al., 2008). Thus, anti-inflammatory cells may become dysfunctional during disease pathogenesis. Further research is needed to determine how the function of eosinophils may be affected during hypertension in HIV.

The results from this study suggest a possible association between eosinophils, potentially driven by plasma IL-5 in hypertensive PWH. This association is present but may be abrogated by BMI in HIV-negative hypertensives. The findings that eosinophils and their maturation cytokine IL-5 are high in hypertensive PWH are interesting but only hypothesis-generating and do not confirm any new pathogenesis of hypertension. However, these two related findings provide a pragmatic and strong rationale to study this pathway further in hypertensive PWH. Even though eosinophils were higher in hypertensive compared to the normotensive participants, this study is unable to conclude on the role eosinophils play in hypertension.

A summary of the probable mechanism linking findings from AIAC cohort and the two control cohorts (VUMC and VSDD) regarding eosinophils, inflammatory markers, macrophage activation markers and other cells in contributing to hypertension in HIV positive individuals on ART is shown in figure 32. It is proposed that HIV infection and/or antiretroviral therapy is/are associated with increased endothelial dysfunction with increased expression of vascular cell adhesion protein 1 (VCAM-1) and intracellular adhesion molecule 1 (ICAM-1). This increases the propensity of activated monocytes, with increased production of sCD163, to diapedese into tissues where they encounter dysfunctional adipose tissue and convert into activated macrophages and dendritic cells (DCs). The activated antigen-presenting cells including macrophages and DCs produce monocyte chemoattractant protein 1 (MCP1) which further increases migration and infiltration of monocyte/macrophages into tissues. They also produce chemotactic cytokine Macrophage Inflammatory Protein-1α (MIP-1α) which activates eosinophils and induce the release of IL-6 and TNF-α from macrophages and DCs. These antigen-presenting cells activate T cells to produce IL-17 which is thought to contribute to hypertension. T cells also possibly produce IL-5 which induces differentiations of eosinophils. The causal relationship between increased IL-5 and eosinophils in inducing hypertension is not known.

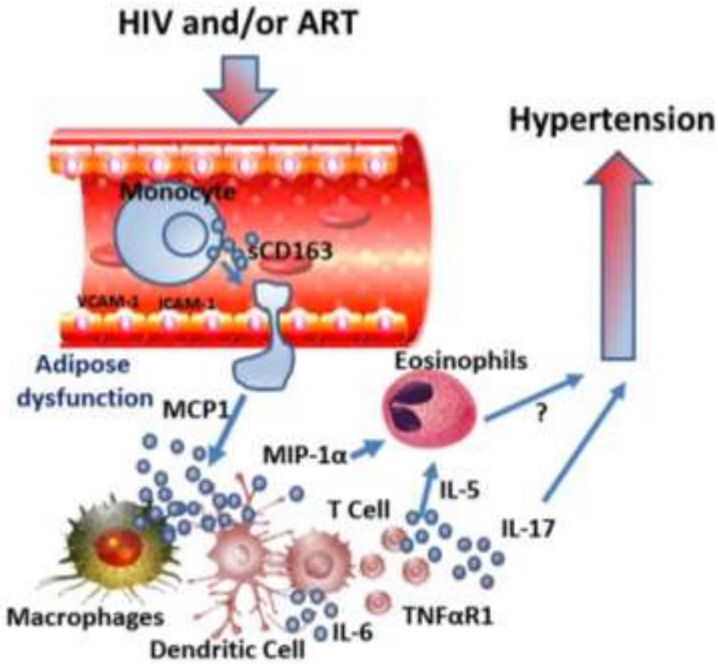


Figure 32. Hypothesized model summarizing all the inflammatory components associated with hypertension in people living with human immunodeficiency virus. In PWH on ART, inflammation and immune activation possibly contributes to hypertension. However, sCD163, IL-6, eosinophils and MIP-1α were within the normal range. VCAM-1, Vascular cell adhesion protein 1; ICAM-1, intracellular adhesion molecule 1; MCP-1, monocyte chemoattractant protein 1; MIP-1α, macrophage inflammatory proteins-1 alpha; TNF-α, tumor necrosis factor alpha

5.3.0 HIV, Immune-activation Salt-sensitive hypertension study

5.3.1 Aim three: Subcohort 3

In order to assess the effect of excess dietary salt on BP and immune cell activation/inflammation, a group of hypertensive and normotensive HIV positive individuals from Livingstone Central Hospital were instructed to follow a one week of low dietary salt followed by one week of high dietary salt then BP and inflammatory cytokines in the supernatant were assayed.

The majority of hypertensive individuals for both HIV positive and HIV negative had their BP raised by high salt intake compared to normotensive HIV positive and HIV negative participants (Figure 28). It was found that the underlying reason behind BP modulation in the majority of the hypertensive was due to the fact that these individuals were salt sensitive and the individuals whose BP did not change were salt resistant. As seen in Figure 28 (B, C, D, F, G, H), the SBP and DBP of some individuals (both salt resistant and salt sensitive) slightly reduced. Salt resistant individuals sometimes exhibit a small reduction in blood pressure while salt sensitive individuals can also have a significant reduction in blood pressure, however, this phenomen is uncommon (Nierenberg et al., 2017).

5.3.1.1 Prevalence of salt sensitivity in HIV positive and HIV negative participants

In the HIV positive group, 96% among the hypertensive and 10% among the normotensive were salt sensitive while the prevalence of salt sensitivity was 71% and 29% among the hypertensive and the normotensive HIV negative controls respectively (Table 30). Although the prevalence of salt sensitive hypertension (SSH) was high, this correlates with other studies suggesting several mechanisms that may be involved, such as genetic predisposition (Luzardo et al., 2015), that link Africans with more susceptibility to SSH (Jones and Rayner, 2020; Tu and Pratt, 2013). Specifically, these mechanisms include genetic variations affecting activity of sodium transport proteins, kidney damage mediated by inflammation, the aldosterone mineralocorticoid receptor pathway and neuronal alterations, among others (Johnson et al., 2015). Among the neuro-endocrine factors involved in the salt sensitivity of blood pressure (SSBP) are the renin-angiotensin-aldosterone system (RAAS), the sympathetic nervous system, natriuretic peptides, insulin, leptin and various endothelial effectors with endocrine and/or paracrine activity. Most of these affect the regulation of tubular sodium and water reabsorption and, thus, volume homeostasis (Elijovich et al., 2016; Mattson, 2019; Reisin and Jack, 2009; Richardson et al., 2013; Sanchez et al., 2015). Another factor that may exacerbate hypertension in HIV-infected people is that greater CD8 activation is associated with less arterial distensibility and flow-mediated dilation which is indicative of endothelial dysfunction (Grome et al., 2017). These data suggest that salt sensitivity phenotype is a risk factor for the development of hypertension and may worsen already existing hypertension among HIV positive individuals.

The prevalence of SSH in the general population is approximately 50% in hypertensive and 25% in the normotensive and salt sensitivity has been linked to endothelial dysfunction and adverse cardiovascular events (Richardson et al., 2013; Weinberger et al., 2001). The prevalence of SSH was high in this current study. It is, however, difficult to make direct comparisons to other studies due to differences in protocols (de la Sierra et al., 2002; Weinberger et al., 2001; Yatabe et al., 2010) used to diagnose salt sensitivity. Moreover, the main aim of this study was not to determine salt sensitivity prevalence but to explore the effects of salt loading on nocturnal BP dipping. These results should therefore be understood in this context.

5.3.1.2 Factors associated with salt sensitivity in HIV positive and HIV negative participants

HIV positive and HIV negative participants were grouped based on salt sensitivity status (Table 32-34). In the HIV positive group, salt sensitivity was associated with non-dipping blood pressure on low and high salt diets (Table 32) and increased random 24-hr sodium and chloride excretion, 24-hr sodium excretion on high salt diet, elevated IL-6, monocyte count and isoLG percentage in monocytes adjusted for sex, age, body mass index and duration on ART, $p<0.05$ (Table 35). However, the monocyte count was within the expected range and this association may have no clinical implications. In the HIV negative group, only nocturnal sodium excretion on low salt diets were associated with salt sensitivity adjusted for similar variables without duration on ART (Table 36). High salt diets therefore, may possibly elicit an inflammatory milieu and is a possible risk factor for the development of hypertension and may worsen already existing hypertension among PWH.

5.3.1.3 Salt intake and renal excretion

Excretion of sodium follows a diurnal rhythm where excretion reaches a maximum during the day and a minimum at night during sleep owing to a drop in blood pressure (pressure-natriuresis concept). However, in this cohort study, it was found that low and high salt intake was associated with higher nocturnal natriuresis in both salt-sensitive and hypertensive HIV positive (Table 32) and HIV negative individuals (Table 33). This phenomenon is not uncommon as it has been reported in a systematic review (Sachdeva and Weder , 2006). Normotensive salt-resistant individuals have the expected nocturnal decrease in sodium excretion. The underlying mechanism behind this is increase in blood pressure seen in hypertensive and in salt-sensitivity. In the HIV positive and HIV negative group, a random 24-hr sodium excretion was higher in salt sensitive participants when compared with normotensive reflecting a higher dietary intake in hypertensives (Table 33 and 34 respectively). First morning urine secretion of sodium was high in HIV positive salt sensitive compared to the salt resistant counterparts. Segregated by hypertension status (Table 38 and 39), hypertensives excreted more sodium and chloride at night, a term referred to as nocturnal natriuresis (applies to sodium excretion alone) on both low and high salt diets. Thus, circadian sodium excretion capability is impared with excess dietary salt especially in salt sensitive individuals resulting in elevated blood pressure during the night (Kimura et al., 2010). Pressure natriuresis is therefore enhanced at night to compensate for low excretion of sodium during the day (Fukuda and Kimura, 2012; Kimura et al., 2010). SSH, correlates with increasing age and is linked to an increased risk for the development of left ventricular hypertrophy, proteinuria, and a blunted nocturnal decline in blood pressure ("non-dipping") (Rodríguez Castellanos, 2006).

5.3.1.4 Blood pressure control

Control mechanisms for blood pressure are many including arterial baroreceptors and chemoreceptors, the central nervous system ischemic response, the renin–angiotensin system, and capillary fluid shift. However, all these mechanisms are overridden by pressure–natriuresis (Sachdeva and Weder , 2006), a concept where a rise or fall in blood pressure correlates positively with natriuresis. The intake of salt therefore, is key in blood pressure modulating.

5.3.1.5 Salt intake and dipping status

Dipping is a physiological phenomenon where blood pressure declines >10% during night sleep (Figure 26). For a non-dipper therefore, night systolic blood pressure decline does not exceed 10% that of day (Figure 27). Non-dipping status has been associated with target organ damage, left ventricular hypertrophy, microalbuminuria and cerebrovascular disease (Sachdeva and Weder, 2006). Non-dipping in this study was associated with increased salt intake, particularly salt sensitivity as observed in one study (Fukuda and Kimura, 2012). In this cohort, salt-sensitive non-dippers were more prevalent in the HIV positive group (n=21, 88%) than the HIV negative (n=19, 76%) and the shift from dipping to non-dipping status of individuals on switching from low to high salt diet was higher in PWH (by 9%) than the HIV negative (by 4%) (Table 32). There is evidence from studies conducted in Sub-Saharan Africa that non-dipping blood pressure prevalence is high in PWH (Borkum et al., 2017). Results from a systematic review reported that an abnormal diurnal blood pressure pattern may be more common among PWH compared to HIV negative individuals, i.e PWH are more likely to have non-dipping status compared with the HIV negative (Kent et al., 2016). The mechanisms underlying non-dipping status in the HIV positive contrasting that of HIV negative are currently unknown. However, a plausible explanation is that chronic inflammation and HIV infection contributes to endothelial dysfunction. This is further exacerbated by ART. All together, this may be the underlying mechanism resulting in dysregulation of the cardiovascular rhythm responsible for dipping (Borkum et al., 2014). Other factors associated with non-dipping status in HIV include but not limited to high psychosocial burden, high prevalence of sleep disturbance, and autonomic dysfunction (Kent et al., 2016) as summarized in Figure 33 but these were not included in this study.

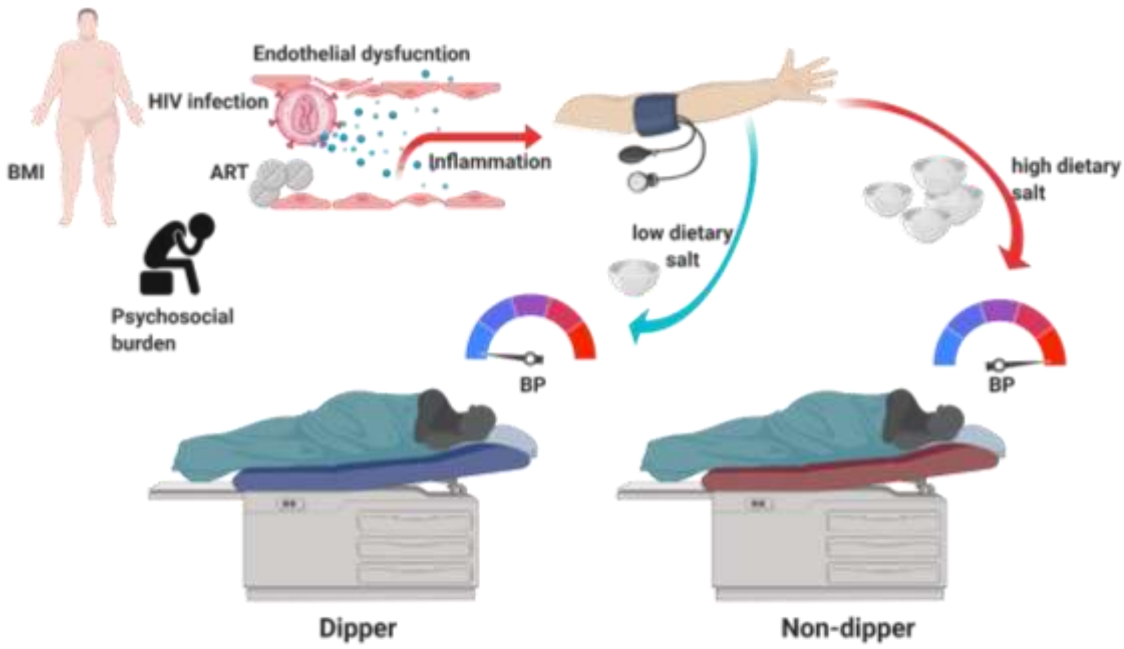


Figure 33. Correlates of dipping status in HIV
It is hypothesized that dipping status may be affected by high salt-intake, inflammation, high BMI, HIV-infection and antiretroviral therapy (ART), psychosocial burden faced in PWH and chronic inflammation. HIV-infection and antiretroviral therapy (ART) may have effects on propagating endothelial dysfunction. All these factors modulate the cardiovascular system and mechanisms involved in dipping. Biorender.com was used to create this figure.

Salt loading results in increased blood pressure response in salt-sensitive individuals. Vasodilation is the underpinning concept behind this. Salt-sensitive individuals do not vasodilate to accommodate fluid load that follows high salt loading hence an increase in blood pressure results whereas salt-resistant individuals vasodilate (Sachdeva and Weder , 2006). As a consequence of this, renal blood flow (RBF) is lowered and renal vascular resistance (RVR) increased in high salt diets resulting in higher blood pressure.

5.3.1.6 **Dietary salt, salt sensitivity and inflammation**

Salt sensitivity in HIV was associated with IL-6, monocyte count and isoLG percentage in monocytes in multivariate logistic regression (Table 35). Since the monocyte count was within the expected range, this possible association may have no clinical value. In HIV negative, salt sensitivity was only associated with nocturnal natriuresis (Table 36). This is likely the first study to report isoLG as it relates to salt sensitivity in PWH.

High salt intake causes high BP in some people by its role in activating the RAAS resulting in fluid overload (Kirabo, 2017). However, salt can also raise BP by eliciting an inflammatory condition that results in direct vessel damage and constriction from activated macrophages, T cells and the effect of inflammatory cytokines and vasoconstrictors secreted such as endothelin and reduction in endothelial nitric oxide (Barbaro et al., 2017; Kirabo et al., 2014; Van Beusecum et al., 2019). Dendritic cells and T cells of hypertensive mice fed with high salt produced higher inflammatory cytokines IL-17, IL-6, interferon-gamma and other cytokines compared with the healthy controls (Barbaro et al., 2017; Kirabo et al., 2014; Norlander et al., 2017; Van Beusecum et al., 2019). As most studies are conducted in murine models with dearth of literature of human studies employing salt and its effect on inflammation, this study tested the hypothesis that high dietary salt is associated with elevated inflammatory markers by measuring levels of cytokines in the plasma of hypertensive and normotensive individuals at the end of low- and high-salt diet (Table 40). Plasma cytokine levels did not differ between the two salt phases. However, surprisingly, the HIV positive normotensive group recorded higher IL-2 (p=0.013) on a high salt diet while in the HIV negative hypertensive group IL-21 was lowered (p=0.017). IL-2 was raised in the HIV-infected hypertensive group and not in the HIV negative while IL-21 was lowered in the HIV negative in contrast to the HIV-infected group. IL-2 is not a specific cytokine. IL-2 was shown to be increased with increased levels of CD4+ T cell immune activation correlating positively with cycling expression during acute infection and this was associated with less decline of CD4+ T cell after 2 years of infection (Xia et al., 2018). IL-2 is a γ chain cytokine produced by activated T cells and plays a critical protective role in the maintenance of immune tolerance by controlling the survival and proliferation of T-regulatory cells (Diallo et al., 2011). IL-2 is known to prevent apoptosis of HIV-infected cells (Diallo et al., 2011). The role that IL-2 plays in hypertension remains unknown, however, IL-2 production is increased in hypertension compared to normotensive individuals (Madej et al., 2018) suggesting a protective anti-inflammatory role. IL-21 was lowered on a high salt diet in HIV-negative individuals from this study. The role and implication of these findings in hypertension is unknown. Taken all together, these data suggest that short term high dietary salt may not be associated with inflammation, however further study is needed.

To understand the role of dietary salt on inflammatory cytokines, there is need for longer follow-up studies to validate this study's findings. This current study followed hypertensive and normotensive patients for a consecutive one week on low and high salt diets which may be too short to elicit an inflammatory milieu. Unlike studies conducted in mice, human studies require longer periods of intervention.

A summary of the concepts surrounding salt sensitivity underlying mechanisms are postulated in Figure 34.

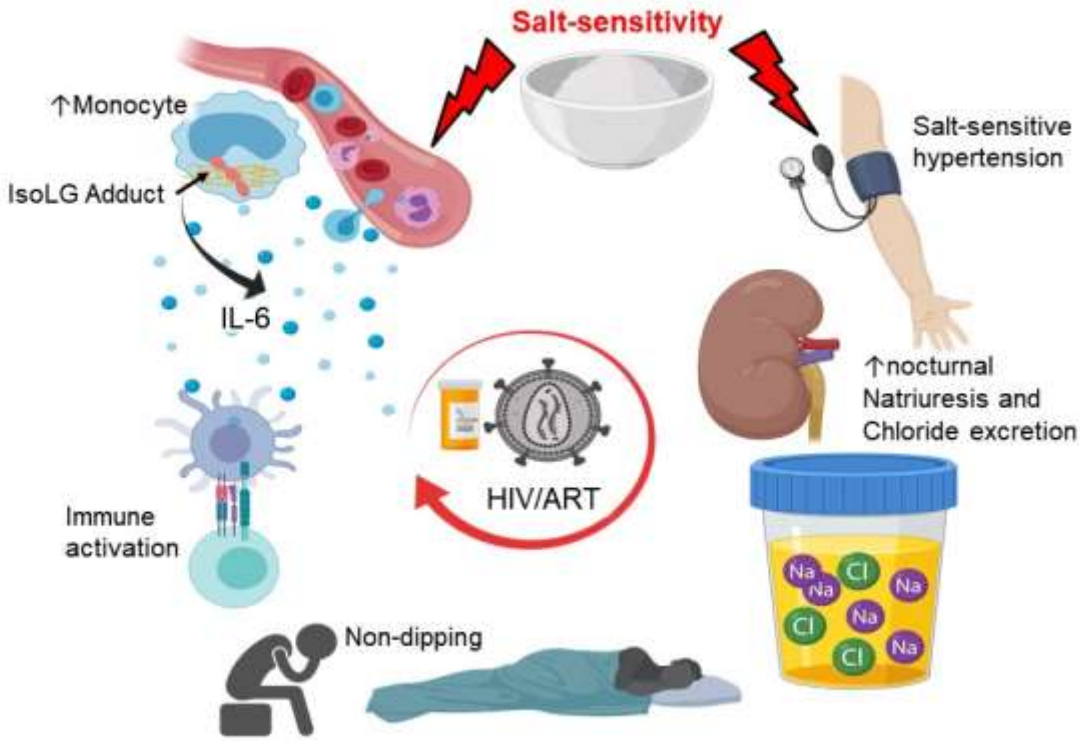


Figure 34. Salt-sensitivity, immune activation/inflammation in HIV
Salt-sensitivity in HIV was associated with vascular monocyte count, isoLevugandin adducts inside monocytes, IL-6, non-dipping blood pressure, nocturnal pressure-natriuresis and sodium excretion and hypertension. Biorender.com was used to create this figure.

5.4.0 The underlying Philosophy of "Hypertension as an inflammatory condition"

Essential hypertension, so-called, due to no clear single identifiable cause, occurs in 95% of all cases of hypertension (Carretero and Oparil, 2000). Several interrelated factors and related systems associated with blood pressure maintenance and regulation have been studied including cardiac function, nervous system, kidney, genetic predisposition, salt sensitivity and circadian physiology of dipping (Beevers et al., 2001; Elijovich et al., 2016; Fahme et al., 2018; Kirabo, 2017). Interestingly, immune cells with inflammatory roles have been linked to these systems suggesting that inflammation may be at the core in the pathogenesis of hypertension (Harrison, 2014). For example, stimulation of peripheral blood monocytes with angiotensin II and lipopolysaccharide induces cells from hypertensive persons to produce significant amounts of IL-1β and TNF-α which increase adhesion molecule expression and induce other cells to produce pro-inflammatory cytokines. Angiotensin II alone is a potential active player in the in-vivo activation and adhesion of circulating monocytes in essential hypertension. The mechanisms involve activation of the nuclear factor-kappa B (NF-κB), a transcription factor that regulates gene expression of inflammatory cytokines (Montecucco et al., 2011). A review of current evidence on the pathophysiology of hypertension challenges the textbook knowledge that we have held for many years. The immune mechanisms involving monocyte, macrophage, and T-cell activation have the potential to induce and propagate hypertension through the production of cytokines including TNF-α, IL-17, IFN-γ and IL-6, likely by promoting vasoconstriction, prolonged production of reactive oxygen species above that which cells can control, and sodium reabsorption in the kidney (Harrison, 2014; Kirabo et al., 2014). Immune cells and mediators cause morphological abnormalities in the vasculature and renal parenchyma that induce renal vasoconstriction, ischemia and injury consequently sustaining systemic hypertension. The inflammatory process can cause a vicious cycle by the production of reactive oxygen species that further injures and exacerbates the process. There is evidence that inflammatory processes contributes to blood pressure rises though the mechanisms are not yet clear (Montecucco et al., 2011). HIV infection and use of ART have also been implicated in the development of hypertension (Fahme et al., 2018). These pieces of evidence suggest that there is need to take a paradigm shift from previously held theories and embrace the idea that hypertension is a potential multifactorial ‘inflammatory’ disease (Montecucco et al., 2011) as postulated in Figure 35 below. The recent discoveries of the role of IsoLGs in the formation of neoantigens inside dendritic cells (Barbaro et al., 2017; Kirabo et al., 2014) with consequential involvement and activation of the adaptive immune system further suggests hypertension to have an autoimmune face.

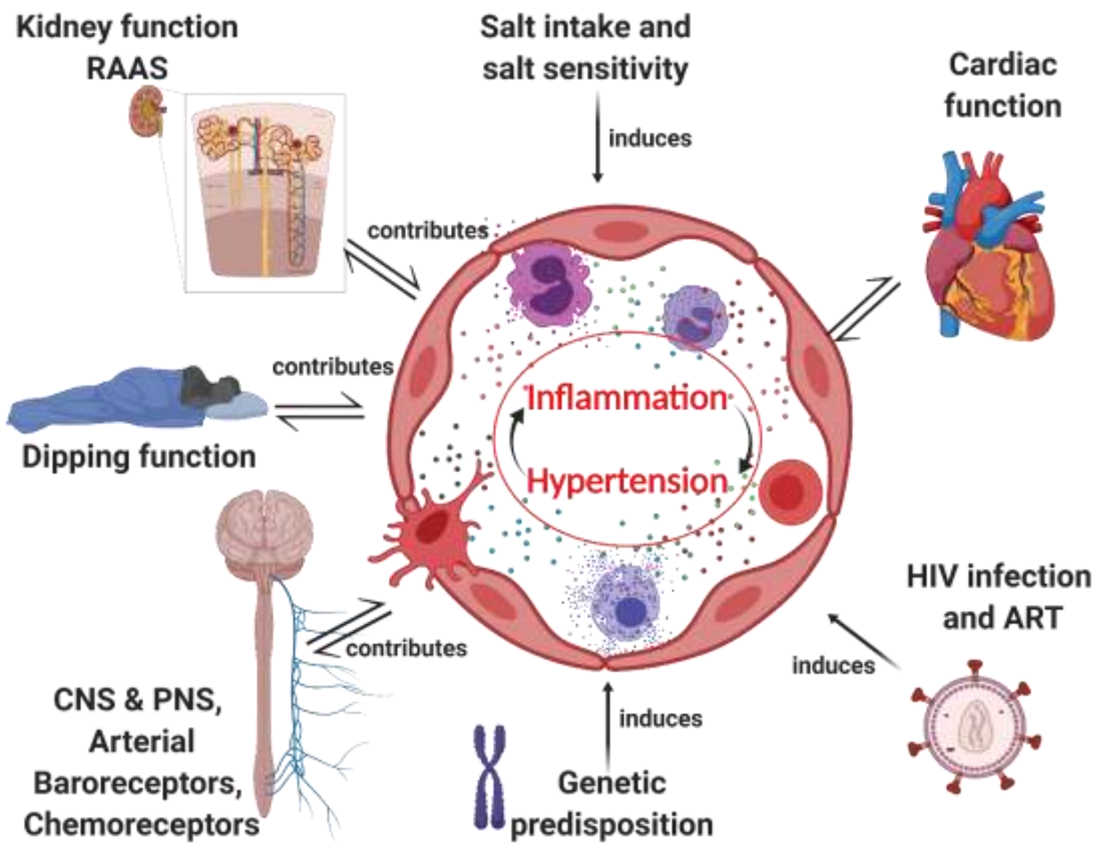


Figure 35. Philosophical concept postulating "Hypertension as an inflammatory condition": The pathophysiology of hypertension

Immune activation and inflammation are at the core of hypertension pathogenesis and progression regardless of the multifactorial contributors and regulators of blood pressure. Biorender.com was used to create this figure.

5.5 Strengths and Limitations of the Study

1. This is likely, the first study known to us from the literature that has elucidated relationships between hypertension and inflammatory markers. This is novel to the existing textbook knowledge that we have held for years.
2. Since the underlying factors associated with HIV and hypertension are multifactorial with other conditions specific to individuals, many factors not sort for are likely to confound the results of the study. However, the experimental nature of the study minimized confounding.
3. Hypertension, inflammation and HIV are all risk factors for CVD. Knowledge about a possible interaction in this research was of clinical interest.
4. Dietary salt intake was not monitored rigorously. Although participants were advised not to eat processed foods and recorded their daily meals and often called for advice when they were not sure of a particular food, possibilities of

them having taken foods with salt other than the salt they were given cannot be ruled. This could impact salt excretion results. However, the similarities in salt excretion is evident that the majority followed the dietary salt intake.

5. In as much as we observed statistical differences (higher or lower levels between hypertensives and normotensives) in immune markers and cells, the levels may be within normal levels and further exploration in the clinical or biological effects to these differences is warranted.

6.0 CHAPTER SIX: CONCLUSION

The risk factors associated with hypertension in PWH include employment status, dietary salt, fasting blood sugar, physical activity and sedentary hours. This study found that, although within the expected reference range, neutrophils, eosinophils and monocytes are higher in hypertension compared with normotensive HIV positive individuals at Livingstone Central Hospital. HIV-infection and treatment are likely to be associated with elevated levels of IL-6, IL-10, IL-17A, TNF-α, isoLevuglandins, eosinophils and markers of immune activation. The study has also shown evidence and confirmed that high intake of dietary salt in HIV positive individuals at Livingstone Central Hospital, elicits a hypertensive stimulus and modulates immune-activation and inflammation status, sodium excretion and dipping status in hypertension.

6.1 Novel findings

This study found several aspects novel to current literature and these aspcets are listed below:

1. HIV-infected/ART-treated hypertensive individuals have higher fasting blood sugar and at greater risk of developing diabetes as compared to the normotensive at Livingstone Central Hospital.
2. Neutrophilia, an inflammatory surrogate, may be associated with hypertension in PWH
3. ART-treated PWH have higher red blood cell counts suggesting that functional characteristics of erythrocytes may be modulated in hypertension.
4. Hypertensive PWH on antiretroviral therapy (ART) with long-term viral suppression exhibit higher levels of circulating eosinophils and IL-5 compared to normotensive PWH. However, eosinophil levels were within the expected range.
5. IL-10 is elevated in some HIV-infected/ART-treated individuals reflecting a positive correlation with IL-6. This may reflect a response mechanism modulated by IL-6 in HIV.
6. IsoLevuglandin adducts in monocytes are reflective of dietary salt intake and inflammation regardless of HIV status and are increased in hypertension. IsoLGs are inflammatory cytokine secretagogues. Targeting IsoLG adducts is

potential for therapy and management of hypertension. However, there is need to study the role IsoLGs may play in other conditions.

7. Salt-sensitivity is likely to be associated with high IL-6, monocytes and isoLG adducts in monocytes. However, IL-6 and monocyte count were within the expected range.
8. One week of high salt does not significantly raise Inflammatory cytokines (IL-5, IL-13, IL-2, IL-6, IL-9, IL-10, IFN-γ, TNF-α, IL-17A, IL-17F, IL-4, IL-21, IL-22). This study suggests that a longer period may be required.

6.2 Recommendations

1. PWLH are at increased risk of cardiovascular disease compared to HIV-negative persons, and the management of hypertension is essential to reducing disease burden in this population.
2. Traditional non-routine risk factors such as employment status, dietary salt, fasting blood sugar, physical activity and sedentary hours were associated with hypertension, hence, there is much need for intensifying monitoring and incorporating additional modifiable non-routine risk factors for hypertension in HIV care
3. National guidelines for accurate diagnosis and management of hypertension in HIV adapted from AHA/ACC are necessary for improving care.
4. Salt-sensitivity tests could be introduced in the ART clinic for personalized prevention and care for hypertension comorbidity.
5. Ambulatory blood pressure monitoring should be introduced to confirm hypertension, assess blood pressure control on antihypertensive and to diagnose non-dipping status.
6. Factors associated with non-dipping status should be assessed in the clinic to help prevent adverse outcomes in hypertensive patients.
7. To determine the effect of using a Salt-reduction and Hypertension Education Integrated Personalized Clinician-patient Counselling and Close-follow-up (SHEDIPeC3) model to improve blood pressure control and adherence to antihypertensive medication among people living with HIV.

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7.1 Appendices

Appendix A Protocol for measuring inflammatory cytokines using Legendplex^{TM} multi-analyte flow Assay kit cat 740722

Principle of the Assay

BioLegend’s LEGENDplexTM assays are bead-based immunoassays using the same basic principle as sandwich immunoassays.

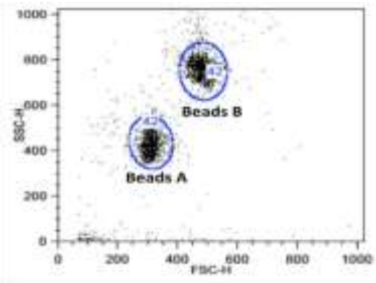
Beads are differentiated by size and internal fluorescence intensities. Each bead set is conjugated with a specific antibody on its surface and serves as the capture beads for that particular analyte. When a selected panel of capture beads is mixed and incubated with a sample containing target analytes specific to the capture antibodies, each analyte will bind to its specific capture beads. After washing, a biotinylated detection antibody cocktail is added, and each detection antibody in the cocktail will bind to its specific analyte bound on the capture beads, thus forming capture bead-analyte-detection antibody sandwiches. Streptavidin-phycoerythrin (SA-PE) is subsequently added, which will bind to the biotinylated detection antibodies, providing fluorescent signal intensities in proportion to the number of bound analytes.

Since the beads are differentiated by size and internal fluorescence intensity on a flow cytometer, analyte-specific populations can be segregated and PE fluorescent signal quantified. The concentration of a particular analyte is determined using a standard curve generated in the same assay.

Beads Usage

The Human Th Cytokine Panel uses two sets of beads. Each set has a unique size that can be identified based on their forward scatter (FSC) and side scatter (SSC) profiles (Beads A and Beads B, Figure 1). Each bead set can be further resolved based on their internal fluorescence intensities. The internal dye can be detected using FL3, FL4, or APC channel, depending on the type of flow cytometer used. The smaller Beads A consists of 6 bead populations and the larger Beads B consists of 7 bead populations (Figure 2-3).

Using a total of 13 bead populations distinguished by size and internal fluorescent dye, the Human Panel allows simultaneous detection of 13 cytokines in a single sample. Each analyte is associated with a particular bead set as indicated (Figures 2-3 and Table 1).



Beads A = smaller beads

Beads B = larger beads

Figure 1. Beads Differentiated by Size

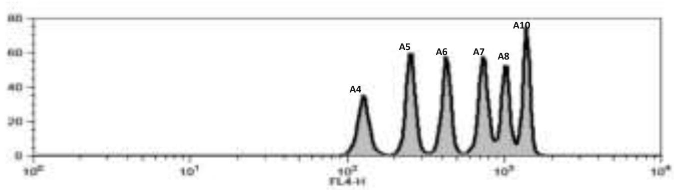


Figure 2. Beads A Classification by FL4

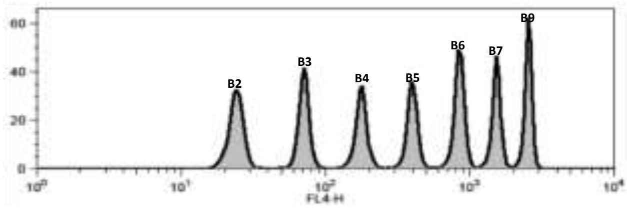


Figure 3. Beads B Classification by FL4

Materials Supplied

The LEGENDplex^{TM} kit contains reagents for 100 tests, listed in the table below. When assayed in duplicate, this is enough for an 8-point standard curve and 40 samples.

Table 1. Kit components

Kit Components	Quantity	Volume	Part #
Setup Beads 1: FITC Beads	1 vial	1 mL	77840
Setup Beads 2: PE Beads	1 vial	1 mL	77842
Setup Beads 3: Raw Beads	1 vial	2 mL	77844
Capture Beads* (see tables below for more information)	**varies**	**varies**	**Varies***
Human Th Panel Detection Antibodies	**1 bottle**	**3.5 mL**	**Varies***
Human Th Panel Standard Cocktail, Lyophilized	**1 vial**	**lyophi-lized**	**Varies***
LEGENDplex™ SA-PE	1 bottle	3.5 mL	77743
LEGENDplex™ Matrix B, Lyophilized	1 vial	lyophi-lized	77549
LEGENDplex™ Assay Buffer	1 bottle	25 mL	77562
LEGENDplex™ Wash Buffer, 20X	1 bottle	25 mL	77564
Filter Plate** or V-bottom Plate***	1 plate		76187** or 76883***
Plate Sealers	4 sheets		78101
Data Analysis Software Dongle	1		21217
Human Th Panel Manual	1		75475

* For full panel, premixed beads are provided ready-to-use. For subpanels, individual beads are provided at 13X concentration. For Standard and Detection Antibodies, full panels use part numbers and subpanels use catalog numbers (See tables below for details).

** For kit with filter plate. *** For kit with V-bottom plate. Only one plate is provided for each kit.

Table 2. For Th Panel (Full Panel):

Kit Components	Quantity	Volume	Part #
Human Th Panel Premixed Beads	1 bottle	3.5 mL	76328
Human Th Panel Detection Antibodies	1 bottle	3.5 mL	76330
Human Th Standard Cocktail, Lyophilized	1 vial	lyophilized	75473

Table 3. For Th Subpanels:

Kit Components	Quantity	Volume	Cat. #
Human IL-5 beads, 13X	1 vial	270 µL	740043
Human IL-13 Beads, 13X	1 vial	270 µL	740047
Human IL-2 Beads, 13X	1 vial	270 µL	740717
Human IL-6 Beads, 13X	1 vial	270 µL	740044
Human IL-9 Beads, 13X	1 vial	270 µL	740045
Human IL-10 Beads, 13X	1 vial	270 µL	740046
Human IFN-γ Beads, 13X	1 vial	270 µL	740052
Human TNF-α Beads, 13X	1 vial	270 µLL	740053
Human IL-17A Beads, 13X	1 vial	270 µL	740546
Human IL-17F Beads, 13X	1 vial	270 µL	740718
Human IL-4 Beads, 13X	1 vial	270 µL	740042
Human IL-21 Beads, 13X	1 vial	270 µL	740719
Human IL-22 Beads, 13X	1 vial	270 µL	740720
Human Th Cytokine Panel Detection Antibodies	1 bottle	3.5 mL	740758
Human Th Cytokine Panel Standard Cocktail, Lyophilized	1 vial	lyophilized	740759

Materials to be Provided by the End-User

• A flow cytometer equipped with two lasers (e.g., a 488 nm blue laser or 532 nm green laser and a 633-635 nm red laser) capable of distinguishing

575 nm and 660 nm or a flow cytometer equipped with one laser (e.g., 488 nm blue laser) capable of distinguishing 575 nm and 670 nm.

Multichannel pipettes capable of dispensing 5 µL to 200 µL

Reagent reservoirs for multichannel pipette

Polypropylene microfuge tubes (1.5 mL)

Laboratory vortex mixer

Sonicator bath (e.g., Branson Ultrasonic Cleaner model #B200, or equivalent)

Aluminum foil

Absorbent pads or paper towels

Plate shaker (e.g., Lab-Line Instruments model #4625, or equivalent)

Tabletop centrifuges (e.g., Eppendorf centrifuge 5415 C, or equivalent)

1.1 mL polypropylene micro FACS tubes, in 96-tube rack (e.g., National Scientific Supply Co, catalog # TN0946-01R, or equivalent).

ASSAY PREPARATION

<u>Reagent Preparation</u>

Preparation of Antibody-Immobilized Beads

Pre-mixed beads bottle was sonicated for 1 minute in a sonicator bath and then vortexed for 30 seconds prior to use.

Preparation of Wash Buffer

The 20X Wash Buffer was brought to room temperature and mixed to bring all salts into solution. 25 mL of 20X Wash Buffer was diluted with 475 mL deionized water. Unused portions were stored between 2°C and 8°C for up to one month.

Preparation of Matrix B (for Serum Samples Only)

5.0 mL LEGENDplex^{TM} Assay Buffer was added to the bottle containing lyophilized. For matrix B, it was incubated for atleast 15 minutes for complete reconstitution and vortexed to mix well and the left over Matrix B stored at -20^{0}C up to one month.

<u>Standard Preparation</u>

Prior to use, the lyophilized Human Th Panel Standard Cocktail was reconstituted with 250 µL Assay Buffer.

It was then mixed and the vial allowed to sit at room temperature for 10 minutes, and then transferred the standard to an appropriately labeled polypropylene microcentrifuge tube. This was used as the top standard C7.

Note: The top standard concentrations of analytes in this panel was set at various concentrations, but may be subject to change from lot to lot (see lot-specific Certificate of Analysis provided in the kit box for details).

6 polypropylene microcentrifuge tubes were labelled as C6, C5, C4, C3, C2 and C1, respectively.

75 µL of Assay Buffer was added to each of the six tubes. 1:4 dilution of the top standard was prepared by transferring 25 µL of the top standard C7 to the C6 tube and mixed well. This was the C6 standard.

In the same manner, serial 1:4 dilutions to obtain C5, C4, C3, C2 and C1 standards were performed (**see the table 4 below using the top standard at 10,000 pg/mL as an example**). Assay Buffer was used as the 0 pg/mL standard (C0). =0

Table 4. Standard and serial dilutions

Tube/Standard ID	Serial Dilution	Assay Buffer to add (µL)	Standard to add	Final Conc. (pg/mL)
C7	--	--	--	10,000
C6	1:4	75	25 µL of C7	2,500
C5	1:16	75	25 µL of C6	625
C4	1:64	75	25 µL of C5	156.3
C3	1:256	75	25 µL of C4	39.1
C2	1:1024	75	25 µL of C3	9.8
C1	1:4096	75	25 µL of C2	2.4
C0	--	75	--	0

<u>Sample Dilution</u>

Serum samples were diluted 2-fold with Assay Buffer before being tested (e.g. dilute 50 µL of sample with 50 µL of Assay Buffer).

If further sample dilution was desired, dilution was done with Matrix B to ensure accurate measurement.

Adding serum samples without dilution usually results in low assay accuracy and possibly, clogging of the filter plate.

ASSAY PROCEDURE USING A V-BOTTOM PLATE

All reagents were allowed to warm to room temperature (20-25°C) before use. The plate was kept upright during the entire assay procedure, including the washing steps, to avoid losing beads. The plate was also placed in the dark or wrapped with aluminum foil for all incubation steps.

Standards and samples were run in duplicate and arranged on the plate in a vertical configuration convenient for data acquisition and analysis (as shown in attached PLATE MAP, page 33). Standards were loaded in the first two columns.

For measuring cell culture supernatant samples, the plate was loaded as shown in the table 5 below (in the order from left to right):

Table 5. plate loading for cell culture supernatant samples

	Assay Buffer	Matrix B	Standard	Sample*
Standard Wells	25 µL	---	25 µL	---
Sample wells	25 µL	---	---	25 µL

For measuring serum samples, the plate was loaded as shown in the table 6 below (in the order from left to right):

Table 6. plate loading for serum samples

	Assay Buffer	Matrix B	Standard	Sample*
Standard Wells	---	25 µL	25 µL	---
Sample wells	25 µL	---	---	25 µL

*See **Sample Dilution**

Mixed beads were vortexed for 30 seconds. 25 µL of mixed beads was added to each well. The total volume was 75 µL in each well after beads addition. (Note: During beads addition, mixed beads bottle was shaken intermittently to avoid bead settling).

The plate was sealed with a plate sealer. The entire plate was then covered with aluminum foil to protect the plate from light and shaken at 800 rpm on a plate shaker for 2 hours at room temperature **(Depending on the shaker, the speed was adjusted.**

The optimal speed is one that is high enough to keep beads in suspension during incubation, but not too high so it causes spill from the wells).

The plate was centrifuged at 1050 rpm (~250 g) for 5 minutes, using a swinging bucket rotor (G.H 3.8) with microplate adaptor. Excessive centrifugation speed was avoided as it may make it harder to resuspend beads in later steps. Caution was taken to make sure the timer of the centrifuge works properly and standby to make sure the centrifuge reaches preset speed.

Immediately after centrifugation, supernatant was dumped into a sink by quickly inverting and flicking the plate **in one continuous and forceful motion**. The plate was then blotted on a stack of clean paper towel and the remaining liquid drained from the well as much as possible. Care was taken not to disturb the bead pellet.

Alternatively, removal of the supernatant was completed using a multichannel pipette set at 75 µL removing as much liquid as possible without removing any beads. Pipette tips were changed between each row or column.

The plate was then washed by dispensing 200 µL of 1X Wash Buffer into each well and incubated for one minute. This step was repeated.

25 µL of Detection Antibodies was added to each well and then the plate was sealed with a new plate sealer. The entire plate was covered with aluminum foil to protect the plate from light and shaken at 800 rpm on a plate shaker for 1 hour at room temperature.

The plate was not washed. 25 µL of SA-PE was then added to each well directly and then the platewas sealed with a new plate sealer. The entire plate was then wrapped with aluminum foil and the plate shaken on a plate shaker at approximate 800 rpm for 30 minutes at room temperature.

Immediately after centrifugation, supernatant was dumped into a sink by quickly inverting and flicking the plate **in one continuous and forceful motion**. The plate was then blotted on a stack of clean paper towel and the remaining liquid drained from the well as much as possible. Care was taken not to disturb the bead pellet.

The plate was then washed by dispensing 200 µL of 1X Wash Buffer into each well and incubated for one minute.

The plate was then washed by dispensing 200 µL of 1X Wash Buffer into each well and incubated for one minute. Immediately after centrifugation, supernatant was dumped into a sink by quickly inverting and flicking the plate **in one continuous and forceful motion**. The plate was then blotted on a stack of clean paper towel and the remaining liquid drained from the well as much as possible. Care was taken not to disturb the bead pellet.

The plate was then washed by dispensing 200 µL of 1X Wash Buffer into each well and incubated for one minute. This washing step is optional but helps to reduce the background.

150 µL of 1X Wash Buffer was then added to each well. The beads then were resuspend by pipetting.

Samples were then read on a flow cytometer immediately within the same day of the assay (Note: Prolonged sample storage can lead to reduced signal).

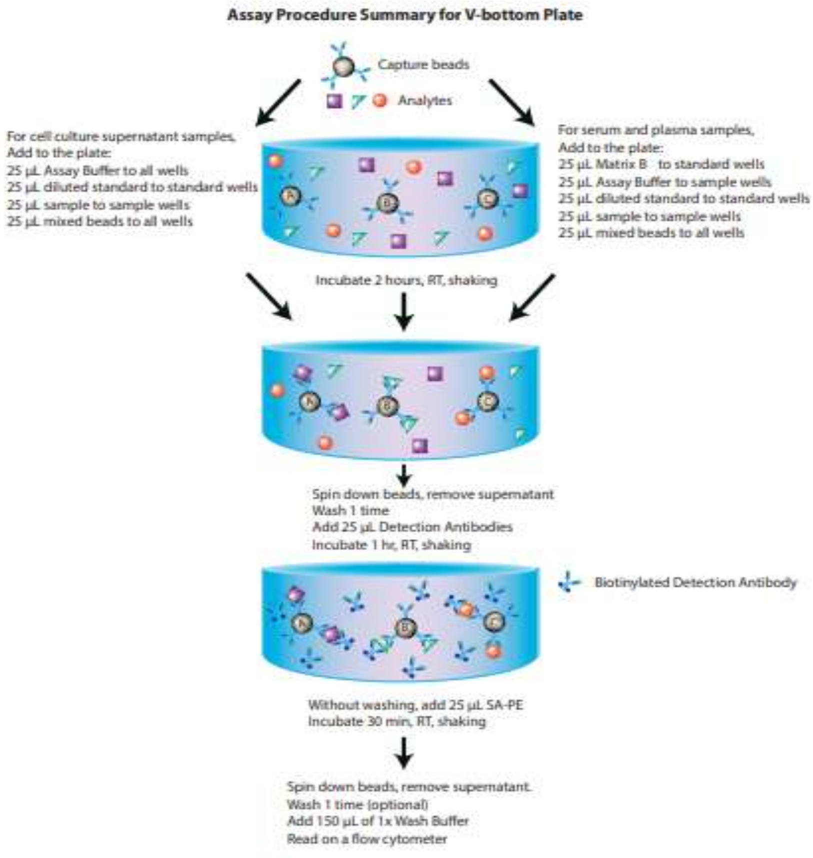


Figure 4. Procedure summary

FLOW CYTOMETER SETUP

In order to generate reliable data, the flow cytometer was set up properly before data acquisition.

DATA ACQUISITION AND ANALYSIS

<u>Data Acquisition</u>

A new template was created on the flow cytometer computer.

Each sample was vortexed for 5 seconds before analysis.

The flow rate was set to low. The number of beads were set to be acquired to about 300 per analyte (e.g., acquire 2,400 beads for an 8-plex assay or 4000 beads for a 13-plex assay). A large gate was created to include both Beads A and Beads B (gate A+B) and set to acquire the number of events in gate A + B. This excluded majority of the debris. Samples were then acquired.

When reading samples, the flow cytometer was set to setup mode first and waiting until bead population was stabilized before recording or switching to acquisition mode. When naming data files, a simple naming with a consecutive numbering for easy data analysiswas employed (e.g. for standards, C0.001, C0.002, C1.003, C1.004, C2.005, C2.006, C3.007, C3.008, ... C7.015, C7.016; for samples, S1.017, S1.018, S2.019, S2.020, S3.021, S3.022…)

All FCS files were stored in the same folder for each assay. If running multiple assays, a separate folder was created for each assay.

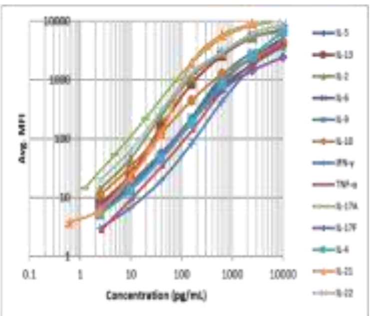
Data Analysis

The FCS file generated on a flow cytometer was analyzed using BioLegend's LEGENDplex^{TM} Data Analysis Software. The software was installed on a PC running Windows 10 and uses it in conjunction with the Data Analysis Software Dongle included in the kit. The dongle had a license key stored in it and was needed to run the software. To use the dongle, it was simply plugged in the USB port of the computer on which the data analysis software was installed, prior to launching the software.

ASSAY CHARACTERIZATION

Representative Standard Curve

This standard curve was generated using the LEGENDplex^{TM} Human Th Cytokine Panel for demonstration purposes only. A standard curve must be run with each assay.



Assay Sensitivity

The minimum detectable concentration (MDC) is the theoretical limit of detection calculated using the LEGENDplex^{TM} Data Analysis Software by applying a 5-parameter curve fitting algorithm. Assay sensitivity presented here is MDC + 2x STDEV.

Table 7. Assay sensitivity in serum and culture

Analyte	Sensitivity in Serum (pg/mL)	Sensitivity in Cell Culture Medium (pg/mL)
Human IL-5	1.2 + 1.3	1.3 + 1.2
Human IL-13	1.4 + 0.7	1.4 + 0.5
Human IL-2	1.4 + 0.4	1.4 + 0.5
Human IL-6	1.0 + 0.8	1.1 + 1.0
Human IL-9	1.7 + 1.4	1.5 + 1.2
Human IL-10	0.7 + 0.4	0.9 + 0.4
Human IFN-γ	1.1 + 0.7	1.3 + 1.1
Human TNF-α	0.7 + 0.5	0.9 + 0.6
Human IL-17A	1.9 + 0.6	2.0 + 0.6
Human IL-17F	0.8 + 0.7	1.0 + 0.7
Human IL-4	1.0 + 0.8	0.9 + 0.8
Human IL-21	6.0 + 3.0	6.0 + 3.0
Human IL-22	1.5 + 0.5	1.5 + 0.7

Cross-Reactivity

The following recombinant proteins were tested at 50 ng/mL using the LEGENDplex™ Human Th Panel. The IL-17A and IL-17F assays also detects human IL-17A/F. No or negligible cross-reactivity was found for all other analytes.

Table 8. cross-reactivity data

hIL-2	hIL-4	hIL-5	hIL-6	hIL-9	hIL-10
hIL-17A	hIL-17F	hIL-21	hIL-13	hIL-22	hTNF-α
hIFN-γ	IL-17A/F	hIL-27	hIL-1α	hIL-1β	hIL-23
hIL-3	hIL-33	hIL-7	hIL-8	hTGF-β1	hTGF-β2
hMIP-1α	hMIP-2	hMCP-1	hRANTES	hEotaxin	hTARC
hMIP-3α	hGRO-α	hCXCL17	hMIG	hITAC	hIL-12p70
hIL-15	hENA-78	hIP-10	mTNF-α	m IFN-γ	mIL-17AF
mIL-2	mIL-4	mIL-5	mIL-6	mIL-9	mIL-10
mIL-17A	mIL-17F	mIL-21	mIL-13	mIL-22	

Accuracy (Spike Recovery)

For spike recovery in serum, target proteins with known concentrations were spiked into human serum at three different levels within the assay range. The spiked samples were then assayed, and the measured concentrations were compared with the expected values.

Table 9. spike recovery in serum data

Analyte	% of Recovery in Serum	Analyte	% of Recovery in Serum
Human IL-5	97%	Human IFN-γ	100%
Human IL-13	100%	Human IL-17A	86%
Human IL-2	119%	Human IL-17F	93%
Human IL-6	80%	Human IL-4	123%
Human IL-9	69%	Human IL-21	98%
Human IL-10	100%	Human IL-22	94%
Human TNF-α	111%		

Linearity of Dilution

For testing linearity of dilution, serum samples were first diluted two-fold with Assay Buffer, then serially diluted 1:2, 1:4, 1:8 with Matrix B and assayed. The measured concentrations of serially diluted samples were then compared with that of the two-fold diluted samples.

Table 10. Linearity of Dilution

Analyte	Linearity of Dilution	Analyte	Linearity of Dilution
Human IL-5	76%	Human IFN-γ	103%
Human IL-13	106%	Human IL-17A	95%
Human IL-2	106%	Human IL-17F	111%
Human IL-6	115%	Human IL-4	102%
Human IL-9	122%	Human IL-21	110%
Human IL-10	122%	Human IL-22	101%
Human TNF-α	103%		

Intra-Assay Precision

Two samples with different concentrations of target proteins were analyzed in one assay with 16 replicates for each sample. The intra-assay precision was calculated as below.

Table 11. Intra Assay precision

Analyte	Sample	Mean (pg/mL)	STDEV	%CV
Human IL-5	Sample 1	79.0	4.4	5%
	Sample 2	325.3	21.3	7%
Human IL-13	Sample 1	35.1	2.0	6%
	Sample 2	125.2	8.9	7%
Human IL-2	Sample 1	37.5	3.4	9%
	Sample 2	140.4	13.9	10%
Human IL-6	Sample 1	49.6	3.5	7%
	Sample 2	198.5	11.9	6%
Human IL-9	Sample 1	66.8	3.7	5%
	Sample 2	217.1	13.9	6%
Human IL-10	Sample 1	23.9	1.9	8%
	Sample 2	91.5	4.4	5%
Human IFN-γ	Sample 1	23.2	2.4	10%
	Sample 2	85.1	5.7	7%
Human TNF-α	Sample 1	50.0	3.3	7%
	Sample 2	183.0	7.0	4%
Human IL-17A	Sample 1	108.0	5.3	5%
	Sample 2	520.3	40.0	8%
Human IL-17F	Sample 1	33.4	1.9	6%
	Sample 2	143.5	10.1	7%
Human IL-4	Sample 1	29.2	1.7	6%
	Sample 2	116.6	5.4	5%
Human IL-21	Sample 1	131.5	6.5	5%
	Sample 2	1013.9	51.6	7%
Human IL-22	Sample 1	56.1	4.1	7%
	Sample 2	212.6	15.3	7%

Inter-Assay Precision

Two samples with different concentrations of target proteins were analyzed in three independent assays with 3 replicates for each sample. The interassay precision was calculated as below.

Table 12. Inter-Assay Precision

Analyte	Sample	Mean (pg/mL)	STDEV	%CV
Human IL-5	Sample 1	82.8	10.3	12%
	Sample 2	362.4	45.6	13%
Human IL-13	Sample 1	36.1	5.9	16%
	Sample 2	131.6	14.7	11%
Human IL-2	Sample 1	38.3	7.6	20%
	Sample 2	158.5	28.1	18%
Human IL-6	Sample 1	57.5	6.4	11%
	Sample 2	225.0	22.9	10%
Human IL-9	Sample 1	69.2	11.9	17%
	Sample 2	231.1	23.8	10%
Human IL-10	Sample 1	24.2	4.5	18%
	Sample 2	94.0	9.3	10%
Human IFN-γ	Sample 1	17.9	3.7	21%
	Sample 2	85.6	10.1	12%
Human TNF-α	Sample 1	48.7	8.5	18%
	Sample 2	197.3	22.8	12%
Human IL-17A	Sample 1	119.5	11.3	9%
	Sample 2	590.5	67.1	11%
Human IL-17F	Sample 1	32.3	4.9	15%
	Sample 2	140.0	67.1	9%
Human IL-4	Sample 1	30.1	4.1	13%
	Sample 2	123.0	14.3	12%
Human IL-21	Sample 1	144.8	25.9	18%
	Sample 2	730.8	182.7	25%
Human IL-22	Sample 1	53.8	6.0	11%
	Sample 2	206.8	14.6	7%

Biological Samples

Serum

Normal human serum samples (n=16) were tested for endogenous levels of the Th related cytokines. The concentrations measured are shown below.

Table 13. Expected values in serum

Analyte	Range (pg/ml)	No. of Detectable	% of Detectable	Mean (pg/mL)
Human IL-5	ND-12.7	11	69%	3.9
Human IL-13	1.4 - 17.3	16	100%	5.1
Human IL-2	ND - 79.4	4	25%	39.1
Human IL-6	ND - 18.4	9	56%	12.9
Human IL-9	ND -5.8	4	25%	3.9
Human IL-10	ND-1.3	3	19%	1.1
Human IFN-γ	ND - 39.4	10	63%	11.5
Human TNF-α	ND - 14.0	8	50%	5.9
Human IL-17A	ND	0	0%	ND
Human IL-17F	ND - 107.0	8	50%	29.0
Human IL-4	ND-56.5	7	44%	18.1
Human IL-21	ND-133.2	10	63%	32.7
Human IL-22	ND - 15.2	6	38%	6.4

ND = Non-detectable

Cell Culture Supernatant

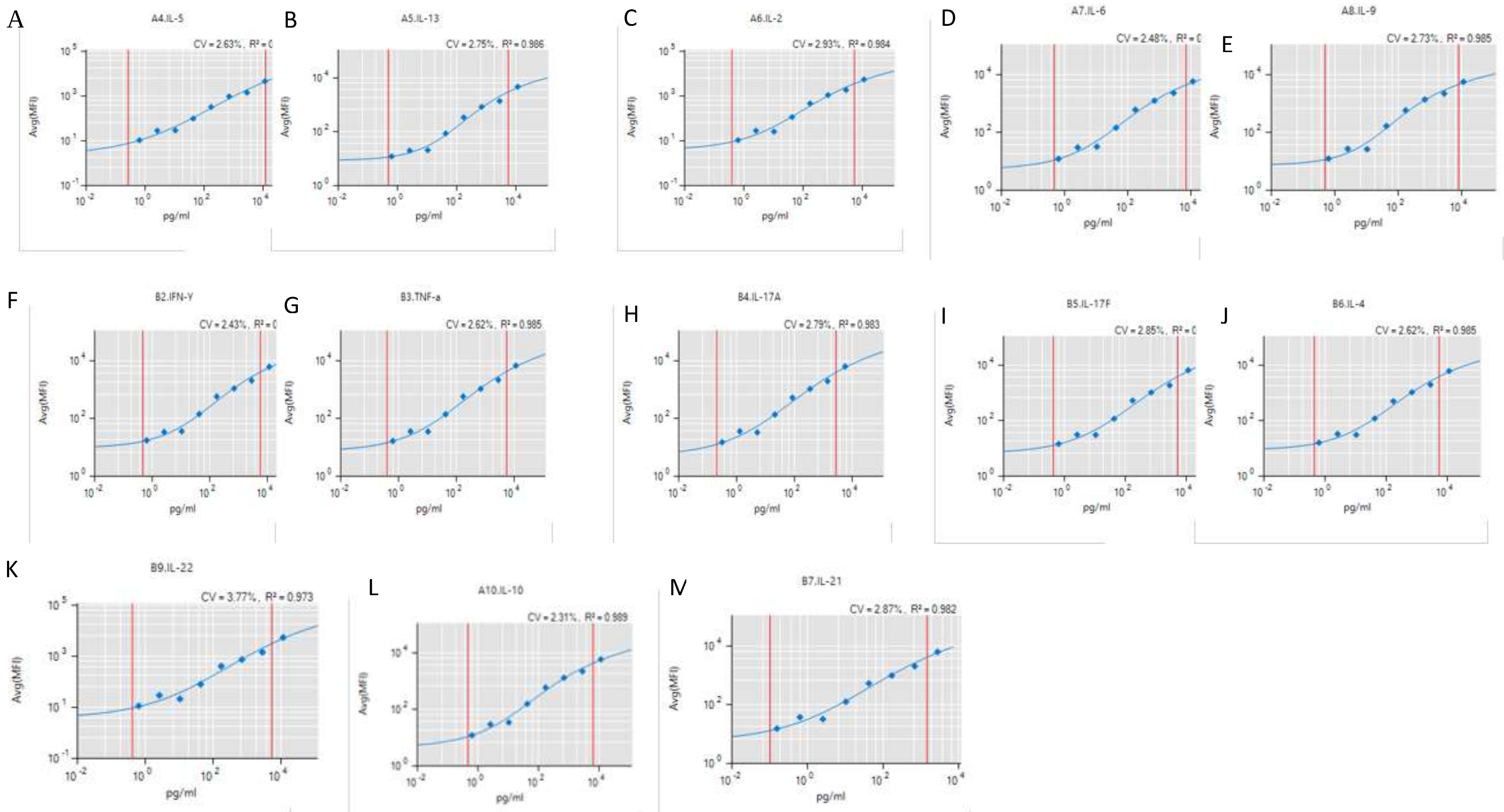
Human PBMCs (1 x 10^6 cells/mL) were cultured under various conditions (LPS, 100 ng/mL; CD3, 1 µg/mL plate-coated; CD28, 1 µg/mL soluble; PMA, 20 ng/mL; Ionomycin, 500 ng/mL). Supernatants were collected after the time intervals designated below and assayed with the LEGENDplex™ Human Th Cytokine Panel. The results (all in pg/mL) are summarized below.

Table 14: Highest secreted levels in cell Culture supanants for a maximum of 96 hours

Analyte	Control	LPS, 96 hours	CD3 + CD28, 24 hours	PMA + Ionomycin, 96 hours
Human IL-5	ND	2	1765	5952
Human IL-13	ND	12	399	1844
Human IL-2	3	12	1737	>10000
Human IL-6	3	>10000	>10000	>10000
Human IL-9	ND	4	360	511
Human IL-10	ND	440	603	22
Human IFN-γ	3	360	>10000	>10000
Human TNF-α	10	23	>10000	8267
Human IL-17A	ND	37	265	340
Human IL-17F	ND	27	143	221
Human IL-4	ND	ND	133	22
Human IL-21	ND	ND	20	185
Human IL-22	ND	47	369	32

ND = Non-detectable

Appendix B. Expected Standard curves for the Legendplex assay protocol



Standard curves for IL-5 (A), IL-13 (B), IL-2 (C), IL-6 (D), IL-9 (E), interferon-gamma (IFN-γ) (F), tumor necrosis factor-alpha (TNF-α)(G), IL-17A (H), IL-17F (I), IL-4 (J), IL-22 (K), IL-10 (L) and IL-21 (M). Average mean fluorescence intensity (Avg MFI) is plotted against cytokine concentration in picograms/mililiter (pg/ml). CV, coefficient of variation; R^2, coefficient of determination. Red lines denotes lower and upper detection limits.

Appendix C. Supplimentary results to Graphs

Table 1. Supplimentary data for figures 11-13

	Hypertension (n, 41) Median (IQR)	Normotensive (n, 175) Median (IQR)	P-value
Nadir CD4, *cell/mm³*	160 (76, 292)	202 (98, 362)	0.250
CD4%	25 (17, 32)	28 (20, 36)	**0.040**
WBC, *x10⁹/L*	5.5 (4.5, 6.6)	4.9 (3.9, 5.7)	**0.029**
Neutrophils, *x10⁹/L*	2.46 (2.09, 3.82)	2.27 (1.78, 2.87)	**<0001**
Lymphocytes, *x10⁹/L*	2.53 (2.09, 3.48)	2.14 (1.68, 2.54)	**0.001**
Monocytes, *x10⁹/L*	0.38 (0.31, 0.62)	0.35 (0.28, 0.42)	**0.003**
Platelets, *x10⁹/L*	262 (235, 342)	256 (215, 310)	0.517
ESR (males), *mm/hr*	23 (10, 46)	32 (18, 55)	**0.024**
ESR (females), *mm/hr*	52 (31, 66)	37 (19, 58)	0.120
RBC, *x10⁹/L*	4.71 (4.04, 4.90)	4.40 (4.05, 4.89)	**0.013**
Hemoglobin (males), *g/dl*	14.8 (13.9, 15.4)	13.9 (12.7, 15.3)	0.141
Hemoglobin (females), *g/dl*	12.2 (11.0, 13.2)	12.5 (11.6, 13.3)	0.346
NTL	1.35 (0.94, 1.89)	1.09 (0.78, 1.40)	**0.010**
FBS, *mmol/L*	5.2 (4.6, 6.0)	5.0 (4.7, 5.3)	0.070
LDL, *mmol/L*	2.55 (1.60, 3.64)	2.29 (2.00, 3.00)	0.932
Tryglycerides, *mmol/L*	1.0 (1.0, 1.0)	1.0 (1.0, 1.0)	0.746
HDL, *mmol/L*	1.2 (0.9, 1.3)	1.3 (1.1, 1.5)	0.274
Total cholesterol, *mmol/L*	4.93 (4.35, 5.69)	4.69 (3.54, 5.53)	0.101
ALT, *mmol/L*	14.5 (10.5, 25.6)	20.4 (15.1, 25.2)	0.114
Urea, *mmol/L*	3.44 (2.76, 4.15)	3.22 (2.52)	0.379
Creatinine, *mmol/l*	99(80, 128)	82 (72, 97)	**0.034**
Estimated glomerular filtration rate, *mL/min/1.73m²*	78 (60, 85)	86 (78, 103)	**0.008**
Creatinine clearance, *mL/min*	99 (80, 128)	82 (71, 95)	**0.023**
Spot urine potassium, *mmol/l*	36 (27, 49)	43 (20, 71)	0.872
Spot urine potassium, *mmol/l*	110 (78, 129)	103 (60, 158)	0.788
Spot urine potassium, *mmol/l*	255 (142, 490)	278 (112, 690)	0.838

IQR, interquatile range; WBC, white blood cells; ESR, erythrocyte sedimentation rate; RBC, red blood cells; NTL, neutrophil to lymphocyte ratio; FBS, fasting blood sugar; LDL, low density lipoprotein; HDL, high density lipoprotein; ALT, alanine transferase. Statistically significant results are in bold. Wilcoxon rank-sum test used.

Table 2. Supplimentary data for figure 15 and 16

	Hypertension (n, 22) Median (IQR)	Normotensive (n, 21) Median (IQR)	P-value
HIV positive group			
IL-5, *pg/ml*	4.05 (2.05, 5.87)	3.51 (2.42, 6.90)	0.761
IL-13, *pg/ml*	2.11 (0.99, 4.10)	3.04 (1.07, 5.04)	0.470
IL-2, *pg/ml*	2.33 (1.73, 3.40)	2.84 (2.32, 3.40)	0.324
IL-6, *pg/ml*	3.81 (2.37, 5.54)	1.91 (1.20, 2.37)	<0.001
IL-9, *pg/ml*	1.19 (1.00, 2.02)	1.42 (1.14, 1.99)	0.304
IL-10, *pg/ml*	2.87 (1.53, 5.89)	2.09 (1.66, 2.61)	0.031
Interferon-gamma, *pg/ml*	2.57 (1.62, 3.39)	2.56 (1.78, 4.09)	0.770
Tumor necrosis factor-alpha, *pg/ml*	2.47 (1.45, 4.45)	2.00 (1.35, 3.30)	0.313
IL-17A, *pg/ml*	2.04 (1.27, 3.09)	1.23 (0.91, 1.55)	0.014
IL-17F, *pg/ml*	2.18 (1.20, 3.37)	2.06 (1.35, 3.11)	0.941
IL-4, *pg/ml*	1.34 (0.89, 2.61)	1.71 (1.05, 2.56)	0.464
IL-21, *pg/ml*	0.54 (0.33, 0.93)	0.60 (0.43, 0.74)	0.903
IL-22, *pg/ml*	5.19 (3.00, 7.61)	5.29 (3.82, 6.60)	0.465
HIV negative group			
IL-5, *pg/ml*	2.46 (1.25, 5.10)	2.57 (1.45, 3.71)	0.801
IL-13, *pg/ml*	2.54 (1.66, 4.70)	3.54 (2.44, 5.77)	0.247
IL-2, *pg/ml*	2.76 (2.15, 3.63)	2.80 (2.28, 3.71)	0.801
IL-6, *pg/ml*	2.65 (1.70, 3.71)	1.76 (0.59, 2.59)	0.021
IL-9, *pg/ml*	1.45 (1.34, 1.85)	1.56 (1.32, 2.02)	0.959
IL-10, *pg/ml*	2.60 (1.54, 2.86)	2.30 (1.59, 3.33)	0.860
Interferon-gamma, *pg/ml*	3.53 (2.55, 5.03)	2.44 (1.81, 3.59)	0.018
Tumor necrosis factor-alpha, *pg/ml*	4.39 (2.13, 6.86)	2.14 (1.35, 3.76)	0.007
IL-17A, *pg/ml*	2.13 (1.37, 3.22)	1.32 (0.54, 2.33)	0.071
IL-17F, *pg/ml*	2.42 (1.34, 3.20)	2.10 (1.31, 2.66)	0.450
IL-4, *pg/ml*	1.88 (1.56, 2.52)	1.94 (1.43, 2.53)	0.650
IL-21, *pg/ml*	1.21 (0.68, 1.94)	0.76 (0.62, 0.99)	0.060
IL-22, *pg/ml*	4.30 (1.39, 6.41)	4.30 (2.70, 5.66)	0.979

Table 3. Supplimentary data for figure 22-24

	Hypertension (n, 31) Median (IQR)	Normotensive (n, 39) Median (IQR)	P-value
sCD14, *pg/ml*	1670500 (1499000, 1966500)	1702500 (1459000, 1946500)	0.81
sCD163, n*g/ml*	604 (452, 787)	500 (390, 645)	0.03
MIP-1α, *pg/ml*	183 (124, 241)	157 (95, 183)	0.01
MCP, *pg/ml*	520 (457, 582)	453 (404, 560)	0.04
VCAM-1, *pg/ml*	608145 (469773, 718587)	564623 (443384, 619580)	0.03
ICAM-1, *pg/ml*	570547 (498876, 697882)	489530 (428704, 606579)	0.02
IL-17, *pg/ml*	67 (36, 120)	56 (25, 81)	0.04
IL-6, *pg/ml*	3.65 (2.59, 7.05)	2.71 (2.00, 4.44)	0.02
IL-10, *pg/ml*	0.91 (0.57, 1.17)	0.91 (0.66, 1.45)	0.48
TNF-α, *pg/ml*	9.02 (8.13, 10.80)	8.32 (7.43, 10.18)	0.37
TNF-αR2, *pg/ml*	16477 (15426, 18048)	16344 (15217, 17479)	0.22
TNF-αR1, *pg/ml*	12737 (10977, 15680)	10654 (9141, 12211)	<0.01
Eosinophil %	2.10 (1.20, 3.30)	1.20 (1.00, 2.00)	<0.01
Eosinophil count, $x10^3$µl	0.13 (0.09, 0.21)	0.08 (0.05, 0.13)	<0.01
IL-5, *pg/ml*			<0.01

Appendix D. Analysis of p values of Model Likelihood Ratio chi-square Test and Nagelkerke R^2 index.

Most of the interesting variables have p<0.01 and R^2>0.3, which indicated the goodness of fit for the HIV-positive cohort.

```
Logistic Regression Model
 lrm(formula = formulaForModel, data = mergedData)
                       Model Likelihood     Discrimination    Rank Discrim.
                          Ratio Test           Indexes           Indexes
 Obs           119    LR chi2      41.81    R2       0.395    C       0.816
  0             63    d.f.             5    g        1.784    Dxy     0.632
  1             56    Pr(> chi2) <0.0001    gr       5.956    gamma   0.633
 max |deriv| 3e-10                          gp       0.323    tau-a   0.318
                                            Brier    0.175
                 Coef    S.E.   Wald Z Pr(>|Z|)
 Intercept     -8.2948 1.7554 -4.73  <0.0001
 Eos            0.4176 0.2150  1.94  0.0521
 Sex=Male       0.6987 0.4926  1.42  0.1561
 age            0.0614 0.0236  2.59  0.0095
 BMI            0.1628 0.0373  4.36  <0.0001
Cohort=HIV     -0.9177 0.4918 -1.87  0.0621
```

Appendix E. Analysis of C-index to measure goodness of fit.

All variables of interest have C-index >0.75 indicating goodness of fit. See last column (C) of each Table.

Formula	Variable of interest	Effect (One Unit)	P	OR (One Unit)	OR (Lower 95%)	OR (Upper 95%)	Value (25% Quantile)	Value (75% Quantile)	Value Diff (75%-25%)	Odds Ratio (Diff: 75%-25%)	OR (Diff, Lower 95%)	OR (Diff, Upper 95%)	P	R2	C
lrm (HTN01~ IL5pgml +Sex+age+FMI)	IL5pgml	0.143	0.0588	1.154	0.995	1.338	4.645	9.448	4.803	1.988	0.975	4.055	0	0.356	0.795
lrm (HTN01~ IL6pgml +Sex+age+FMI)	IL6pgml	0.018	0.8741	1.018	0.815	1.272	2.282	5.743	3.46	1.064	0.492	2.301	0.002	0.298	0.779
lrm (HTN01 ~ TNFaR1pgml +Sex+age+FMI)	TNFaR1pgml	0	0.1681	1	1	1	9927.125	13650.625	3723.5	1.616	0.817	3.196	0.001	0.329	0.781
lrm (HTN01 ~ MIP1apgml+Sex+age+FMI)	MIP1apgml	0.008	0.0648	1.008	0.999	1.017	121.75	199.75	78	1.905	0.961	3.775	0	0.378	0.809
lrm (HTN01 ~ MCP1pgml +Sex+age+FMI)	MCP1pgml	0.002	0.3134	1.002	0.998	1.007	421.75	574.5	152.75	1.458	0.7	3.036	0.001	0.314	0.768
lrm (HTN01 ~ ICAM1pgml +Sex+age+FMI)	ICAM1pgml	0	0.1406	1	1	1	445708	646911.125	201203.125	1.814	0.821	4.008	0.001	0.333	0.794
lrm (HTN01~ sCD163ngml +Sex+age+FMI)	sCD163ngml	0.002	0.1719	1.002	0.999	1.004	416.975	729.025	312.05	1.733	0.787	3.815	0.001	0.327	0.774

Formula	Variable of interest	Effect (One Unit)	P	OR (One Unit)	OR (Lower 95%)	OR (Upper 95%)	Value (25% Quantile)	Value (75% Quantile)	Value Diff (75%-25%)	Odds Ratio (Diff: 75%-25%)	OR (Diff, Lower 95%)	OR (Diff, Upper 95%)	P	R2	C
lrm (HTN01 ~ CD4CD38 +Sex+age+FMI)	CD4CD38	-0.134	0.0718	0.875	0.757	1.012	5.3	10.9	5.6	0.473	0.21	1.069	0.001	0.354	0.81
lrm (HTN01 ~ Eos+Sex +age+FMI)	Eos_percent	0.643	0.0299	1.902	1.065	3.398	1.1	2.7	1.6	2.797	1.106	7.078	0	0.373	0.808
lrm (HTN01 ~ IL17pgml +Sex + age+FMI)	IL17pgml	0.007	0.3334	1.007	0.993	1.02	36.25	87	50.75	1.4	0.708	2.766	0.001	0.313	0.773
lrm (HTN01 ~ VCAM1pgml +Sex+age+FMI)	VCAM1pgml	0	0.2444	1	1	1	471585.5	650258.5	178673	1.466	0.77	2.794	0.001	0.32	0.774

Formula	Variable of interest	Effect (One Unit)	P	Odds Ratio (One Unit)	OR (Lower 95%)	OR (Upper 95%)	Value (25% Quantile)	Value (75% Quantile)	Value Diff (75%-25%)	Odds Ratio (Diff: 75%-25%)	OR (Diff, Lower 95%)	OR (Diff, Upper 95%)	P	R2	C
lrm (HTN01 ~ IL5pgml)	IL5pgml	0.167	0.0128	1.182	1.036	1.348	4.645	9.448	4.803	2.23	1.186	4.194	0.007	0.131	0.638
lrm (HTN01 ~ IL6pgml)	IL6pgml	0.152	0.0904	1.164	0.976	1.388	2.282	5.743	3.46	1.692	0.92	3.11	0.074	0.06	0.656
lrm (HTN01 ~ TNFaR1pgml)	TNFaR1pgml	0	0.0051	1	1	1	9927.125	13650.625	3723.5	2.447	1.308	4.577	0.001	0.187	0.715
lrm (HTN01 ~ MIP1apgml)	MIP1apgml	0.01	0.0255	1.01	1.001	1.018	121.75	199.75	78	2.123	1.097	4.111	0.002	0.167	0.658

Formula	Variable of interest	Effect (One Unit)	P	Odds Ratio (One Unit)	OR (Lower 95%)	OR (Upper 95%)	Value (25% Quantile)	Value (75% Quantile)	Value Diff (75%-25%)	Odds Ratio (Diff: 75%-25%)	OR (Diff, Lower 95%)	OR (Diff, Upper 95%)	P	R2	C
lrm (HTN01 ~ MCP1pgml)	MCP1pgml	0.004	0.0935	1.004	0.999	1.008	421.75	574.5	152.75	1.767	0.908	3.436	0.083	0.056	0.64
lrm (HTN01 ~ ICAM1pgml)	ICAM1pgml	0	0.0334	1	1	1	445708	646911.125	201203.125	2.077	1.059	4.073	0.024	0.094	0.674
lrm (HTN01 ~ sCD163ngml)	sCD163ngml	0.002	0.0399	1.002	1	1.004	416.975	729.025	312.05	1.995	1.032	3.856	0.031	0.086	0.629
lrm (HTN01 ~ CD4CD38)	CD4CD38	-0.194	0.006	0.823	0.717	0.946	5.3	10.9	5.6	0.337	0.155	0.732	0.002	0.181	0.726
lrm (HTN01 ~ Eos)	Eos	0.706	0.0074	2.025	1.209	3.394	1.1	2.7	1.6	3.093	1.355	7.064	0.005	0.145	0.689
lrm (HTN01 ~ IL17pgml)	IL17pgml	0.01	0.0556	1.01	1	1.021	36.25	87	50.75	1.695	0.988	2.911	0.041	0.077	0.589
lrm (HTN01 ~ VCAM1pgml)	VCAM1pgml	0	0.0473	1	1	1	471585.5	650258.5	178673	1.735	1.007	2.99	0.028	0.089	0.61

Appendix F. Participant Information Sheet

CROSS SECTIONAL STUDY

Title: Immunopathology of Hypertension in HIV-Infected Patients

Principle Investigator (PI): Masenga, Sepiso K.

The PI is a Biomedical Scientist/Clinical Biochemist currently working at Livingstone Central Hospital in the pathology laboratory. He is a PhD student at the University of Zambia and the study he is conducting is a requirement for completion of his doctorate degree.

Purpose and Background

This is a study to assess the risk factors that can affect the person's heart and general health. It will help in explaining some of the factors related to high blood pressure by examining certain substances in the blood. This study involves both HIV positive and HIV negative individuals.

Description of the study

You will be required to undergo medical screening and examinations. Medical records will also be used to access data where applicable. You will also be required to answer some questions during a 15 minutes interview. In case, there will be questions that you will not feel comfortable to answer, you are free not to. Questions will be asked in the following areas: diet, demographic, physical exercise and clinical health. In-case you are asked to come on a certain day, we will refund your transport. This study has been approved by the University of Zambia Health Sciences Research Ethics Committee (UNZAHSREC).

Procedures

This study will involve the collection of not more than 40mls of blood from your vein that will be sent to the local laboratory for analysis; to assess if various cells of your immune system produce inflammatory substances that maybe harmuful for your blood vessels.

Voluntary participation (Right to Refuse or Withdraw)

Before you decide whether or not you should participate in this study, I would like to explain to you about voluntary participation, the risks and benefits involved, what is expected of you and also what you should expect.

Your participation in the study and giving consent is voluntary and therefore, you are free to withdraw from the study at any time you wish to do so. You have a right to seek any clarification from the researcher whenever you wish to do so. Withdraw from the study will be of no negative consequence for you and you are therefore free to withdraw at any point in time without affecting or jeopardizing your future medical care.

Benefits

Upon request you may be availed and explained to, your results. As a result of your participation, the aggregated results will provide valuable information that will assist how we manage patients with hypertension in future. Thus your participation in this study may help thousands of people as far as in many years to come.
In case you are found with any new medical problems, you will be recommended for standard medical attention and attended to appropriately and routinely as per hospital care.

Risks (unwanted circumstance)

You may have some uncomfortable pain when blood is being drawn from your vein not different from any routine blood collection, however, this pain does not last long.
Should you however feel unbearable pain or discomfort, testing will be stopped and you will be observed. The option to continue with the study will be given after full recovery but it will be of no consequence if you do not wish to continue.
Every effort will be made to reduce the pain that you feel as the blood is collected. It is also important to understand that obtaining a blood sample from some of the participants may be more difficult than from others.

Confidentiality

Please note that all the information gathered will be highly confidential and privacy will be maintained at all times. Except for this disclosure, all information obtained in this study will be considered confidential and used only for research purposes. Your identity and any identify will be kept confidential.
Your name and other personal identifiers will be removed from the questionnaire, and only a study number will be used to connect your name and your answers without identifying you. The test results will be given to you on request and you will be referred to Livingstone Central hospital for further investigation if found to have results needing urgent medical attention otherwise, they will be withheld.

If you agree to participate in this study, you will be asked to sign the consent form provided with this information sheet.

If you wish to seek any clarification, please contact the following: -

Mr Sepiso K. Masenga
Laboratory Department,
Livingstone Central Hospital,
P.O. Box 60091,
Lusaka, Zambia
Cell.no. +260-977-674774

The chairperson
The University of Zambia
Health Sciences Research Ethics
Committee, P.O. Box 50110
Lusaka, Zambia
Email: s.munsaka@unza.zm
Tel.no. +260977925304

INTERVENTIONAL STUDY

Title: Immunopathology of Hypertension in HIV-Infected Patients

Principle Investigator (PI): Masenga, Sepiso K.

The PI is a Biomedical Scientist/Clinical Biochemist currently working at Livingstone Central Hospital in the pathology laboratory. He is a PhD student at the University of Zambia and the study he is conducting is a requirement for completion of his doctorate degree.

Purpose and Background

This is a study to assess the risk factors that can affect the person's heart and general health. It will help in explaining some of the factors related to high blood pressure by examining certain substances in the blood. This study involves both HIV positive and HIV negative individuals.

Description of the study

You will be required to undergo medical screening and examinations. Medical records will also be used to access data where applicable. You will also be required to answer some questions during a 15 minutes interview. In case, there will be questions that you will not feel comfortable to answer, you are free not to. Questions will be asked in the following areas: diet, demographic, physical exercise and clinical health. This study will require you to be seen at least 3 times, once every week and In-case you are asked to come to the hospital on a certain day, we will refund your transport. This study has been approved by the University of Zambia Health Sciences Research Ethics Committee (UNZAHSREC).

Procedures

This study will involve the collection of not more than 20mls of blood from your vein that will be sent to the local laboratory for analysis; to assess if various cells of your immune system produce inflammatory substances that maybe harmful for your blood vessels with increased salt intake. In order to do some Salt sensitivity tests to help us know if you are sensitive to salt or not by monitoring your blood pressure, I will provide you with premeasured dietary salt. In the 1st week (7 days), I will request you to reduce your salt intake and record your daily diet; In the second week you will be given the WHO recommended low salt (4g), and in the third week, high salt (9g/day). During this period, I will monitor your BP 24hrs each day using an ambulatory BP monitoring device.

Voluntary participation (Right to Refuse or Withdraw)

Before you decide whether or not you should participate in this study, I would like to explain to you about voluntary participation, the risks and benefits involved, what is expected of you and also what you should expect**.**

Your participation in the study and giving consent is voluntary and therefore, you are free to withdraw from the study at any time you wish to do so. You have a right to seek any clarification from the researcher whenever you wish to do so. Withdraw from the study will be of no negative consequence for you and you are therefore free to withdraw at any point in time without affecting or jeopardizing your future medical care.

Benefits

Upon request you may be availed and explained to, your results. You will know if you are sensitive to salt and this will directly be beneficial to your blood pressure control knowledge. As a result of your participation, the aggregated results will provide valuable information that will assist how we manage patients with hypertension in future. Thus, your participation in this study may help thousands of people as far as in many years to come.

In case you are found with any new medical problems, you will be recommended for standard medical attention and attended to appropriately and routinely as per hospital care.

Risks (unwanted circumstance)

You may have some uncomfortable pain when blood is being drawn from your vein not different from any routine blood collection, however, this pain does not last long.

Should you however feel unbearable pain or discomfort, testing will be stopped and you will be observed. You blood pressure may rise with increased salt intake; however, you will be monitored to avoid the blood pressure rising to an extent of clinical concern. The option to continue with the study will be given after full recovery should you fall sick from other causes but it will be of no consequence if you do not wish to continue.

Every effort will be made to reduce the pain that you feel as the blood is collected. It is also important to understand that obtaining a blood sample from some of the participants may be more difficult than from others.

Confidentiality

Please note that all the information gathered will be highly confidential and privacy will be maintained at all times. Except for this disclosure, all information obtained in this study will be considered confidential and used only for research purposes. Your identity and any identifies will be kept confidential.

Your name and other personal identifiers will be removed from the questionnaire, and only a study number will be used to connect your name and your answers without identifying you. The test results will be given to you on request and you will be referred to Livingstone Central hospital for further investigation if found to have results needing urgent medical attention otherwise, they will be withheld.

If you agree to participate in this study, you will be asked to sign the consent form provided with this information sheet.

If you wish to seek any clarification, please contact the following: -

Mr Sepiso K. Masenga
Laboratory Department,
Livingstone Central Hospital,
P.O. Box 60091,
Lusaka, Zambia
Cell.no. +260-977-674774

The chairperson
The University of Zambia
Health Sciences Research Ethics
Committee, P.O. Box 50110
Lusaka, Zambia
Email: s.munsaka@unza.zm
Tel.no. +260977925304

Appendix G. Consent forms

CONSENT TO PARTICIPATE IN A RESEARCH STUDY

STUDY TITLE**: Immunopathology of Hypertension in HIV-Infected Patients**

By signing my name below, I ………………………………………….…….. Confirm the following:

- I have read (or had read to me) this entire consent document and all of my questions have been answered adequately.
- The study's purpose, procedures, risks and possible benefits have been explained to me.
- I freely and voluntarily choose to participate.
- I understand that participating or not will not affect my health care or that of my family members.
- I understand that my rights and privacy will be maintained.
- I also agree to be followed up (recalled) for further additional investigations/research in future. Contact no: 09…………………………..Email……………………………………
-

Participant signature ………………………… Date……………………..

Thumb print if participant can't sign………………………….

…………………………. ……………………………….

Witness (Name and Signature) Date

Declaration by member of research team/taking consent:

I have given a verbal explanation of the research project to the participant, and have answered the participant's questions about it to the best of my ability.

I believe that the participant understands the study and has given informed consent to participate**.**

Researcher's Name:	
Signature:	**Date:**

NOTE: *The participant will be provided with a signed and dated copy of this consent form. It will help him remember what we discussed today.*

Contact Details

In case you have any more questions about this study at any time, please feel free to contact any of the numbers below.

Mr Sepiso K. Masenga
Laboratory Department,
Livingstone Central Hospital,
P.O. Box 60091,
Lusaka, Zambia
Cell.no. +260-977-674774
Email Address: sepisomasenga@gmail.com

The Chairperson
University of Zambia Health Sciences Research Ethics Committee
P.O .Box 50110
Lusaka
Contact Number: +260977925304

Appendix H. Data collection tools

INTERVIWER STRUCTURED QUESTIONAIRE

Adapted from (Riley et al., 2016)

Study number: ______________ **Date: _____/_____/____**

Study site (Specify name) ……………………………………………………….

PART 1: SOCIAL-DEMOGRAPHIC CHARACTERISTICS
This section will ask for your social and demographic related information such as your age, income, education and employment status. Tick OR write a number OR words were applicable.

1. Date of Birth (*mwaka wakuzyalwa)*: ___day (*buzuba*)/____month *(mwezi)* /_____year*(mwaka)*

2. Gender of the patient: Male ☐ Female ☐

3. What is your Marital status *(sena mulikwete*?): tick one that is applicable
   - Currently Married *(ndikwete)* ☐ Divorced *(twakalekana)* ☐
   - Single *(tandikwete)* ☐ Widowed *(ndakafwidwa)* ☐
   - Never married *(taakwe ndindakakwete)* ☐ Separated *(twakaandana)* ☐
   - Cohabitating *(ndijisi syamwali)* ☐ Refused *(ndakaka)* ☐

4. Which of the following best describes your main work/life status over the past 12 months? *(ninchito nzi njomucita ku mwezi ikumi atubili yainda)*
   - Government employee *(mubelesi wamfulumende)* ☐
   - Non-government employee *(nseli wamfulumende)* ☐
   - Self-employed *(ndalibelekela ndilike)* ☐
   - Non-paid *(tandivwoli)* ☐
   - Student *(ndimwana wacikolo)* ☐
   - Retired *(ndakaleka ncito)* ☐
   - Unemployed (able to work) *(tandibeleki pe pesi ndacikonzya)* ☐
   - Unemployed (unable to work) (tandikonzi kubeleka pe) ☐
   - Refused *(ndakaka)* ☐

5. What is the highest level of education you have completed *(Ino lwiiyo lwanu lusikila ali)?*
   - No formal schooling *(tindakayiya pe)* ☐
   - Less than primary school *(tindakamanizya plaimali)* ☐

- Primary school completed *(ku plaimali)* ☐
- Secondary school completed *(ndakamana sekondali)* ☐
- College/University completed *(ndaka manizya ku college/university)* ☐
- Post graduate degree *(ndakainda ku lwiiyo lwa university)* ☐
- Refused *(ndakaka)* ☐

PART 2: LIFESTYLE/DIETARY RISK FACTOR ASSESSMENT PROFILE

This section will ask for Dietary related information such as salt intake, vegetable intake, fruit intake, alcohol consumption and smoking status. Tick OR write a number OR words were applicable. *(Eci cibeela tuyanda kuzyiba twaambo tujatikizya zyakulya mbuli munyo, cisyu ca matu, micelo, bukoko alimwi akufweba. Amuchonge na mupe twaambo)*

1. what foods does your typical meal include *(mulya cakulya nzi lyoonse)* ___________________________
2. In a typical week, on how many days do you eat fruit? *(mu mvwiki yomwe, ma zuba ongaye nomulya miceelo)* ______days on average per week *(mazuba mvwiki anwiki)*
3. How many fruits do you eat on one of those days? *(mulya micelo yongaye, mumazuba ayo)* ______fruits on average
4. In a typical week, on how many days do you eat vegetables? *(Mu mwiki yomwe, mazuba ongaye nomulya cisyu ca mateu (ma vegi)* ______days on average per week

With the next questions, we would like to learn more about salt in your diet. Dietary salt includes ordinary table salt, unrefined salt such as sea salt, iodized salt, salty stock cubes and powders, and salty sauces such as soya sauce or fish sauce. The following questions are on adding salt to the food right before you eat it, on how food is prepared in your home, on eating processed foods that are high in salt such as [Crisps, chipsy, soya mince], and questions on controlling your salt intake. Please answer the questions even if you consider yourself to eat a diet low in salt
(mibizyo itobela, tuyanda kuzyiba munyo ngomubelesya. Munyo mbuli wa busu, wa mabwe alimwi a sautu wakabilwa iodine kubilikilizya a sautu ubikwa mu soya alimwi answi. Mibuzyo eeyi iguminizya mbomubelesya mu cakulya canu ambomujika cakulya kubelesya sautu mung'anda yanu, kutaluba ambomulya zyakulya zijisi sautu munji mbuli ma crypsy, ma chipusi alimwi a nyama soya)

5. How often do you add salt or a salty sauce/spice to your food right before you eat it or as you are eating it? *(ziindi zyongaye zyomubika sautu alimwi a sipaisi mucakulya canu nomulya)*
   - Always *(ciindi coonse)* ☐ Often *(muzimwi zindi)* ☐
   - Sometimes *(fumbwa biyo)* ☐ Rarely *(nkacekace)* ☐
   - Never *(tandibiki)* ☐ Don't know *(tandizyi)* ☐

-

6. How often is salt, salty seasoning or a salty sauce added in cooking or preparing foods in your household? *(ziindi zyongaye, zyomubelesya na kubika sautu azyipangwa ku sautu nomujika cakulya mung'anda yanu.)*
   - Always *(ciindi coonse)* ☐ Often *(muzimwi zindi)* ☐
   - Sometimes *(fumbwa biyo)* ☐ Rarely *(nkacekace)* ☐
   - Never *(tandibiki)* ☐ Don't know *(tandizyi)* ☐

7. How often do you eat processed food high in salt? By processed food high in salt, I mean foods that have been altered from their natural state, such as packaged salty snacks, canned salty food including pickles and preserves, salty food prepared at a fast food restaurant, cheese, bacon, processed meat, Crisps, chipsy, soya mince etc .

   (muzyiinda zilibuti zyomulya zyakulya zijikidwe zyijisi sautu munji. Awa twaamba zyakulya zipindudwe mbuli ma sinaki, zyamumagabba alimwi azyipangwa mu ma resteaurant mbuli chiizi, ba tunyama twa ngulube, nyama yakugaya, ma cripusi, ma chipusi a nyama soya)

   - Far too much *(tulabelesya kwindililа maningi)* ☐ Too much *(maningi)* ☐
   - Just the right amount *(tubelesya bweelede)* ☐ Too little *(asyoonto)* ☐
   - Far too little *(asyoonto maningi)* ☐ Don't know *(tandizyi)* ☐

Alcohol consumption

8. Have you ever consumed any alcohol such as beer, wine, spirits, tujilili, Kachasu? *(sena mwakanywa makoko, waini, masipirit, tujilijili alimwi akachasu)*
1. Yes *(inzya)* ☐ No *(taakwe)* ☐ I don't know *(tandizyi)* ☐ refused (ndakaka) ☐

9. Have you consumed any alcohol within the past 12 months? *(sena mwakanywa kale bukoko mu myezi ili ikumi atubili yainda).*
2. Yes *(inzya)* ☐ No *(taakwe)* ☐ I don't know *(tandizyi)* ☐ Refused *(ndakaka)* ☐

10. Have you consumed any alcohol within the past 30 days? *(sena mwakanywa bukoko kumazuba alimwi makumi otatwe yainda)*
3. Yes *(inzya)* ☐ No *(takwe)* ☐ I don't know *(tandizyi)* ☐ Refused *(ndakaka)* ☐

11. Have you consumed any alcohol within the past 7 days? *(sena mwakanywa bukoko kumazuba ali ciloba yainda)*
4. Yes *(inzya)* ☐ No *(takwe)* ☐ I don't know *(tandizyi)* ☐ Refused *(ndakaka)* ☐

12. During the past 7 days, on how many occasions did you have at least one standard alcoholic drink? *(kumazuba aliciloba yainda, ziindi zyongaye nimwakanywa bukoko bweelede nokuba botolo na cigabba chomwe)*

5. Number *(ziindi):*______ refused (ndakaka)☐ I don't know (tandizyi) ☐

Smoking status (kufweba)

13. Are you a Current smoker? *(sena mulafweba)*

6. Yes *(inzya)*☐ No *(takwe)*☐ I don't know *(tandizyi)*☐ Refused *(ndakaka)* ☐

14. If yes, on average per day, how many cigarettes do you smoke? *(naa mulafweba, misanga yongaye njomufweba abuzuba)*___________

15. If NO, Have you smoked tobacco, cigarette or any other material before? *(kuti na tamufwebi, sena mwakafweba musanga na tombwe ili yoonse)*

7. Yes *(inzya)* ☐ No *(takwe)*☐ I don't know *(tandizyi)* ☐

16. If yes, on average per day, how many cigarettes did you smoke? *(namwazumina, misanga yongaye njimwakafweba abuzuba)*___________

17. Estimate the Period since you stopped smoking *(kwayinda ciindi cilibuti nimwakaleka kufweba*): _____days *(mazuba)*_____ ;months *(mwezi);*______*;* years *(myaka)*

Note: Go to the IPAQ before continuing with part 3

PART 3: CLINICAL ASSESSMENT PROFILE (MAKANI A NSEBA YANU)

(specify or tick or write N/A were applicable)

With the next questions or examinations, we would like to assess your health. Your attending doctor or health personnel will ask some health related questions, make some measurements and examine you.
(kumibizyo itobela, tuyanda kuzyiba nseba yanu. Ba nseba balamubuzya mibuzyo ijatilizya buumi bwanu akumi pima)

Note: *Some parts do not require translation as information will be gotten from patient files, However, some have still been translated.*

1. HIV Status:

- HIV Sero-positive☐ HIV Sero-Negative☐ HIV positive (self-reported) ☐

2. ART regimen (drugs patient taking): tick applicable
   - 3TC lamivudine ☐ ABC abacavir ☐
   - AZT azidovudine (also known as zidovudine, or ZDV) ☐
   - ddI didanosine ☐ EFV efavirenz ☐
   - FTC emtricitabine ☐ LPV lopinavir ☐
   - NVP nevirapine ☐ TDF tenofovir disoproxil fumarate ☐
   - XTC ☐ d4T (stavudine ☐

3. Date patient started ART: ______/______/______

4. Adherence issues *(makani akunywa musamu):*
   - How many ARV drugs do you take per day? *(misamu yongaye yakazunda njomunywa abuzuba)*
     ______drugs per day
   - In the past three days excluding today, have you taken all the ARV drugs? *(kumazuba otatwe ayinda, sena mwakanya misamu yoonse)*
     YES *(inzya)*☐ NO *(pepe)*☐ I don't know *(tandizyi)*☐
   - If No, how many ARV drugs have you not taken in total? (kuti na mwakaka, misamu yongaye antomwe njimwataka nywa)
     ______drugs not taken

5. Blood pressure

- Systolic pressure (mm hg): 1._____R_____L;2._____R_____L;3_____R_____L;4_____R_____L;5_____R_____L
- Diastolic pressure(mm hg): 1._____R_____L;2._____R_____L;3_____R_____L;4_____R_____L;5_____R_____L
- Pulse rate (beats per minute):

  1._____R_____L;2._____R_____L;3_____R_____L;4_____R_____L;5_____R_____L

History of Raised Blood Pressure

- Have you ever had your blood pressure measured by a doctor or other health worker? *(sena mwaka pimwa kale bulowa mbobuenda mumubili na BP aba silisi)*
  YES *(inzya)*☐ NO *(taakwe)*☐ I don't know (*tandizyi)* ☐

- Have you ever been told by a doctor or other health worker that you have raised blood pressure or hypertension? *(Sena ba nseba baka mwaambila kale kuti mulijisi bulwazi bwa kumoyo na BP)*
  YES *(inzya)*☐ NO *(taakwe)*☐ I don't know (*tandizyi)* ☐

IF YES, Have you been told in the past 12 months? *(na mwazumina, sena sena bakamwambila ku myezi ili ikumi atubili yainda)*
YES *(inzya)* ☐ NO *(taakwe)* ☐ I don't know (*tandizyi)* ☐

- In the past two weeks, have you taken any drugs (medication) for raised blood pressure prescribed by a doctor or other health worker? *Ku mwiki zyobile zyainda, sena mwakanywa musamu wa moyo na BP wakalebwa aba nseba).*
  YES *(inzya)* ☐ NO *(taakwe)* ☐ I don't know (*tandizyi)* ☐

- Have you taken any herbal or traditional medicine for raised blood pressure or hypertension? (sena kulimusamu wacintu cisiya ngomwakanwa ku bulwazi bwa moyo na BP)
  YES *(inzya)* ☐ NO *(taakwe)* ☐ I don't know (*tandizyi)* ☐

- Are you currently taking any herbal or traditional remedy for your raised blood pressure*? (sena kuli musamu wa cintu cisiya ngomunywa kulwazi bwa moyo na BP)*
  YES *(inzya)* ☐ NO *(taakwe)* ☐ I don't know (*tandizyi)* ☐

6. Anti-hypertensive: TICK APPLICABLE
   - thiazide diuretics ☐ calcium channel blockers ☐
   - ACE inhibitors ☐ angiotensin II receptor antagonists (ARBs) ☐
   - beta blockers ☐ OTHER: ___________________________

7. How long have you had hypertension? *(Mwakala myaka yongaye abulwazi bwa moyo na BP*) ____________months
8. History of Diabetes
   - Have you ever had your blood sugar measured by a doctor or other health worker? *(sena mwakapimwa kale bulwazi bwa sugar)*
     YES *(inzya)* ☐ NO *(taakwe)* ☐ I don't know (*tandizyi)* ☐
   - Have you ever been told by a doctor or other health worker that you have diabetes? (sena bakamwambila ba nseba kuti mulijisi bulwazi bwa sugar)
     YES *(inzya)* ☐ NO *(taakwe)* ☐ I don't know (*tandizyi)* ☐
   - Is there anyone with diabetes in a first- or second-degree relative (your family line) *(sena kuli uciswa bulwazi bwa sugar mumukwasyi wenu)*
     YES *(inzya)* ☐ NO *(taakwe)* ☐ I don't know (*tandizyi)* ☐
9. Other drugs you are taking *(misamu imbi njemunywa):* ____________________

10. BMI/ Obesity/Waist-to-hip ratio
    - Height in meters : _________
    - Weight in Kgs : _________
    - Waist circumference in centimetres: _________
    - Hip circumference in centimetres: _________

PART 4: LABORATORY PROFILE

1. CD4 count (cells/µl): _________ Viral load (copies/ml): _________
2. FBC:
   __Lymp________Neu________PLT________Mon________________________
3. ESR_______________________
4. Glucose (mmol/l): _________
5. Lipid & Lipoprotein profile
   - LDL-c (mmol/l): _________ Triglyceride: _________
   - Cholesterol (mmol/l): _________
6. Electrolytes (mmol/l): K+________ Na+________ Cl- ________ Na+/K+_____
7. Kidney function (mmol/l): Creatinine: ________ Urea: ________
8. Liver function (mmol/l):
   - AST: ________ ALT: ________
9. **9.** Urinalysis: are the following abnormally present in the urine sample?
   - Blood ☐ Glucose ☐ Albumin ☐
   - Ketones ☐ protein ☐ Nitrites ☐
   - Bilirubin ☐ Urobilirubin ☐
   - What is the PH: ________ Specific gravity: ________
   - Urine sodium___________potassium____________ Na+/K+_____
10. Urine Microscopy: ____
11. Cytokines (attach results):
    ____

END OF DATA COLLECTION

International Physical Activity Questionnaire (IPAQ)

Adopted from IPAQ long form and used with slight modifications (adapted or localized)

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.
(Tuyanda kuzyiba misobano icitwa mukuzunganya mibili mubumi bwanu. Tuyanda kuzyiba musobano njomwatide kwa mvwiki (mazuba asikila ciloba) yainda. Amuvwiile mubuzyo nokubakuti tamusobani maningi.)

Think about all the **vigorous** and **moderate** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal.

(amuyeye misobano yankasaalo ayimwi misyoonto njommwali kucita kwa mvwiki yainda na mazuba kusikila a ciloba. Misobano yankasaalo njiyeeyo itamupi kuyoya mbuli lyoonse. Misobano misyonto njiyeeyo iizungaanya biyo mibili akuyooya buyo ashonto pesi kuinda ambomuyoya lyoonse.)

PART 1: JOB-RELATED PHYSICAL ACTIVITY (INCITO IZUNGANYA MIBILI)

The first section is about your work. This includes paid jobs, farming, volunteer work, course work, and any other unpaid work that you did outside your home. Do not include unpaid work you might do around your home, like housework, yard work, general maintenance, and caring for your family. These are asked in Part 3.
(cibeela cakusanguna ncha nchito yanu. Eyi ibikilizya nchito ivolwa mali, kulima, kulyaaba, nchito yakubala, anchito biyo njomucita itali yaa ng'anda. Nutabikilizyi nchito yaang'anda ita volwi mali, mbuli kupyaanga mung'anda akusa kazya lubuwa akugwasya mukwasyi.)

1. Do you currently have a job or do any unpaid work outside your home? *(sena kuli nchito njomucita itali yakung'anda kwanu)*

☐ Yes

☐ No → ***Skip to PART 2: TRANSPORTATION***

The next questions are about all the physical activity you did in the **last 7 days** as part of your paid or unpaid work. This does not include traveling to and from work.
(mibuzyo itobela ijatikizya nchito yakumubili njomwa kacita mvwiki yainda ivolwa na itavolwi. Mutabikilizyi lweendo lwakuya a kujoka kunchito)

2. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, heavy construction, or climbing up stairs **as part of your work**? Think about only those physical activities that you did for at least 10 minutes at a time.
(Mu mvwiki yainda, mazuba ongaye ngumwakacita nchito igusya nkasaalo mbuli kunyamuna zilema kusya, kuyaka na kutanta mujulu anchito. Amuuyeye nchito njomwakacita kwiinda ituzuzumina tuinda ikkumi)

_____ **days per week *(mazuba a mvwiki)***

☐ No vigorous job-related physical activity *(kwiina nchito yankasaalo njondakacita)* → ***Skip to question 4***

3. How much time did you usually spend on one of those days doing **vigorous** physical activities as part of your work? *(ciindi cilamfu buti ncomwakabelesya kunchito igusya nkasaalo kuciindi eeco.)*

_____ **hours per day *(ma oola abuzuba)***

_____ **minutes per day *(ituzuzumina abuzuba)***

4. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads **as part of your work**? Please do not include walking. *(alimwi amuyeeye nchito njomwakacita kwa ituzuzumina tusikila ikumi akuindilizya. Kwa mvwiki(mazuba a ciloba yainda) yainda, mazuba ongaye ngomwakacita nchito yeelede itapinkasalo mbuli kunyamuna kabbudu katalemi anchito. Mutabikilizyi kweenda.)*

_____ **days per week *(mazuba munvwiki)***

☐ No moderate job-related physical activity → ***Skip to question 6***
Kwiina nchito isyonto njondakacita

5. How much time did you usually spend on one of those days doing **moderate** physical activities as part of your work? *(chiindi cilamfu buti ncomwakabelesya kucita nchito insyoonto anchito)*

_____ **hours per day *(ma oola abuzuba)***
_____ **minutes per day *(itizuzumina abuzuba)***

6. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **as part of your work**? Please do not count any walking you did to travel to or from work. *(kwa mvwiki yainda na mazuba asiki aciloba, mazuba ongaye ngo mwakeenda kuya kunchito kwiinda ituzuzumina tulikumi akwindilila)*

_____ **days per week *(mazuba mu mvwiki)***

☐ No job-related walking → ***Skip to PART 2: TRANSPORTATION***
(kwiina ninda kayenda pe)

7. How much time did you usually spend on one of those days **walking** as part of your work? *(nchiindi cilamfu buti ncomwakeenda anchito yanu njomuchita)*

_____ **hours per day *(ma oola abuzuba)***
_____ **minutes per day *(ituzuzumina abuzuba)***

PART 2: TRANSPORTATION PHYSICAL ACTIVITY

These questions are about how you traveled from place to place, including to places like work, stores, movies, and so on.
(Eeyi mibuzyo itobela, ijatikizya kweenda kwanu muma sena asiyene mbuli anchito, kumantoolo, ku mabonwababonwa azimwi biyo.)

8. During the **last 7 days**, on how many days did you **travel in a motor vehicle** like a train, bus, car? *(kwa mvwiki yainda na mazuba a ciloba, mazuba ongaye ngomwakeenda mu mootokala na citima na mubbasi)*

_____ **days per week *(mazuba mumvwiki)***

☐ No traveling in a motor vehicle *(Taakwe nindakabeelesya mootoka)* ➞ ***Skip to question 10***

9. How much time did you usually spend on one of those days **traveling** in a train, bus, car, or other kind of motor vehicle? *(cindi cilamfubuti nchomwakabelesya kweenda mu citima, bassi a cimbayambaya cilicoonse)*

_____ **hours per day *(ma oola abuzuba)***
_____ **minutes per day *(ituzuzumina abuzuba)***

Now think only about the **bicycling** and **walking** you might have done to travel to and from work, to do errands, or to go from place to place. *(lino amuyeeye kubelesya nchiinga na kweenda ansi nkomwakabelesya kuunka alimwi akujoka ku nchito, na kuya kumasena asiyene.)*

10. During the **last 7 days**, on how many days did you **bicycle** for at least 10 minutes at a time to go **from place to place**? *(kwa mvwiki yainda na mazuba asikila a ciloba, mazuba ongaye ngomwaka chova nchinga kwiinda ituzuzumina tulikkumi kuya ku masena asiyene suyene)*

_____ **days per week *(mazuba a mvwiki)***

☐ No bicycling from place to place *(Kwiina nindakabelesya nchinga)* ➞ ***Skip to question 12***

11. How much time did you usually spend on one of those days to **bicycle** from place to place? *(nciindi cilamfu buti nchomwaka belesya nchiinga kuya kumasena asiyenisiyene)*

_____ **hours per day *(ma oola abuzuba)***
_____ **minutes per day *(ituzuzumina abuzuba)***

12. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time to go **from place to place**? (kwa mvwiki yainda *na mazuba asikila a ciloba*, mazuba ongaye ngomwakeenda kwa ituzuzumina tuyinda ikkumi kuya mumasena ayindene)

_____ **days per week *(mazuba amvwiki)***

☐ No walking from place to place *(kwiina nindakayenda pe)*

→ ***Skip to PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY***

13. How much time did you usually spend on one of those days **walking** from place to place? *(nchiindi cilamfubuti ncomwakabelesya kweenda kumasena asiyene)*

_____ **hours per day *(ma oola abuzuba)***
_____ **minutes per day *(ituzuzumina abuzuba)***

PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY

This section is about some of the physical activities you might have done in the **last 7 days** in and around your home, like housework, gardening, yard work, general maintenance work, and caring for your family. *(eci cibeela cijatikizya nchito njomywaka cita kwa mvwiki yainda ang'anda yanu mbuli kubotya ng'anda, kulima cisyu, kubotyang'anda alimwi akubamba mukwasyi)*

14. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, chopping wood, or digging **in the garden or yard**? *(kamuyeeya nchito njomwakacita ituzuzumina tuinda ku kkumi. Kwa mvwiki yainda na asikila ciloba, mazuba ongaye ngomwakacita nchito njumu, mbuli kunyamuna zilema, kugonka nkuni na kusya mu gadeni)*

_____ **days per week**

☐ No vigorous activity in garden or yard (kwiina nchito njondakacita) → ***Skip to question 16***

15. How much time did you usually spend on one of those days doing **vigorous** physical activities in the garden or yard? (nchiindi cilamfu buti nchomwakabelesya amazzuba na buzuba obo anchito yankasaalo ang'anda yenu na mu gadeni)

_____ **hours per day *(ma oola abuzuba)***
_____ **minutes per day *(ituzuzumina abuzuba)***

16. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, sweeping, washing windows, and raking **in the garden or yard**? *(alimwi amuyeeye nchito njommwakacita ituzuzumina tuyinda ku kkumi. Mu mvwiki yainda na mazuba asikila ciloba, mazuba ongaye ngomwaka beleka nchito yeelede mbuli kupyanga, kubotya ma window akusowa matu)*

_____ **days per week**

☐

No moderate activity in garden or yard → ***Skip to question 18***
(kwiina nchito yeelede njondakacita mugadeni na ang'anda)

17. How much time did you usually spend on one of those days doing **moderate** physical activities in the garden or yard? *(ciindi cilamfu buti njomwakabelesya kuma zuba ayo kunchito yeelede ang'anda yanu.)*

_____ hours per day *(ma oola abuzuba)*
_____ minutes per day *(ituzuzumina abuzuba)*

18. Once again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, washing windows, scrubbing floors and sweeping **inside your home**? *(alimwi amuyeeye nchito njomwakacita kwiinda ituzuzumina tuli kkumi akwiinda. Mumvwiki yainda, mazuba ongaye ongomwakabelesya ku nchito yeelede mbuli kunyamuna zitalemi, kubotya ma windo, kubotya ansi ang'anda alimwi kupyanga ang'anda)*

_____ days per week

☐ No moderate activity inside home *(kunyina nchito yeelede njondaka cita)*
→ ***Skip to PART 4: RECREATION, SPORT AND LEISURE-TIME PHYSICAL ACTIVITY***

19. How much time did you usually spend on one of those days doing **moderate** physical activities inside your home? *(ciindi cilamfu buti nchomwakabelesya kumazuba ayo mu mvwiki kubeleka nchito yeelede ang'anda)*

_____ hours per day *(ma oola abuzuba)*
_____ minutes per day *(ituzuzumina abuzuba)*

PART 4: RECREATION, SPORT, AND LEISURE-TIME PHYSICAL ACTIVITY

This section is about all the physical activities that you did in the **last 7 days** solely for recreation, sport, exercise or leisure. Please do not include any activities you have already mentioned. *(eci cibeela cijatikizya nchito yakumubili njo mwakacita kwa mvwiki yinda mbuli kulikkomanisya, misobano, kusotoka. Mutayinduluki zimwa lemba na zimwaamba kale)*

20. Not counting any walking you have already mentioned, during the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **in your leisure time**? *(kutaamba kweenda nkomwamba kale, mu mwiki yainda na mazuba asikila ciloba, mazuba ongaye ngomwaka yenda kwiinda ituzuzumina tuli kkumi mu ciindi nchomupumuna)*

_____ **days per week**

☐ No walking in leisure time → ***Skip to question 22***
(kwiina nindakayenda pe)

21. How much time did you usually spend on one of those days **walking** in your leisure time? *(nchiindi cilamfu buti nchomwakabelesya mu mazuba ayo kweenda aciindi cakupumuna kwanu)*

_____ **hours per day *(ma oola abuzuba)***
_____ **minutes per day *(ituzuzumina abuzuba)***

22. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like aerobics, running, fast bicycling, or fast swimming **in your leisure time**? *(amuyeeye nchito njomwakacita aciindi iinda ituzuzumina tuli kkumi. Mu mvwiki yainda, mazuba ongaye ngomwaka cita nchito yankasaalo mbuli kusootoka, kubalika, kuchovwa nchiinga alimwi akuyamba mumeenda muciindi canu cakulikkomanisya)*

_____ **days per week (mazuba mu mvwiki)**

☐ No vigorous activity in leisure time → ***Skip to question 24***
(kwiina nchito yankasaalo njindakacita)

23. How much time did you usually spend on one of those days doing **vigorous** physical activities in your leisure time? (nchiindi cilamfu buti ncomwakabelesya munchito yankasaalo muciindi cakupumuna kwenu)

_____ **hours per day *(ma oola abuzuba)***
_____ **minutes per day *(ituzuzumina abuzuba)***

24. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like bicycling at a regular pace, swimming at a regular pace, and doubles tennis **in your leisure time**? *(alimwi amuyeeye biyo nchito njomwakacita iinda ituzuzumina tulikkumi. Mu mvwiki yainda mazuba ongaye ngomwacita nchito itaminyi mbuli kuchova nchinga asyoonto, kuyamba asyonto alimwi akusobanya kabbola kasyonto aciindi cakulookezya kwanu)*

_____ **days per week (mazuba mu mvwiki)**

☐ No moderate activity in leisure time → ***Skip to PART 5: TIME SPENT SITTING***
(kwiina nchito isyoonto njondakabeleka)

25. How much time did you usually spend on one of those days doing **moderate** physical activities in your leisure time? *(nchindi cilamfu buti nchomwakabelesya muma zuba ayo kubeleka nchito iitakatalwi mu ciindi caku lyookezya kwanu)*

_____ hours per day *(ma oola abuzuba)*
_____ minutes per day *(ituzuzumina abuzuba)*

PART 5: TIME SPENT SITTING

The last questions are about the time you spend sitting while at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading or sitting or lying down to watch television. Do not include any time spent sitting in a motor vehicle that you have already told me about. (*mibuzyo yakumanizya ciindi nchomupumuna anchito, ang'anda, ciindi nomucita nchito yakubala alimwi aciindi cakulikkomanisya. Eci cibikkilizya ciindi ncomusowa akukkala chuuno, kuswaya bayandwa, kubala, kukkala, kubuwa chipekupeku, Mutaambi ciindi nchomusowa mu mootokalape)*

26. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekday**? *(mu mvwiki yainda na mazuba asikila ciloba, ciindi cilamfu buti nchomwakabelesya kukkala biyo abuzuba mu mvwiki yomwe)*

_____ hours per day *(ma oola abuzuba)*
_____ minutes per day *(ituzuzumina abuzuba)*

27. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekend day**? *(mu mvwiki yainda na mazuba asikila ciloba, nchiindi cilamfu buti ncomwakabelesya kukkala biyo ku mamanino amvwiki)*

_____ hours per day *(ma oola abuzuba)*
_____ minutes per day *(ituzuzumina abuzuba)*

28. OVERALL QUESTION: Generally, how long do you think these physical activity trends (questions asked) for the past 7 days have been going on? (mukweezyamisya biyo, ciindi cilamfu buti nchomuyeeya kuti nchito yakumubili ibeelekwa mu mvwiki yainda yali kucitika, hena mwalikucita ciindi na mazuba na myezi na mu myaka zyongaye)

_____ Days *(mazuba)*
_____ Months *(myezi)*
_____ Years *(myaka)*

This is the end of the questionnaire, thank you for participating.

TYPE 2 DIABETES RISK ASSESSMENT FORM

Circle the right alternative and add up your points.

1. Age

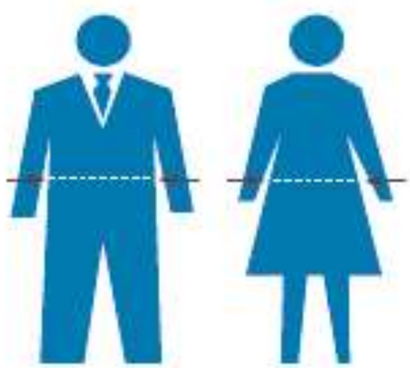
0 p.	Under 45 years
2 p.	45–54 years
3 p.	55–64 years
4 p.	Over 64 years

2. Body-mass index
(See reverse of form)

0 p.	Lower than 25 kg/m^2
1 p.	25–30 kg/m^2
3 p.	Higher than 30 kg/m^2

3. Waist circumference measured below the ribs (usually at the level of the navel)

	MEN	WOMEN
0 p.	Less than 94 cm	Less than 80 cm
3 p.	94–102 cm	80–88 cm
4 p.	More than 102 cm	More than 88 cm



4. Do you usually have daily at least 30 minutes of physical activity at work and/or during leisure time (including normal daily activity)?

0 p.	Yes
2 p.	No

5. How often do you eat vegetables, fruit or berries?

0 p.	Every day
1 p.	Not every day

6. Have you ever taken medication for high blood pressure on regular basis?

0 p.	No
2 p.	Yes

7. Have you ever been found to have high blood glucose (eg in a health examination, during an illness, during pregnancy)?

0 p.	No
5 p.	Yes

8. Have any of the members of your immediate family or other relatives been diagnosed with diabetes (type 1 or type 2)?

0 p.	No
3 p.	Yes: grandparent, aunt, uncle or first cousin (but no own parent, brother, sister or child)
5 p.	Yes: parent, brother, sister or own child

Total Risk Score

☐ **The risk of developing type 2 diabetes within 10 years is**

Lower than 7	**Low:** estimated 1 in 100 will develop disease
7–11	**Slightly elevated:** estimated 1 in 25 will develop disease
12–14	**Moderate:** estimated 1 in 6 will develop disease
15–20	**High:** estimated 1 in 3 will develop disease
Higher than 20	**Very high:** estimated 1 in 2 will develop disease

"Guidelines for Data Processing and Analysis of the International Physical Activity Questionnaire (IPAQ)" used

Guidelines for Data Processing and Analysis of the International Physical Activity Questionnaire (IPAQ) - Short Form, Version 2.0. April 2004

Introduction

This document provides an revision to the outline for scoring the short form of the International Physical Activity Questionnaire (IPAQ) . This is available on the website www.ipaq.ki.se.

There are many different ways to analyse physical activity data, but to-date there is no consensus on a "correct" method for defining or describing levels of activity based on self-report surveys. The use of different scoring protocols makes it very difficult to compare within and between countries, even when the same instrument has been used.

IPAQ is an instrument designed primarily for population surveillance of adults. It has been developed and tested for use in adults (age range of 15-69 years) and until further development and testing is undertaken the use of IPAQ with older and younger age groups is not recommended. IPAQ is being used also as an evaluation tool in some intervention studies, but the range of domains and types of activities included in IPAQ should be carefully noted before using it in this context.

This document describes the *April 2004 revision* to the IPAQ short scoring protocol1. These revisions are have been suggested by the IPAQ scientific group, to examine variation among countries in more detail2. Given the broad range of domains of physical activity asked in IPAQ, new cut-points need to be trialed and developed to express physical activity in the population. These cut-points are preliminary, in the sense that they are not yet supported by epidemiological studies, which have typically used Leisure time physical activity (LTPA) to examine benefits or risks of being active. Hence, 30 minutes of moderate intensity PA on most days of the week was evidence-based, using the estimates of risk (reduction) from these LTPA measures in numerous epidemiological studies.

A new set of suggested cutpoints is based on work in the area of total physical activity, specifically total walking, where recommendations of at least 10,000 steps, and possibly 12,500 steps per day are considered "high active" (Tudor Locke reference). This equates to at least 2 hours of all forms of walking per day, which includes all settings and domains of activity, and could be a population goal for total HEPA (health-enhancing physical activity). With this background, new cutpoints are proposed for expressing physical activity levels in populations using generic physical activity measures such as IPAQ3.

1 The first version of an IPAQ scoring protocol was in August 2003; this is a revised version, April 2004. This revised version does not
change the continuous forms of reporting data, but does suggest a new category for describing the most active groups in populations.
The changes from the August 2003 scoring protocol are indicated in this document.
2 Previous scoring algorithms returned high prevalence rates with limited variation among countries; hence a higher cutpoint is sought,
as the IPAQ instrument measures total PA, including LTPA as well as incidental, occupational and transport related PA all in one
question. This results in much higher prevalence estimates than measures of LTPA alone.
3 This results in changes to the categories used for levels of activity, and to the truncation rules [as greater than two hours per day may
be required as usable data for walking and other physical activity behaviors].

Characteristics of the IPAQ short-form instrument:

1) IPAQ assesses physical activity undertaken across a comprehensive set of domains including leisure time, domestic and gardening (yard) activities, work-related and transport-related activity;

2) The IPAQ short form asks about three specific types of activity undertaken in the three domains introduced above and sitting. The specific types of activity that are assessed are walking, moderate-intensity activities and vigorous intensity activities; frequency (measured
in days per week) and duration (time per day) are collected separately for each specific type
of activity.

3) The items were structured to provide separate scores on walking; moderate-intensity; and
vigorous-intensity activity as well as a combined total score to describe overall level of activity. Computation of the total score requires summation of the duration (in minutes) and
frequency (days) of walking, moderate-intensity and vigorous-intensity activity;

4) Another measure of volume of activity can be computed by weighting each type of activity by its energy requirements defined in METS (METs are multiples of the resting metabolic rate) to yield a score in MET minutes. A MET-minute is computed by multiplying the MET score by the minutes performed. MET-minute scores are equivalent to kilocalories for a 60 kilogram person. Kilocalories may be computed from MET-minutes using the following
equation: MET-min x (weight in kilograms/60 kilograms). The selected MET values were
derived from work undertaken during the IPAQ Reliability Study undertaken in 2000-2001.

Using the Ainsworth et al. Compendium (*Med Sci Sports Med* 2000) an average MET score
was derived for each type of activity. For example; all types of walking were included and an
average MET value for walking was created. The same procedure was undertaken for moderate-intensity activities and vigorous-intensity activities. These following values continue to be used for the analysis of IPAQ data: Walking = 3.3 METs, Moderate PA = 4.0
METs and Vigorous PA = 8.0 METs 4.

Analysis of IPAQ

Both categorical and continuous indicators of physical activity are possible from the IPAQ short form. However, given the non-normal distribution of energy expenditure in many populations, the Continuous indicator is presented as median minutes or median MET minutes rather than mean minutes or mean MET-minutes.

Categorical score

Regular participation is a key concept included in current public health guidelines for physical activity.5 Therefore, both the total volume and the number of day/sessions are included in the IPAQ analysis algorithms. There are three levels of physical activity suggested for classifying

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4 Note that there is still some debate about whether 8 Mets for vigorous is sustainable, in occupational settings for
several hours; we have no data on this, but it is likely to be less than that, maybe 7 METs or even less; however, for the
moment, we suggest keeping with the compendium value of * METs.

5 Pate RR, Pratt M, Blair SN, Haskell WL , Macera CA, Bouchard C et al. Physical activity and public health. A
recommendation from the Centers for Disease Control and Prevention and the American College of Sports Medicine.
Journal of Amercian Medical Association 1995; 273(5):402-7. and U.S.Department of Health and Human Services.
Physical Activity and Health: A Report of the Surgeon General. Department of Health and Human Services, Centers for
Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, The Presidents'
Council on Physical Fitness and Sports: Atlanta, GA:USA. 1996.

populations; these are the new proposed levels, which take account of the concept of total physical activity of all domains. The proposed levels are:

[i] Inactive

[ii] Minimally active.6

[iii] HEPA active. (health enhancing physical activity; a high active category).

The criteria for these three levels are shown below.

1. Inactive (CATEGORY 1)
This is the lowest level of physical activity. Those individuals who not meet criteria for Categories 2 or 3 are considered .**insufficiently active’** [CATEGORY 1].

2. Minimally Active (CATEGORY 2)
The minimum pattern of activity to be classified as .sufficiently active. is any one of the following 3 criteria:
a) 3 or more days of vigorous activity of at least 20 minutes per day **OR**
b) 5 or more days of moderate-intensity activity or walking of at least 30 minutes per day **OR**
c) 5 or more days of any combination of walking, moderate-intensity or vigorous intensity activities achieving a minimum of at least 600 MET-min/week.
Individuals meeting at least one of the above criteria would be defined as achieving the minimum recommended to be considered .**minimally active’** [CATEGORY 2]. This category is more than the minimum level of activity recommended for adults in current public health recommendations, but is not enough for “total PA” when all domains are considered. IPAQ measures total physical activity whereas the recommendations are based on activity (usually leisure-time or recreational) over and above usual daily activities.

3. HEPA active (CATEGORY 3)
A separate category labeled .**HEPA’** level, which is a more active category [CATEGORY 3] can be computed for people who exceed the minimum public health physical activity recommendations, and are accumulating enough activity for a healthy lifestyle. This is a useful indicator because it is known that higher levels of participation can provide greater health benefits, although there is no consensus on the exact amount of activity for maximal benefit. Also, in considering lifestyle physical activity, this is a total volume of being active which reflects a healthy lifestyle. It is at least 1.5 to 2 hours of “being active” throughout the day, which is more than the LTPA-based recommendations of 30 minutes7.
In the absence of any established criteria, the IPAQ scientific group proposes this new cut-point, which equates to approximately at least 1.5 -2 hours of total activity per day, of at least moderate intensity activity. It is desirable to have a “HEPA” activity category, because in some populations, a large proportion of the population may be classified as .minimally active. because the IPAQ instrument assess all domains of activity. Category 3 sets a higher threshold of activity and provides a useful mechanism to distinguish variation in sub-population groups.

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6 “Minimally active” implies some physical activity but is not an optimal level of total HEPA.
7 As Tudor-Locke and others have indicated, there is a basal level of around 1 hour of activity just in activity of daily
living, and an additional 0.5 – 1 hour of LTPA makes a healthy lifestyle amount of total PA – hence, these new cutpoints
are still consistent with the general LTPA based public health recommendations of at least half an hour per day of

additional activity or exercise.

The two criteria for classification as HEPA active. are:

a) vigorous-intensity activity on at least 3 days achieving a minimum of at least 1500 MET-minutes/week **OR**

b) 7 or more days of any combination of walking, moderate-intensity or vigorous intensity activities achieving a minimum of at least 3000 MET-minutes/week8

Continuous score

Data collected with IPAQ can be reported as a continuous measure and reported as median METminutes. Median values can be computed for walking (W), moderate-intensity activities (M), and vigorous-intensity activities (V) using the following formulas:

MET values and Formula for computation of Met-minutes

Walking MET-minutes/week = 3.3 * walking minutes * walking .days.

Moderate MET-minutes/week = 4.0 * moderate-intensity activity minutes * moderate days

Vigorous MET-minutes/week = 8.0 * vigorous-intensity activity minutes * vigorous-intensity days

A combined total physical activity MET-min/week can be computed as the sum of Walking +

Moderate + Vigorous MET-min/week scores.

The MET values used in the above formula were derived from the IPAQ validity and reliability study undertaken in 2000-2001 9. A brief summary of the method is provided above (see first page)

As there are no established thresholds for presenting MET-minutes, the IPAQ Research Committee proposes that these data are reported as comparisons of median values and interquartile ranges for different populations.

IPAQ Sitting Question

The IPAQ sitting question is an additional indicator variable and is not included as part of any summary score of physical activity. Data on sitting should be reported as median values and interquartile range. To-date there are few data on sedentary (sitting) behaviors and no wellaccepted thresholds for data presented as categorical levels.

Data Processing Rules

In addition to a standardized approach to computing categorical and continuous measures of

physical activity, it is necessary to undertake standard methods for the cleaning and treatment of IPAQ datasets. The use of different approaches and rules would introduce variability and reduce the comparability of data.

There are no established rules for data cleaning and processing on physical activity. Thus, to allow more accurate comparisons across studies IPAQ has established and recommends the following guidelines:

1. Data cleaning

• time should be converted from hours and minutes into minutes

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8 Note: this replaces the previous IPAQ short form cutpoint of 1500 mets.mins/ week

9 Craig *CL,Marshall A , Sjostrom M* et al. International Physical Activity Questionnaire: 12 country reliability and validity
Med Sci Sports Exerc 2003;August.

• ensure that responses in 'minutes' were not entered in the 'hours' column by mistake during
self-completion or during data entry process, values of "15", "30", "45", "60" and "90" in the
'hours' column should be converted to "15", "30", "45", "60" and "90" minutes, respectively, in the minutes column.

• Time should be converted to daily time [usually is reported as daily time, but a few cases
will be reported as optional weekly time . eg. VWHRS, VWMINS - convert to daily time]

•convert time to mets-mins [see above; days x daily time]

• must have the number of days for the day variables; for the 'time' variables, either daily or
weekly time is needed if 'don't know' or 'refused ' or data are missing in walking, moderate
or vigorous days or minutes, then that case is removed from analysis

2. Maximum Values for excluding outliers: This rule is to exclude data which are unreasonably high; these data are to be considered outliers and thus are excluded from analysis. All Walking, Moderate and Vigorous time variables which total at least or greater than .16 hours. should be excluded from the analysis.

The 'days' variables can take the range 0-7 days, or 8,9 (don.t know or refused); values greater than 9 should not be allowed and those data excluded from analysis.

3. Truncation of data rules: This rule is concerned with data truncation and attempts to normalize the distribution of levels of activity which are usually skewed in national or large population data sets. It is recommended that all Walking, Moderate and Vigorous time variables exceeding '4 hours' or '240 minutes' are truncated (that is re-coded) to be equal to '240 minutes' in a new variable10. This rule permits a maximum of 28 hours of activity in a week to be reported for each category of physical activity.

This rule requires further testing, but is an initial manner proposed for classifying these population data.

When analysing IPAQ data and presenting the results in categorical variables, this rule has the important effect of preventing misclassification in the 'high active' category. For example, an individual who reports walking for 2.5 hours every day and nothing else would be classified as 'HEPA active' (reaching the threshold of 7 days, and ≥ 3000 MET.mins. Similarly, someone who reported walking for 90 minutes on 5 days, and 4 hours (240 mins) of moderate activity on another day and 70 minutes of vigorous activity on another day, would also be coded as 'HEPA active' because this pattern meets the '7 day' and '3000 MET-min' criteria for 'HEPA active'.

4. Minimum Values for Duration of Activity: Only values of 10 or more minutes of activity will be included in the calculation of summary scores. The rationale being that the scientific evidence indicates that episodes or bouts of at least 10 minutes are required to achieve health benefits. Responses of less than 10 minutes [and their associated days] should be re-coded to 'zero'.

Summary of Data Processing Rules 1- 4 above

Data management rules 2, 3, and 4 deal with first excluding outlier data, then secondly, recoding high values to '4 hours', and finally describing minimum amounts of activity to be included in analyses. These rules will ensure that highly active people remain highly active,

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10 Note that this is a different truncation rule to the earlier scoring protocol; we have previously used 2 hours as a
truncation point for LTPA measures. This higher truncation point is proposed in order to allow people who walk for 2.5
hours per day and do nothing else to be categorized as 'HEPA' active; if data were truncated, these individuals would be
recoded to 2 hours per day, and over 7 days, total 2772 MET.mins, due to the truncation rule. The new truncation rule
allows 2.5 hours to be counted in full. The initial purpose of truncation was to normalize the distributions, and was based
on expert judgments. It is now suggested that 4 hours / day be proposed as a truncation threshold for more inclusive
'lifestyle PA measures' such as IPAQ.

while decreasing the chances that less active individuals are coded as highly active.

5. Calculating Total Days for 'minimally Active' [category 2] and 'HEPA Active' [category 3]

Presenting IPAQ data using categorical variables requires the total number of .days. on which allbphysical activity was undertaken to be assessed. This is difficult because frequency in 'days' is asked separately for walking, moderate-intensity and vigorous-intensity activity, thus allowing the total number of .days. to range from a minimum of 0 to a maximum of 21'days' per week. The IPAQ instrument does not record if different types of activity are undertaken on the same day. In calculating **'minimal activity'**, the primary requirement is to identify those individuals who undertake a combination of walking and/or moderate-intensity activity on at least '5 days'/week. Individuals who meet this criterion should be coded in a new variable called '*at least five days"*.

Below are two examples showing this coding in practice:
i) an individual who reports '2 days of moderate' and '3 days of walking' should be coded as a value indicating '*at least five days"*;
ii) an individual reporting .2 days of vigorous., '2 days walking' and '2 days moderate' should be coded as a value to indicate .*at least five days"* [even though the actual total is 6].
The original frequency of .days. for each type of activity should remain in the data file for use in the other calculations.

The same approach as described above is used to calculate total days for computing the '**HEPA active'** category. The primary requirement according to the stated criteria is to identify those individuals who undertake a combination of walking, moderate-intensity and or vigorous activity on at least 7 days/week. Individuals who meet this criterion should be coded in a value in a new variable to reflect *"at least 7 days*".

Below are two examples showing this coding in practice:
i) an individual who reports '4 days of moderate' and '3 days of walking' should be coded as
the new variable *"at least 7 days*'.
ii) an individual reporting .3 days of vigorous., .3 days walking. and .3 days moderate. should
be coded as *"at least 7 days*. [even though the total adds to 9] .

Summary: The algorithm(s) in Appendix 1 and Appendix 2 to this document show how these rules work in an analysis plan, to develop the categories 1 [inactive], 2 [minimally], and 3 [HEPA] levels of activity. A short form [.at a glance.] and a diagram showing these analytic steps for 'sufficient physical activity' and 'high active' categories are shown as appendix 1 at the end of this document.
IPAQ Research Committee
April 2004

IPAQ Scoring Protocol (Short Versions) Categorical Score- three levels of physical activity are proposed

1. *Inactive*
• No activity is reported **OR**
• Some activity is reported but not enough to meet Categories 2 or 3.

2. Minimally Active
Any one of the following 3 criteria
• 3 or more days of vigorous activity of at least 20 minutes per day **OR**
• 5 or more days of moderate-intensity activity or walking of at least 30 minutes per day

OR
• 5 or more days of any combination of walking, moderate-intensity or vigorous intensity activities achieving a minimum of at least 600 MET-min/week.
3. HEPA active
Any one of the following 2 criteria
• Vigorous-intensity activity on at least 3 days and accumulating at least 1500 METminutes/
week **OR**
• 7 or more days of any combination of walking, moderate-intensity or vigorous intensity activities achieving a minimum of at least 3000 MET-minutes/week

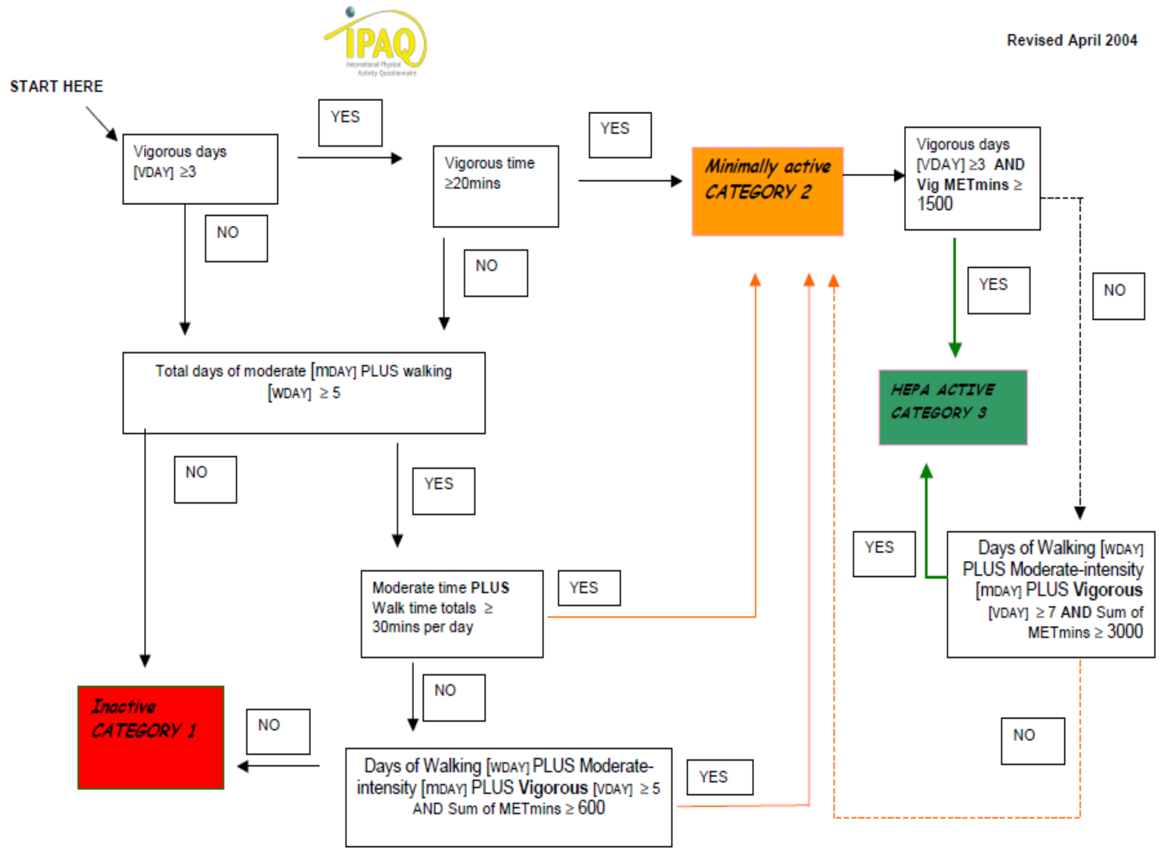
Continuous Score
Expressed as MET-min per week: MET level x minutes of activity x events per week

Sample Calculation

MET levels	**MET-min/week for 30 min episodes, 5 times/week**
Walking = 3.3 METs	3.3*30*5 = 495 MET-min/week
Moderate Intensity = 4.0 METs	4.0*30*5 = 600 MET-min/week
Vigorous Intensity = 8.0 METs	8.0*30*5 = 1,200 MET-min/week
	TOTAL = 2,295 MET-min/week

Total MET-min/week = (Walk METs*min*days) + (Mod METs*min*days) + Vig METs*min*days)

Please review the document "Guidelines for the data processing and analysis of the International Physical Activity Questionnaire (Short Form)" for more detailed description of IPAQ analysis and recommendations for data cleaning and processing [www.ipaq.ki.se].



Flow chart algorithm for the analysis of IPAQ short form

24-hour food intake recall form

Study ID#: Gender: Date:

Breakfast			Time: Place:	
Name	Food description	Estimated Amount	Number and way of Salt tablets consumed	Preparation/ Ingredients
Lunch			Time: Place:	
Dinner			Time: Place:	
Other in-between meals (indicate times for each meal)			Time: Place:	

Appendix I. Definition and explaination of key terms and procedures

Body mass index (BMI) of an individual is calculated as weight in kilograms divided by the square of height in meters.

BP Measurement Definitions adapted from (Whelton et al., 2017)

BP Measurement	**Definition**
SBP	First Korotkoff sound*
DBP	Fifth Korotkoff sound*
Pulse pressure	SBP minus DBP
Mean arterial pressure	DBP plus one third pulse pressure (Calculation assumes normal heart rate.)
Mid-BP	Sum of SBP and DBP, divided by 2

BP indicates blood pressure; DBP, diastolic blood pressure; and SBP, systolic blood pressure.
Korotkoff sound : These are "tapping" sounds associated with the turbulent flow that occur when the cuff pressure is greater than the diastolic pressure and less than the systolic pressure

AHA Categories of BP in Adults

BP Category	**SBP**		**DBP**
Normal	<120 mm Hg	and	<80 mm Hg
Elevated	120–129 mm Hg	and	<80 mm Hg
Hypertension			
Stage 1	130–139 mm Hg	or	80–89 mm Hg
Stage 2	≥140 mm Hg	or	≥90 mm Hg

Note: Individuals with SBP and DBP in 2 categories should be designated to the higher BP category. BP indicates blood pressure (based on an average of ≥2 careful readings obtained on ≥2 occasions); DBP, diastolic blood pressure; and SBP systolic blood pressure.

JNC 7 Categories of BP in Adults

BP Category	**SBP**		**DBP**
Normal	<120 mm Hg	and	<80 mm Hg
Prehypertension	120–139 mm Hg	and	80-89 mm Hg
Hypertension			
Stage 1	140–159 mm Hg	or	90–99 mm Hg
Stage 2	≥160 mm Hg	or	≥ 100 mm Hg

JNC 7, The seventh report of the joint National committee on the prevention, detection, evaluation and treatment of high blood pressure; BP, blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure

Checklist and Guidelines for Accurate Measurement of BP (office measurement)

Key Steps for Proper BP Measurements	**Specific Instructions**
Step 1: Properly prepare the patient	1. Have the patient relax, sitting in a chair (feet on floor, back supported) for >5 min. 2. The patient should avoid caffeine, exercise, and smoking for at least 30 min before measurement. 3. Ensure patient has emptied his/her bladder. 4. Neither the patient nor the observer should talk during the rest period or during the measurement. 5. Remove all clothing covering the location of cuff placement. 6. Measurements made while the patient is sitting or lying on an examining table do not fulfill these criteria.
Step 2: Use proper technique for BP measurements	1. Use a BP measurement device that has been validated, and ensure that the device is calibrated periodically. 2. Support the patient's arm (e.g., resting on a desk). 3. Position the middle of the cuff on the patient's upper arm at the level of the right atrium (the midpoint of the sternum). 4. Use the correct cuff size, such that the bladder encircles 80% of the arm, and note if a larger- or smaller-than-normal cuff size is used. 5. Either the stethoscope diaphragm or bell may be used for auscultatory readings.
Step 3: Take the proper measurements needed for diagnosis and treatment of elevated BP/hypertension	1. At the first visit, record BP in both arms. Use the arm that gives the higher reading for subsequent readings. 2. Separate repeated measurements by 1–2 min. 3. For auscultatory determinations, use a palpated estimate of radial pulse obliteration pressure to estimate SBP. Inflate the cuff 20–30 mm Hg above this level for an auscultatory determination of the BP level. 4. For auscultatory readings, deflate the cuff pressure 2 mm Hg per second, and listen for Korotkoff sounds.
Step 4: Properly document accurate BP readings	1. Record SBP and DBP. If using the auscultatory technique, record SBP and DBP as onset of the first Korotkoff sound and disappearance of all Korotkoff sounds, respectively, using the nearest even number. 2. Note the time of most recent BP medication taken before measurements.
Step 5: Average the readings	Use an average of ≥2 readings obtained on ≥2 occasions to estimate the individual's level of BP.
Step 6: Provide BP readings to patient	Provide patients the SBP/DBP readings both verbally and in writing.

BP indicates blood pressure; DBP, diastolic blood pressure; and SBP, systolic blood pressure

Selection Criteria for BP Cuff Size for Measurement of BP in Adults

Arm Circumference	Usual Cuff Size
22–26 cm	Small adult
27–34 cm	Adult
35–44 cm	Large adult
45–52 cm	Adult thigh

BP Patterns Based on Office and Out-of-Office Measurements

	Office/Clinic/Healthcare Setting	Home/Nonhealthcare/ABPM Setting
Normotensive	No hypertension	No hypertension
Sustained hypertension	Hypertension	Hypertension
Masked hypertension	No hypertension	Hypertension
White coat hypertension	Hypertension	No hypertension

ABPM indicates ambulatory blood pressure monitoring; and BP, blood pressure.

Resistant hypertension - when a patient takes 3 antihypertensive medications with complementary mechanisms of action (a diuretic should be 1 component) but does not achieve control or when BP control is achieved but requires ≥4 medications**. Criteria:** Office SBP/DBP ≥130/80 mm Hg and Patient prescribed ≥3 antihypertensive medications at optimal doses, including a diuretic, if possible or Office SBP/DBP <130/80 mm Hg but patient requires ≥4 antihypertensive medications

Metabolic Equivalent of Task (MET) or metabolic Equivalent - is a physiological measure expressing the energy cost of physical activities and is defined as the ratio of metabolic rate (and therefore the rate of energy consumption) during a specific physical activity to a reference metabolic rate, usually represented by resting metabolic rate. In this case, the variable **MET-min/week** expresses weekly metabolic engagement in walking, and in both moderate and vigorous physical activities practice.

Mechanism - Process by which something takes place or is brought about. Factors that may be linked in contributing to an outcome.

STEPwise approach to surveillance (STEPS) is a simple, standardized method for collecting, analysing and disseminating data in WHO member countries.

STROBE (STrengthening the Reporting of OBservational studies in Epidemiology) Statement is a reporting guideline including a checklist of 22 items that are considered essential for good reporting of observational studies.

Appendix J. Published papers

Elevated Eosinophils as a Feature of Inflammation Associated With Hypertension in Virally Suppressed People Living With HIV

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***Background*—**People living with HIV (PLWH) are at increased risk of cardiovascular disease, including hypertension, which persists despite effective plasma viral suppression on antiretroviral therapy. HIV infection is characterized by long-term alterations in immune function, but the contribution of immune factors to hypertension in PLWH is not fully understood. Prior studies have found that both innate and adaptive immune cell activation contributes to hypertension.

***Methods and Results*—**We hypothesized that chronic inflammation may contribute to hypertension in PLWH. To test this hypothesis, we enrolled a cohort of 70 PLWH (44% hypertensive) on a long-term single antiretroviral therapy regimen for broad phenotyping of inflammation biomarkers. We found that hypertensive PLWH had higher levels of inflammatory cytokines, including tumor necrosis factor-α receptor 1, interleukin-6, interleukin-17, interleukin-5, intercellular adhesion molecule 1 and macrophage inflammatory protein-1α. After adjustment for age, sex, and fat mass index, the circulating eosinophils remained significantly associated with hypertension. On the basis of these results, we assessed the relationship of eosinophils and hypertension in 2 cohorts of 50 and 81 039 similar HIV-negative people; although eosinophil count was associated with prevalent hypertension, this relationship was abrogated by body mass index.

***Conclusions*—**These findings may represent a unique linkage between immune status and cardiovascular physiological characteristics in HIV infection, which should be evaluated further. **(*J Am Heart Assoc*. 2020;9:e011450. DOI: 10.1161/JAHA.118.011450.)**

Key Words: eosinophilia • HIV • hypertension • inflammation • interleukin-5

With the introduction of effective antiretroviral therapy (ART), people living with HIV (PLWH) can now survive decades, but this success is tempered by an increasing burden of cardiovascular disease in this population.[1–4] One contributor to the excess cardiovascular mortality in PLWH may be the increased prevalence of hypertension compared with the general population, which persists despite suppression of plasma viremia with ART.[5] Recent evidence suggests that HIV may contribute to hypertension through direct viral effects on the lining of vessels, and indirectly through eliciting an inflammatory cascade that contributes to the genesis and propagation of hypertension and atherosclerosis.[6]

Over the past 50 years, research in several laboratories, including our own, has demonstrated that both the innate and adaptive immune systems contribute to hypertension. T cells infiltrate the kidneys and perivascular space in response to hypertensive stimuli and release inflammatory cytokines that promote renal and vascular dysfunction, leading to elevated blood pressure and end organ damage.[7] However, the mechanisms of inflammation contributing to hypertension

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Accompanying Figure S1 through S3 and Tables S1 through S12 are available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.118.011450

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Clinical Perspective

What Is New?

- Hypertensive people living with HIV (PLWH) on antiretroviral therapy with long-term viral suppression exhibit higher levels of circulating eosinophils and the eosinophil maturation marker interleukin-5 compared with normotensive PLWH.
- There may be a novel pathway linking eosinophils and hypertension in PLWH, and further studies are warranted to validate and explore this finding.

What Are the Clinical Implications?

- PLWH are at increased risk of cardiovascular disease compared with HIV-negative people, and the management of hypertension is essential to reducing disease burden in this population.
- Targeting inflammation, especially eosinophils and their maturation, may provide therapeutic benefit in treating and/or preventing hypertension in PLWH.

are not well explored in PLWH. In this study, we used a well-characterized cohort of PLWH on long-term ART to investigate the relationship between immune cell subtypes, soluble biomarkers of inflammation, and prevalent hypertension. We also compared our findings in hypertension in HIV-negative participants with or without hypertension.

Methods

Availability of Data and Material

The data sets used and/or analyzed during the current study will be made available on request from the corresponding author.

Human Study Population

We used the Adiposity and Immune Activation Cohort of 70 PLWH recruited at the Vanderbilt Comprehensive Care Clinic from 2013 to 2014, who have been previously described.[8–12] All subjects were on efavirenz, tenofovir, and emtricitabine (ie, the combination pill Atripla) for at least the 6 months before enrollment, and had been on ART with persistent HIV-1 RNA measurements <50 copies/mL for at least the previous 2 years. All participants were nondiabetic, with no history of acute coronary events, stroke, known rheumatologic or inflammatory conditions (aside from HIV), or concomitant comorbidities that might modify eosinophil counts (allergic rhinitis, asthma, dermatoses, and parasitic disease). Use of antihypertensive medication at the time of the study visit and/or >2 sequential outpatient systolic blood pressure measurements >140 mm Hg preceding the visit were used to classify hypertension status. A cohort of 50 HIV-negative people, also from Vanderbilt University Medical Center, served as controls. Data on this cohort were obtained by review of medical records of volunteers who provided consent for participation in research conducted at the Division of Clinical Pharmacology. Data in normotensive and hypertensive HIV-negative subjects with available differential counts of white blood cells were collected, eliminating participants with comorbidities known to modify eosinophil counts, such as allergic rhinitis, asthma, dermatoses, and parasitic disease. Each subject could have ≥1 eosinophil count over time (mean, 3.9±0.4; median, 3). If counts were >1, values were averaged. As an additional HIV-negative control cohort, individuals of interest were sought in the Synthetic Derivative, a Vanderbilt University Medical Center database of ~2.5×10^6 deidentified electronic medical records. The search strategy consisted of identifying subjects without HIV/AIDS and dividing them by the presence versus absence of essential hypertension diagnostic codes. Only subjects containing ≥1 data point on eosinophil counts and body mass indexes (BMIs) were used. Exclusion criteria were all diseases known to produce eosinophilia, including but not limited to respiratory and cutaneous allergic disorders, hematologic malignancies, some infections, collagen vascular disorders, and ages <30 years and >60 years. For subjects with >1 measurement of eosinophils or BMI, all values were averaged. All participants provided written informed consent, and the study was approved by the Vanderbilt Institutional Review Board.

Inflammatory Biomarker Assessment

Venous blood was drawn in the morning between 8 and 11 AM and after a minimum 8-hour fast. Samples were collected in an EDTA-containing vacutainer and centrifuged for 10 minutes at 4°C, and the plasma was removed and immediately frozen at −80°C. Plasma levels of soluble cluster of differentiation (sCD) 14 and sCD163, 2 surface markers released into circulation by activated macrophages, were measured using ELISA (R&D Systems, Minneapolis, MN). Other plasma cytokines and immune biomarkers, including interleukins, MCP1 (monocyte chemoattractant protein-1), MIP-1α/β (macrophage inflammatory protein-1 α and β), tumor necrosis factor-α (TNF-α), and soluble TNF-α receptors 1 and 2 (sTNFR1 and sTNFR2, respectively), as well as vascular cell adhesion molecule-1 and intercellular adhesion molecule-1 (ICAM-1), were measured in duplicate using a standard multiple immunoassay panel (MesoScale, Rockville, MD).

Flow Cytometry

Peripheral blood mononuclear cells were obtained from fasting whole blood samples, as previously described.[10] Flow cytometry was performed using the Fortessa (Becton Dickson Biosciences) flow cytometer to measure activated, senescent, exhausted, and memory T-cell subsets using previously described fluorochrome panels.[8]

Eosinophil Counts

Eosinophil counts and percentages were obtained in normotensive and hypertensive participants with and without HIV from automated differential cell counts performed in the Vanderbilt University Medical Center Clinical Laboratory.

Assessment of Body Composition

A full-body Dual-energy X-ray absorptiometry (GE Lunar Prodigy; GE Healthcare, Little Chalfont, UK) measured total body fat mass to calculate fat mass index (FMI; total fat in kilograms divided by height in meters, squared). FMI is a variant of BMI that accounts for individual variability in the ratio of fat/lean mass.[13] The assessment of FMI is shown in Table S1.

Statistical Analyses

We assessed the normality of data using kurtosis and skewness values as well as graphing using Q-Q plots. Medians and interquartile ranges were calculated for continuous variables, and percentages were calculated for categorical variables. Demographics, clinical characteristics, and the outcome variables were compared between hypertensive and normotensive people using Mann-Whitney *U* test or χ^2 test, as appropriate. We conducted statistical analysis in R software (http://www.r-project.com). Logistic regression was performed to analyze the association between hypertension and inflammation markers, with adjustment of age, sex, and FMI/BMI for the HIV-positive participants and age, sex, and BMI for the HIV-negative participants. Log2 transformation was performed on variables with highly skewed data distribution. Multiple imputation was performed to impute missing data with Hmisc package in R (cran.r-project.org/package=Hmisc). No adjustments were made for multiple comparisons for this exploratory study, although results and the potential for false discovery were interpreted in the context of known biological pathways.[14] P=0.05 was used to infer statistical significance.

Results

Clinical characteristics of the HIV-positive study cohort are shown in Table 1. In unadjusted comparisons, we observed no significant differences among $CD4^+$ and $CD8^+$ naïve and memory T-cell subtypes between hypertensive and normotensive individuals. We also found no significant differences among $CD4^+$ and $CD8^+$ T cells expressing CD57 and Programmed cell death protein 1. However, we found that hypertension was associated with lower $CD4^+$ T cells expressing the CD38 activation marker, but not $CD8^+$ $CD38^+$ T cells, and there were no significant differences in dual-expressing $CD38^+$ and Human Leukocyte Antigen – DR $isotype^+$ cells (Figure S1). The gating strategy to identify T-cell subpopulations is shown in Figure S2.

Increased Macrophage Activation in PLWH Is Associated With Prevalent Hypertension

We have previously shown that cells of the innate immune system, including monocyte-derived dendritic cells, are activated during experimental and human hypertension.[15,16] We observed no significant difference in sCD14 in normotensive compared with hypertensive participants with HIV (Figure 1A). However, we found that hypertension was associated with a marked increase in macrophage activation markers and chemokines, including sCD163 (Figure 1B) and MIP-1α (Figure 1C). In addition, we found that hypertension was associated with a significant increase in MCP1 (Figure 1D) and both vascular cell adhesion molecule-1 (Figure 1E) and ICAM-1 (Figure 1F). These results suggest that increased macrophage activation and expression of vascular adhesion molecules are a feature of inflammation and hypertension among PLWH on ART.

Cytokines Associated With Hypertension in PLWH

Previous studies have found that inflammatory cytokines, including interleukin-17, contribute to the development of hypertension.[17–19] We sought to determine if hypertension in PLWH is associated with increased cytokine production. We found that hypertensive PLWH have significantly higher levels of interleukin-17 (Figure 2A). We also found that hypertension was associated with increased circulating interleukin-6 (Figure 2B). In addition, we observed lower levels of the anti-inflammatory cytokine interleukin-10 in hypertensive participants, but this did not reach statistical significance (Figure 2C). There was no significant difference in plasma TNF-α or sTNFR2, but we found a significant elevation of sTNFR1 (Figure 2D through 2F). Thus, hypertension in PLWH is associated with higher circulating levels of interleukin-17, interleukin-6, and sTNFR1.

Elevated Eosinophils Were Associated With Increased Hypertension in Virally Suppressed PLWH

Prior studies have indicated that eosinophils play a role in several immune-mediated diseases.[20] As shown in Figure 3A,

Table 1. HIV-Positive Participant Characteristics

Variables	Total Participants (n=70)	Normotensive (n=39)	Hypertensive (n=31)	*P* Value
Age, median (IQR), y	42 (35–47)	43 (35–47)	49 (43–52)	0.002*
Women, n (%)	30 (43)	16 (41)	14 (45)	0.810
Duration on treatment, median (IQR), y	6.2 (4.3–10.1)	6.1 (4.3–11.1)	6.4 (4.3–8.2)	0.804
BMI, median (IQR), kg/m^2	32.3 (26.3–37.1)	26.5 (22.8–32.8)	33.9 (28.2–40.0)	0.001*
FMI $\times 10^3$, median (IQR)	12.8 (8.8–16.3)	8.8 (5.7–13.2)	13.3 (9.9–17.5)	0.003*
$CD4^+$ count, median (IQR), cells/μL	701 (540–953)	700 (523–924)	690 (581–969)	0.554
Nadir $CD4^+$ count, median (IQR), cells/μL	257 (140–378)	276 (183–393)	240 (100–371)	0.301
$CD8^+$ count, median (IQR), cells/μL	752 (600–1004)	774 (630–949)	675 (585–1062)	0.582
Smokers, n (%)	25 (36)	14 (36)	11 (35)	1.000
Cigarettes per day (N=69), median (IQR)	0.0 (0.0–4.0)	0.0 (0.0–4.0)	0.0 (0.0–4.0)	0.787
Nonwhite, n (%)	38 (54)	23 (59)	15 (48)	1.000
Hepatitis C infection, n (%)	8 (11)	2 (5)	6 (19)	0.127
Fasting total cholesterol, median (IQR), mg/dL	176 (154–202)	176 (160–203)	171 (142–200)	0.460
Fasting LDL, median (IQR), mg/dL	105 (88–123)	111 (89–123)	101 (85–124)	0.435
Fasting HDL, median (IQR), mg/dL	45 (36–52)	47 (35–55)	43 (36–50)	0.619
Fasting triglycerides, median (IQR), mg/dL	101 (73–139)	98 (80–147)	102 (73–138)	0.953
Obese, n (%)	35 (50)	14 (35.9)	21 (67.7)	0.008*
Average waist circumference, median (IQR), cm	104 (88–122)	96 (82–110)	114 (101–128)	0.001*
Average mid-upper arm circumference, median (IQR), cm	33 (30–36)	31 (28–35)	35 (31–38)	0.006*
Visceral adipose tissue, median (IQR), cm	1554 (630–2219)	906 (381–2061)	1950 (1316–3014)	0.002*
Taking NSAIDS, n (%)	10 (14.3)	5 (12.8)	5 (16.1)	0.694
Taking daily aspirin, n (%)	6 (8.6)	0 (0.0)	6 (19.4)	0.006
Highly sensitive CRP, median (IQR), mg/L	2.4 (1.1–6.2)	2.1 (0.8–3.4)	3.2 (1.5–6.6)	0.05
Amyloid A, median (IQR), pg/mL $\times 10^6$	1.8 (0.9–5.0)	1.4 (0.7–2.2)	4.2 (1.8–9.7)	<0.001*
Leptin, median (IQR), ng/mL	17.3 (8.5–32.1)	11.5 (5.9–30.2)	23.6 (12.4–37.2)	0.02*
Adiponectin, median (IQR), pg/mL $\times 10^6$	9.9 (5.9–14.6)	10.6 (6.2–18.5)	9.5 (5.4–13.8)	0.200

For quantitative variables age, duration on treatment, BMI, FMI, and CD4 and CD8 counts, we used the Wilcoxon rank sum test; and we used the χ^2 test for the remaining binary categorical variables. BMI indicates body mass index; CD, cluster of differentiation; CRP, C-reactive protein; FMI, fat mass index; HDL, high-density lipoprotein; IQR, interquartile range; LDL, low-density lipoprotein.
*$P<0.05$.

we found that among PLWH, participants with hypertension had a significantly higher percentage of eosinophils when compared with the normotensive participants. The absolute numbers of eosinophils were also similarly elevated in hypertensive PLWH when compared with normotensive participants (Figure 3B). The positive correlation between eosinophils and hypertension in virally suppressed PLWH remained significant after adjusting for age, sex, and FMI in a multivariate analysis (Table 2). We repeated a similar analysis using BMI instead of FMI, and eosinophils remained significantly associated with hypertension in PLWH (Table S2). Notably, we found that the eosinophil maturation and differentiation factor interleukin-5 was also associated with hypertension in virally suppressed HIV^+ people in a univariate analysis (Figure 3C). This association was robust but did not reach statistical significance in the adjusted model (Table 2). The finding of both elevated circulating eosinophils and increased plasma levels of a key maturation factor strongly suggests that expansion of the eosinophil population may be a feature of hypertension in HIV. Given the small sample size, we performed goodness-of-fit analysis using the *P* values and the Nagelkerke R^2 as well as the C-index analysis. Both these tests indicated goodness of fit (Tables S3 and S4).

To determine whether hypertension is also associated with elevated eosinophil counts in HIV-negative individuals, we recruited an additional cohort of 50 HIV-negative participants, including 25 normotensive and 25 hypertensive participants. Characteristics of these participants are shown in Table S5.

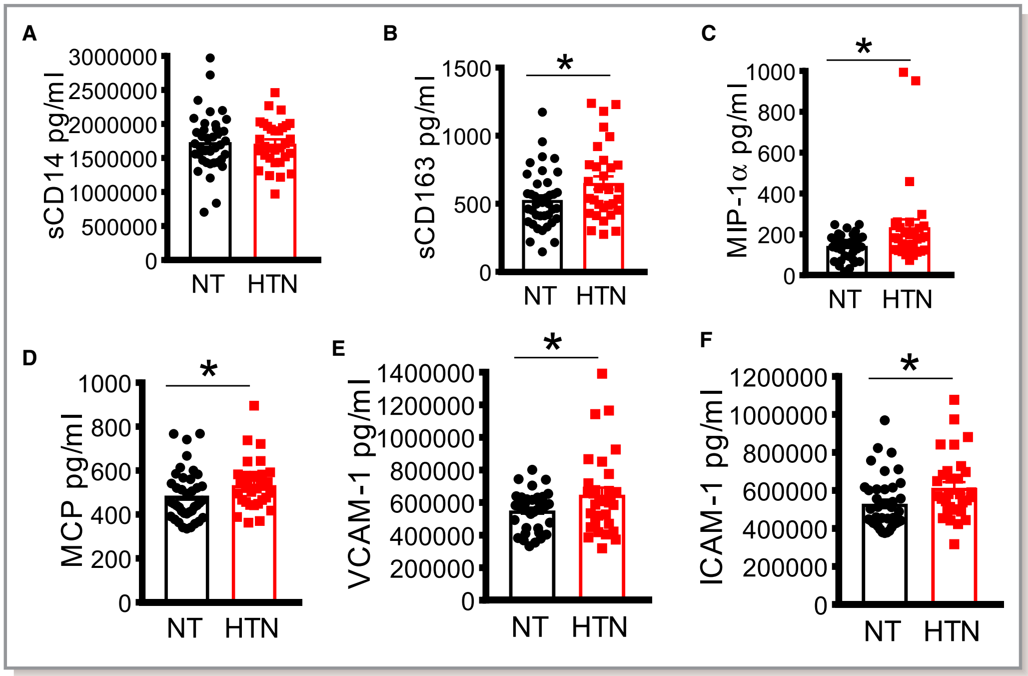

Figure 1. Increased macrophage activation in virally suppressed HIV$^+$ participants on antiretroviral therapy is associated with blood pressure elevation. Analysis of monocyte/macrophage activation in plasma using ELISA assay in people living with HIV showing soluble cluster of differentiation (sCD) 14 (**A**), sCD163 (**B**), MIP (macrophage inflammatory protein) (**C**), MCP (monocyte chemoattractant protein) (**D**) and adhesion molecules, including vascular cell adhesion molecule 1 (VCAM-1) (**E**) and intercellular adhesion molecule 1 (ICAM-1) (**F**). HTN indicates hypertensive; NT, normotensive. **P*<0.05 using the Mann-Whitney *U* test.

The eosinophil counts were obtained by retrospective review of the medical records for differential counts of white blood cells. We found that eosinophil counts were elevated in hypertensive when compared with normotensive people without HIV (Figure 3D). However, in a multivariate analysis after adjusting for sex, age, and BMI, eosinophil counts were not associated with hypertension, but they were significantly associated with increased BMI (Table S6). In addition, we performed a regression analysis on the combined cohort comprising both the 70 HIV-positive and the 50 HIV-negative individuals. The clinical characteristics for the combined group are shown in Table S7. After multivariate analysis of this combined cohort, controlling for age, sex, and BMI, the association between eosinophils and hypertension was robust but did not reach statistical significance (Table S8).

In additional studies, we used the Synthetic Derivative, a Vanderbilt University Medical Center database of ~2.5×10^6 deidentified electronic medical records, to identify additional HIV-negative normotensive and hypertensive participants. We excluded participants who did not have information on eosinophil counts, those who were diabetic, and those with a history of acute coronary events, stroke, known rheumatologic or inflammatory conditions, or concomitant comorbidities that might modify eosinophil counts (allergic rhinitis, asthma, dermatoses, and parasitic disease). Pediatric subjects, who constitute a large number of the Vanderbilt Synthetic Derivative, were also excluded. The clinical characteristics for this cohort are shown in Table S9. We found that hypertensive HIV-negative subjects had a significantly higher eosinophil count when compared with the normotensive subjects (Figure 3D 165.81±1.29 [n=9725 hypertensive subjects] versus 151.97±0.49 [n=71 314 normotensive subjects]; *P*<0.0001). However, like our smaller cohort of 50 HIV-negative participants, higher eosinophil count was significantly corelated with the higher BMI in the hypertensive participants (*P*<0.000001). The eosinophil count remained significantly associated with hypertension after a multivariate analysis (Table S10).

We performed an additional analysis combining the HIV-positive and HIV-negative cohorts to determine the interaction between elevated eosinophils and HIV. This analysis confirmed that elevated eosinophils were significantly associated with hypertension (*P*=0.045) in the HIV-positive cohort, but not significant in HIV-negative cohort (*P*=0.41) (Table S11 and Figure S3). The prediction results with one unit increase of eosinophilia in HIV-positive and HIV-negative cohorts are shown in Table S12. These results suggest that unlike in HIV-negative participants, in whom higher BMI explains the elevated

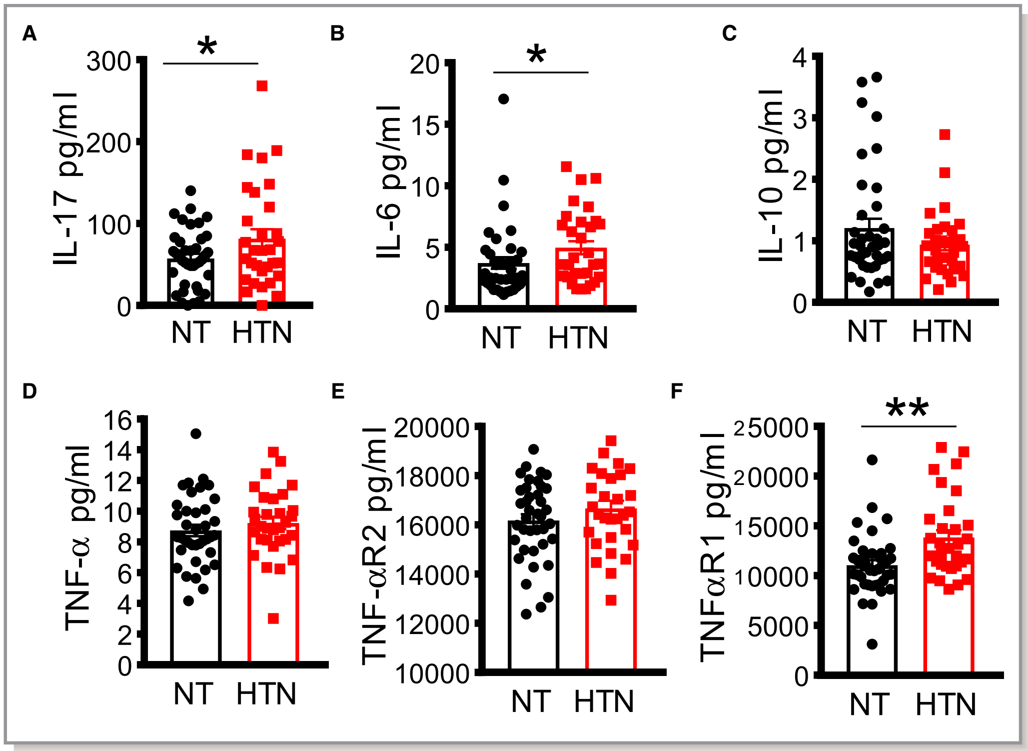

Figure 2. Increased cytokine production in virally suppressed HIV^{+} participants on antiretroviral therapy is associated with hypertension. Cytokine production was analyzed in plasma using ELISA, including interleukin (IL)-17 (**A**), IL-6 (**B**), IL-10 (**C**), tumor necrosis factor (TNF)-α (**D**), TNF-α receptor 2 (TNF-αR2) (**E**), and TNF-α receptor 1 (TNF-αR1) (**F**). HTN indicates hypertensive; NT, normotensive. $^{*}P<0.05$, $^{**}P<0.01$ using the Mann-Whitney *U* test.

eosinophil counts, HIV infection is accompanied by adipose tissue dysfunction that could mimic obesity, and this may increase eosinophil counts in HIV even without increase in BMI.

Discussion

In the current studies, we found that virally suppressed hypertensive PLWH and on ART exhibited higher levels of circulating eosinophils and their maturation marker interleukin-5. They also had higher levels of inflammatory cytokines, including TNF-α1, interleukin-6, and interleukin-17, when compared with normotensive controls. In addition, ICAM-1 and MIP-1α were elevated in hypertensive PLWH. After adjustment for multiple clinical and demographic factors, the circulating eosinophil counts remained significantly associated with hypertension in this population. However, elevated eosinophil counts were not significantly associated with hypertension in HIV-negative participants after adjusting for multiple clinical and demographic factors.

Our results are summarized in Figure 4, in which we propose that HIV infection and/or ART is/are associated with increased endothelial dysfunction, with increased expression of vascular cell adhesion molecule-1 and ICAM-1. This increases the propensity of activated monocytes, with increased production of sCD163, to diapedese into tissues where they encounter dysfunctional adipose tissue and convert into activated macrophages and dendritic cells. The activated antigen-presenting cells, including macrophages and dendritic cells, produce MCP1, which further increases migration and infiltration of monocyte/macrophages into tissues. They also produce chemotactic cytokine MIP-1a, which activates eosinophils and induces release of interleukin-6 and TNF-α from macrophages and dendritic cells. These antigen-presenting cells activate T cells to produce interleukin-17, which contributes to hypertension. They also produce interleukin-5, which induces differentiation of eosinophils. Thus, these findings suggest an association between higher eosinophil levels, potentially driven by elevated plasma interleukin-5, and hypertension in HIV-positive but not HIV-negative people. The causal relationship between increased interleukin-5 and eosinophils in inducing hypertension is not known.

Although ART has significantly increased life expectancy among PLWH, there is now increased risk for hypertension and cardiovascular disease.[5,21] In humans, observational studies have reported a higher prevalence of hypertension in HIV-positive people compared with the general population.[5,22]

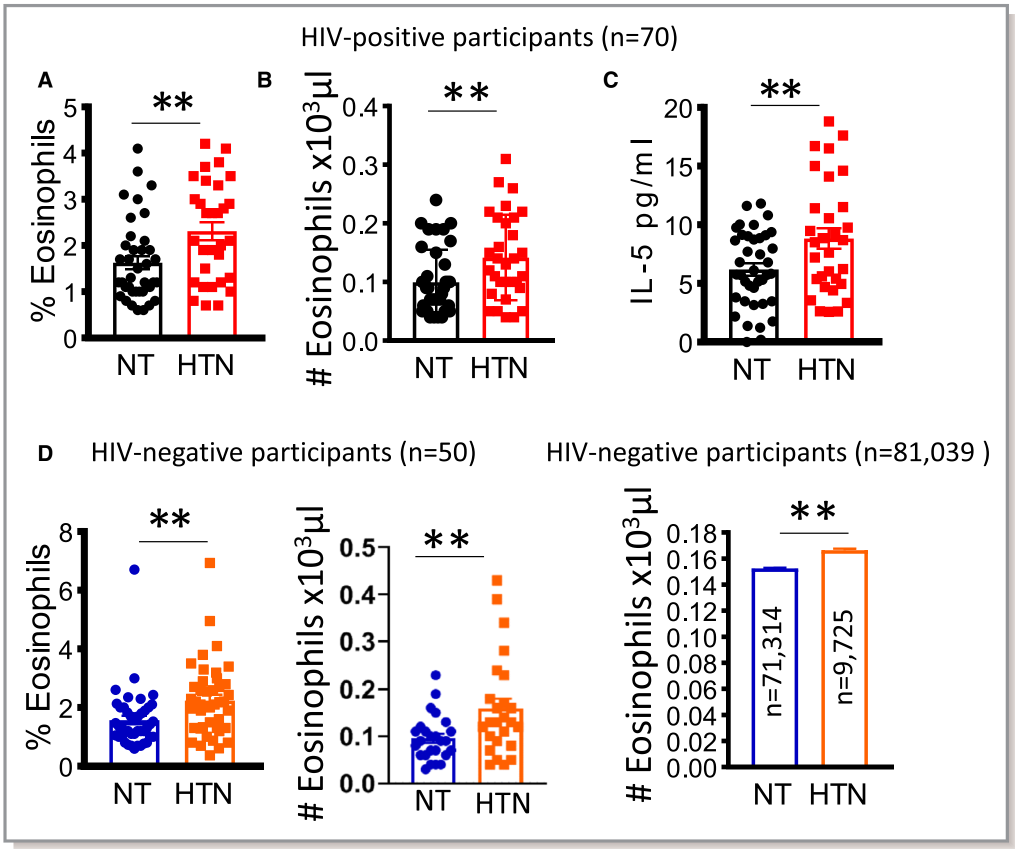

Figure 3. Elevated eosinophils in blood are associated with increased hypertension. Analysis of eosinophils was performed using automated differential count of white blood cells and expressed as percentage (**A**) and absolute counts (**B**) in people living with HIV. Interleukin (IL)-5 was analyzed using ELISA in plasma of people living with HIV (**C**). Eosinophil counts in HIV-negative normotensive (NT) and hypertensive (HTN) participants in two different cohorts (**D**). $^*P<0.01$ using the Mann-Whitney *U* test.

Recent studies suggest that HIV infection and its treatment with ART may play a role in the development of hypertension.[22] One contributor may be the accumulation of central adiposity because of effects of both primary infection and antiretroviral agents on fat distribution. Notably, hypertensive people had similar quantities of fat mass in the lower extremities compared with normotensive people, but significantly higher quantities of android and truncal fat. Together, these findings likely represent a lipodystrophy phenotype characterized by central lipohypertrophy without a pronounced limb lipoatrophy, potentially reflecting the limitation of enrollment to people on efavirenz/tenofovir disoproxil fumarate/emtricitabin as opposed to older thymidine analogue medications (eg, zidovudine or stavudine). Although the accumulation of central adiposity may contribute to inflammation, the HIV-specific inflammatory factors predisposing to hypertension are poorly understood. Herein, we found some similarity as well as unique immune components associated with hypertension in PLWH when compared with the general population.

An interesting finding of the present study is that elevated blood pressure in PLWH was associated with low levels of CD38 expression on $CD4^+$ T cells, which is a transmembrane glycoprotein indicating T-cell activation. Prior studies have found that lower percentages and absolute numbers of $CD4^+CD38^+$ T cells were associated with severe HIV disease pathogenesis and immunologic deterioration in children perinatally infected with HIV and surviving for >5 years.[23] We found no significant difference in expression of CD38 on $CD8^+$ T cells in hypertensive versus normotensive subjects, which has been found to be a marker of residual virus replication in chronically HIV-infected patients receiving Combination Antiretroviral Therapy.[24] Indeed, all participant in the current studies had been virally suppressed (viral load<50 copies/mL limit of detection) for a minimum 12 months before the study. Further research is needed to determine the role of $CD4^+$ T cells expressing CD38 and how they may interact with other immune cells in the setting of hypertension in HIV-positive individuals.

We found that monocyte/macrophage activation was associated with hypertension in PLWH. This was indicated by a marked increase in the shedding of the hemoglobin-haptoglobin scavenger receptor CD163 into the circulation as

Table 2. Association Between Hypertension and Inflammatory Cell Subset/Biomarkers in HIV Using Logistic Regression Adjusted for FMI, Age, and Sex

Variable	25% Quantile	75% Quantile	Value Difference (75%–25%)	*P* Value	Adjusted OR (75%–25%)	Adjusted OR (75%–25%, 95% CI)		*P* Value
					OR (Difference: 75%–25%)	OR (Difference, Lower 95%)	OR (Difference, Upper 95%)	
Interleukin-17	36.25	87	50.75	0.056	1.4	0.708	2.766	0.333
Interleukin-6	2.282	5.743	3.46	0.09	1.064	0.492	2.301	0.874
TNFαR1	9927.125	13 650.625	3723.5	0.005	1.616	0.817	3.196	0.168
MIP-1α	121.75	199.75	78	0.025	1.905	0.961	3.775	0.065
MCP1	421.75	574.5	152.75	0.094	1.458	0.7	3.036	0.313
ICAM-1	445 708	646 911.125	201 203.125	0.033	1.814	0.821	4.008	0.141
VCAM1	471 585.5	729.025	312.05	0.04	1.733	0.787	3.815	0.172
sCD163	416.975	5.743	3.46	0.09	1.064	0.492	2.301	0.874
$CD4^{+}CD3^{+}$ T cells	5.3	10.3	5.6	0.006	0.394	0.158	0.985	0.063
Interleukin-5	4.645	9.448	4.803	0.013	1.988	0.975	4.055	0.059
Eosinophils	1.1	2.7	1.6	0.007	2.797	1.106	7.078	0.03
Eosinophil %	1.787	2.659	0.872	0.009	1.735	0.804	3.746	0.043

CD indicates cluster of differentiation; FMI, fat mass index; ICAM-1, intercellular adhesion molecule 1; MCP1, monocyte chemoattractant protein 1; MIP-1α, macrophage inflammatory protein-1α; OR, odds ratio; sCD, soluble CD; TNFαR1, tumor necrosis factor-α receptor 1; VCAM1, vascular cell adhesion molecule-1.

sCD163 by the activated macrophages and monocytes. In addition, we found increased MIP-1α and MCP in hypertensive when compared with normotensive PLWH. Our current findings are in keeping with increasing evidence indicating that cells of the innate immune system, including monocytes and macrophages, play a role in the pathogenesis of hypertension. Previous studies have found that activated macrophages contribute to hypertension and deletion of monocytes markedly reduces experimental hypertension.[25–27] Macrophages accumulate in the kidney and the vasculature in experimental models of hypertension and promote hypertension.[28,29] Moreover, we found that hypertension in PLWH was associated with increased expression of adhesion molecules, including vascular cell adhesion molecule-1 and ICAM-1, which govern migration and infiltration of immune cells into tissues and have been implicated in the pathogenesis of hypertension in experimental animal models.[30]

A notable finding of our current study is that hypertension in PLWH was associated with increased cytokine production and signaling, including interleukin-6, TNF-α, and interleukin-17. Previous studies have found that T cells and their cytokines contribute to hypertension.[31,32] Mice lacking T cells (recombination-activating gene-$1^{-/-}$ mice) and mice lacking the T-cell cytokine interleukin-17A develop blunted hypertension.[17,33] We previously found that hypertension augments the capacity of dendritic cells to produce interleukin-6 and these polarized T cells to produce interleukin-17 and TNF-α.[16,34] Thus, hypertension in PLWH is associated with some inflammatory components similar to those that have been implicated in hypertension in the general population.

In this cohort of virally suppressed PLWH, eosinophils were significantly associated with hypertension, independent of age, sex, and adiposity. Interleukin-5 and eosinophils have been implicated in several inflammatory conditions, including asthma, drug hypersensitivity, neoplastic disorders, and helminths infections.[35] Interleukin-5 is produced by T helper 2 cells, mast cells, γδT cells, natural killer, and natural killer T cells and other nonhematopoietic cells.[36–40] It was originally identified as a T-cell–derived cytokine involved in antibody production from B cells.[38] Recently, interleukin-5 has been implicated in mediating maturation and differentiation of eosinophils in mice and humans.[38] In addition, eosinophils play a controversial role in obesity-mediated inflammation.[41,42] In the LifeLines Cohort Study of >13 000 members of the general population in Europe, higher eosinophil counts were associated with higher triglycerides, total cholesterol, and hemoglobin A1c, but lower high-density lipoprotein, in addition to a higher odds of obesity and metabolic syndrome.[43] Despite this association between eosinophils and cardiometabolic disease, no prior studies have found any association between eosinophils and hypertension.

Our results indicate that although eosinophil counts are elevated in hypertensive PLWH independent of BMI, they are dependent on BMI in HIV-negative people. Previously, eosinophils were thought to be only associated with Th2 inflammatory disorders, including parasitic infections and allergic reactions.[44] However, recent studies have found that

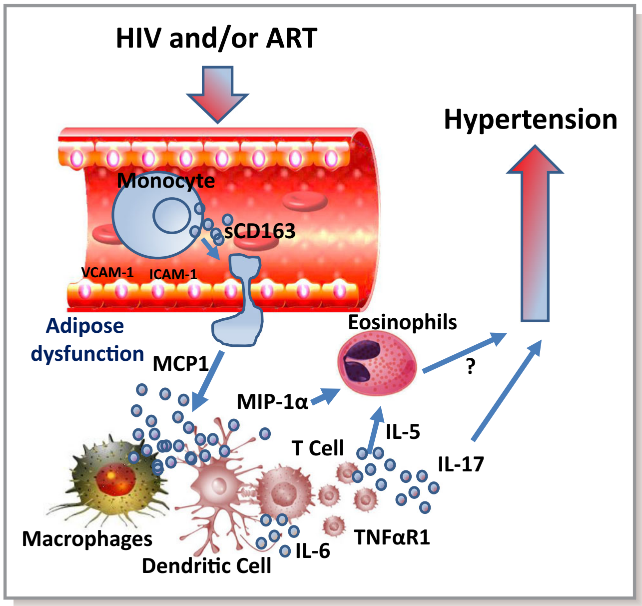

Figure 4. Hypothesized model summarizing all the inflammatory components associated with hypertension in people living with HIV. HIV infection and/or antiretroviral therapy (ART) is/are associated with increased endothelial dysfunction with increased expression of vascular cell adhesion molecule-1 (VCAM-1) and intracellular adhesion molecule 1 (ICAM-1). This increases the propensity of activated monocytes, with increased production of soluble cluster of differentiation (sCD) 163, to diapedese into tissues where they encounter dysfunctional adipose tissue and convert into activated macrophages and dendritic cells. The activated antigen-presenting cells, including macrophages and dendritic cells, produce MCP1 (monocyte chemoattractant protein 1), which further increases migration and infiltration of monocyte/macrophages into tissues. They also produce chemotactic cytokine MIP-1α (macrophage inflammatory protein-1α), which activates eosinophils and induces release of interleukin (IL)-6 and tumor necrosis factor (TNF)-α from macrophages and dendritic cells. These antigen-presenting cells activate T cells to produce IL-17, which contributes to hypertension. They also produce IL-5, which induces differentiation of eosinophils. TNF-αR1 indicates TNF-α receptor 1.

eosinophils can infiltrate adipose tissue and regulate its function.[41] Wither et al found that eosinophils play a role in obesity-related hypertension, where they regulate perivascular adipose tissue and vascular function.[45] Mice lacking eosinophils do not elicit an anticontractile effect resulting from adiponectin and adipocyte-derived NO.[46] This effect mimics the obese phenotype and is restored by reconstitution of eosinophils. The role of eosinophils in hypertension in the setting of HIV infection is not known. Acquired lipodystrophy occurs in 60% to 80% of patients with HIV on cART,[47] and is associated with endothelial dysfunction leading to cardiovascular disease.[48–51] Thus, it is likely that although elevated eosinophil counts were independent of increased BMI in the hypertensive PLWH, they may be associated with acquired lipodystrophy, which creates a metabolic milieu similar to increased BMI. Understanding how eosinophils impact cardiovascular function and hypertension may have important implications for the treatment of metabolic disorders associated with obesity and HIV.

Eosinophils have been found to regulate macrophage activation in adipose tissue. Wu et al[41] found that in mice, eosinophils are major producers of interleukin-4 in white adipose tissues and increase infiltration of alternatively activated macrophages. Mice fed a high-fat diet develop increased body fat, impaired glucose tolerance, and insulin resistance in the absence of eosinophils. These studies suggest that eosinophils play a role in metabolic homeostasis through maintenance of adipose alternatively activated macrophages. Indeed, we found that macrophage activation factors, including MIP-1α, were elevated in hypertensive PLWH. However, unlike in the HIV-negative participants, we found that eosinophil counts are increased in HIV independent of BMI. It is not clear what role these cells play in hypertension associated with HIV and whether they contribute to macrophage activation. Moreover, peripheral blood eosinophilia is often observed in patients with chronic kidney disease, those with acute kidney injury, or patients on renal replacement therapy, and this may be associated with bioincompatibility of the dialysis material, acute allograft rejection, or *Strongyloides* hyperinfection. Further scientific effort is required to determine if eosinophilia is associated with kidney disease in hypertensive PLWH.[52]

Our results suggest an association between higher eosinophil levels, potentially driven by elevated plasma interleukin-5, and hypertension in PLWH. This association is present but abrogated by BMI in HIV-negative hypertensive people. The findings that eosinophils and their maturation cytokine interleukin-5 are elevated in hypertensive PLWH are interesting but only hypothesis generating and do not confirm any new pathogenesis of hypertension. However, these 2 related findings provide a pragmatic and strong rationale to study this pathway further in either hypertension or hypertension in HIV.

There is evidence that $CD8^+$ T cells activated in presence of interleukin-4 can exhibit a Th2-like phenotype and produce cytokines interleukin-4, interleukin-5, interleukin-6, and interleukin-10. The role of $CD8^+$ T cells in the context of the HIV-1 infection is not fully understood; however, it is possible that they are responsible for production of high levels of interleukin-5,[53,54] which may drive the eosinophilia. Although we did not observe any significant differences in $CD8^+$ T cells between normotensive and hypertensive PLWH, previous studies have found that adipose tissue from PLWH is enriched for $CD8^+$ T cells compared with HIV-negative controls; and similar changes seen have been observed in obesity.[55,56] This role for $CD8^+$ T cells in interleukin-5 production does raise the possibility that the elevated eosinophils we observed in hypertensive PLWH may not represent a causal mechanism, but rather be a reflection of a link between the $CD8^{+}$ T-cell antiviral immune response and cardiovascular function. In keeping with this concept, absence of eosinophils has been associated with worsening of cardiometabolic health and restoration of eosinophils leads to increased vascular relaxation and improved glucose homeostasis.[41] However, other studies have failed to demonstrate an effect of these cells in rescuing metabolic impairment,[42] and Amini et al found that higher eosinophil counts were associated with risk factors of metabolic syndrome, including higher triglycerides, total cholesterol, and hemoglobin A1c.[43] Studies have found increased levels of other cells known to be anti-inflammatory, such as T-regulatory cells in disease. For example, T-regulatory cells are increased in the circulation of patients with idiopathic pulmonary arterial hypertension.[57,58] Thus, anti-inflammatory cells may become dysfunctional during disease pathogenesis. Further research is needed to determine how the function of eosinophils may be affected during hypertension in HIV.

In summary, we discovered a significant association between elevated eosinophils and hypertension that might represent a novel pathway leading to hypertension in HIV-infected adults. Further research is needed to determine the specific contribution of the HIV-positive status versus ART on inflammation and how they may contribute to hypertension and cardiovascular disease. We acknowledge that this is a cross-sectional study in a limited cohort of 70 HIV-positive participants and therefore cannot address causality. Although our findings obviously warrant further validation, we believe there is clinical utility for treatment of hypertension in or outside the context of HIV.

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Disclosures

None.

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Supplemental Material

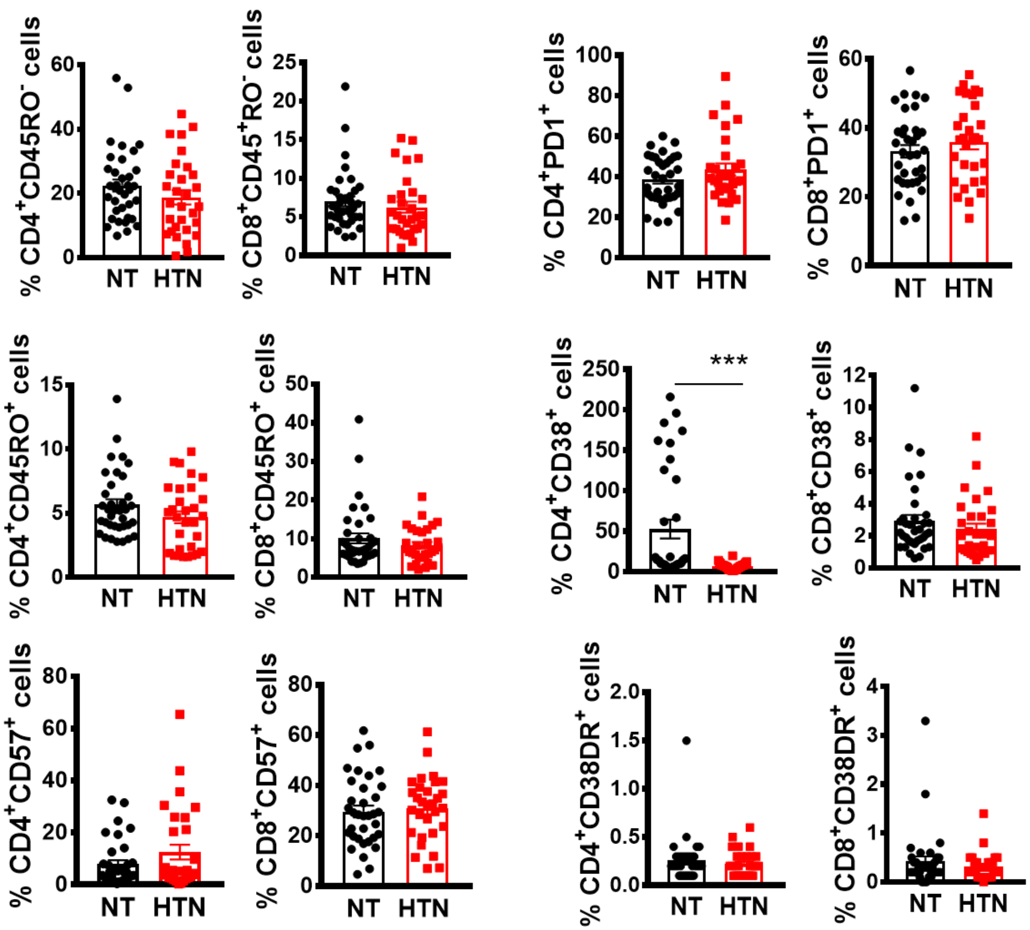

Figure S1. Increased CD4 T cell activation in people living with HIV is negatively associated with hypertension. Flow cytometry analysis of T cell subsets in people living with HIV showing(A)naïve and (B) memory CD4$^+$ and CD8$^+$T cell subtypes, (C) T cells expressing CD57, (D) PD1, (E) CD38and (F) the CD38 receptor in normotensives compared to hypertensives. ***p<0.001.

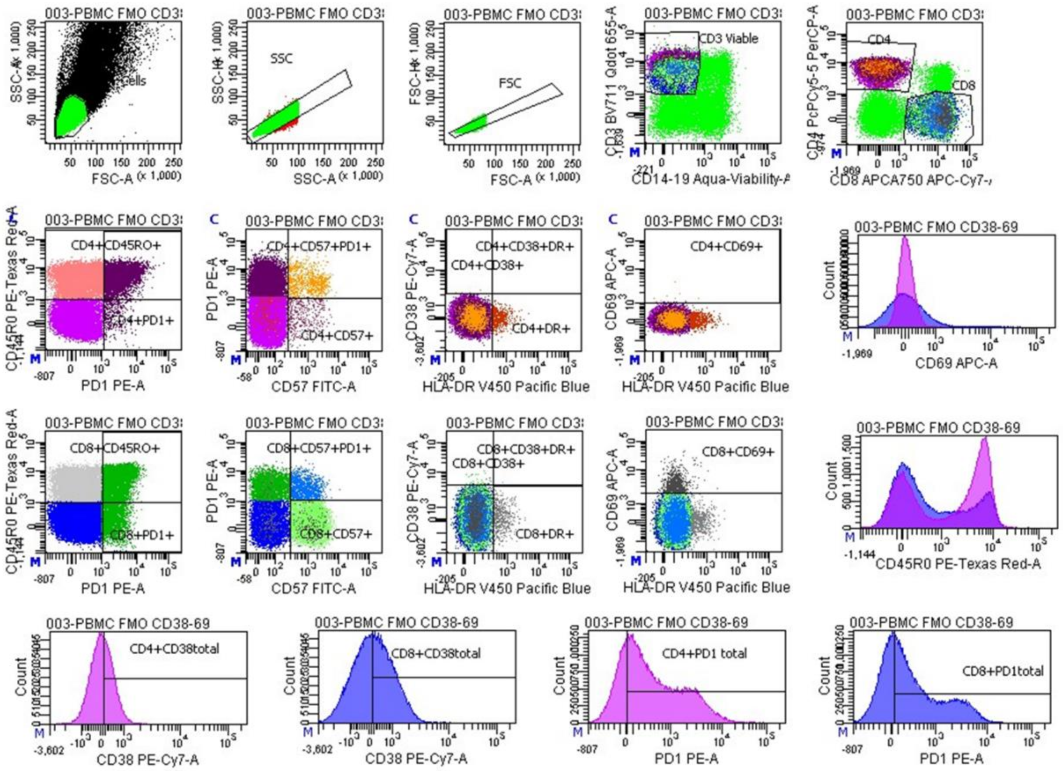

Figure S2. Gating strategy to identify T cell subtypes.

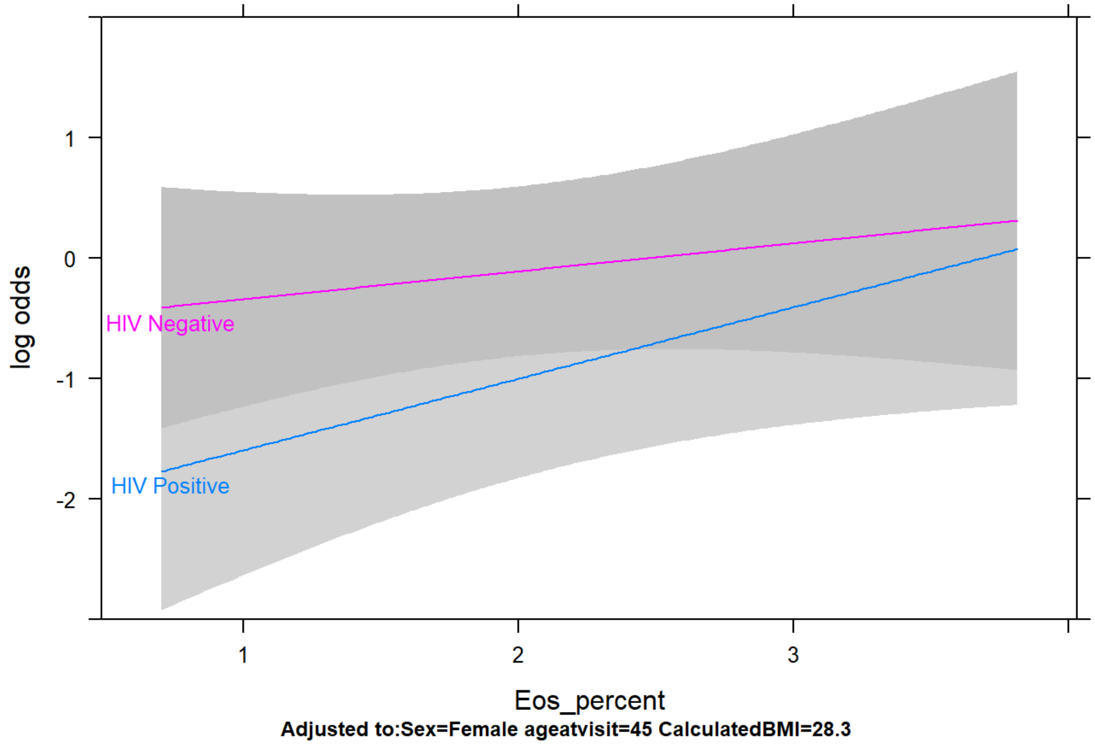

Figure S3. Analysis combining the HIV positive and negative cohorts to determine the Interaction between elevated eosinophils and HIV. Log odds of elevated eosinophils in HIV positive and negative cohort (p=0.045 in the HIV positive cohort and p=0.41 in the HIV negative cohort).

Table S1. Fat Mass measurements between hypertensives and normotensive.

	HIV Positive n=69		p value
	Normotensive n=39 *median (IQR)*	**Hypertensive n=30** *median (IQR)*	
Left Arm FM, kg	1.18 (0.80, 1.53)	1.56 (1.21, 2.15)	**0.003**
Left Leg FM, kg	4.82 (2.90, 6.10)	4.71 (3.26, 7.05)	0.321
Left trunk FM, kg	7.18 (3.60, 10.83)	11.62 (8.88, 15.64)	**<0.001**
Left total FM, kg	13.23 (7.29, 19.33)	17.85 (13.86, 25.58)	**0.003**
Right arm FM, kg	1.25 (0.81, 1.60)	1.59 (1.32, 2.15)	**0.002**
Right leg FM, kg	4.88 (2.96, 6.07)	5.59 (3.55, 7.64)	0.160
Right trunk FM, kg	7.40 (3.49, 11.07)	11.76 (8.73, 15.91)	**0.001**
Right total FM, kg	13.80 (7.89, 19.32)	18.31 (14.24, 25.40)	**0.003**
Arms FM, kg	2.43 (1.60, 3.19)	3.13 (2.53, 4.31)	**0.003**
Legs FM, kg	9.77 (5.85, 12.17)	9.88 (6.82, 14.40)	0.204
Trunk FM, kg	14.09 (7.09, 21.84)	22.81 (17.51, 31.61)	**<0.001**
Android FM, kg	2.58 (1.03, 3.90)	4.14 (3.11, 6.18)	**0.001**
Gynoid FM, kg	4.31 (3.10, 6.49)	5.72 (3.92,7.46)	0.056
Total FM, kg	27.03 (15.04, 38.47)	36.04 (28.10, 51.22)	**0.003**
FMI, kg/m^2	8.8 (5.7, 13.2)	13.3 (9.9, 17.5)	**0.003**

Wilcoxon rank sum test used. FM, fat mass, FMI, fat mass index. P values less than 0.05 are shown in bold.

Table S2. Association between hypertension and inflammatory cell subset/biomarkers in HIV using logistic regression adjusted for body mass Index, age, and sex.

				Adjusted OR (75% to 25%)	**Adjusted OR (75% to 25%, 95% CI)**		
Variable	25% Quantile	75% Quantile	Value Diff (75%-25%)	Odds Ratio (Diff: 75%-25%)	OR (Diff, Lower 95%)	OR (Diff, Upper 95%)	p-value
IL-17	36.25	87	50.75	1.451	0.722	2.917	0.296
IL-6	2.282	5.743	3.46	1.031	0.466	2.285	0.939
TNFαR1	9927.125	13650.625	3723.5	1.665	0.842	3.291	0.143
MIP1-α	121.75	199.75	78	1.99	0.991	3.998	0.053
MCP1	421.75	574.5	152.75	1.432	0.68	3.017	0.345
sCD163	416.975	5.743	3.46	1.78	0.777	4.076	0.173
$CD4^+CD38^+$ T ells	5.3	10.3	5.6	0.394	0.513	1.168	0.112
IL-5	4.645	9.448	4.803	1.975	0.955	4.082	0.066
Eosinophils	1.1	2.7	1.6	2.623	1.024	6.716	0.044

Table S3. Analysis of p values of Model Likelihood Ratio chi-square Test and Nagelkerke R^2 index. Most of the interesting variables have p<0.01 and R^2>0.3, which indicated the goodness of fit for the HIV-positive cohort.

```
Logistic Regression Model


 lrm(formula = formulaForModel, data = mergedData)



                       Model Likelihood     Discrimination    Rank Discrim.
                          Ratio Test           Indexes           Indexes
 Obs            119    LR chi2      41.81    R2       0.395    C       0.816
  0              63    d.f.             5    g        1.784    Dxy     0.632
  1              56    Pr(> chi2) <0.0001    gr       5.956    gamma   0.633
 max |deriv| 3e-10                           gp       0.323    tau-a   0.318
                                             Brier    0.175
                Coef    S.E.   Wald Z Pr(>|Z|)
 Intercept     -8.2948 1.7554 -4.73  <0.0001
 Eos            0.4176 0.2150  1.94  0.0521
 Sex=Male       0.6987 0.4926  1.42  0.1561
 age            0.0614 0.0236  2.59  0.0095
 BMI            0.1628 0.0373  4.36  <0.0001
Cohort=HIV    -0.9177 0.4918 -1.87  0.0621
```

Table S4. **Analysis of C-index to measure goodness of fit. All variables of interest have C-index >0.75 indicating goodness of fit.** See last column (C) of each Table.

Formula	Variable of interest	Effect (One Unit)	P	OR (One Unit)	OR (Lower 95%)	OR (Upper 95%)	Value (25% Quantile)	Value (75% Quantile)	Value Diff (75%-25%)	Odds Ratio (Diff: 75%-25%)	OR (Diff, Lower 95%)	OR (Diff, Upper 95%)	P	R2	C
lrm (HTN01~ IL5pgml +Sex+age+FMI)	IL5pgml	0.143	0.0588	1.154	0.995	1.338	4.645	9.448	4.803	1.988	0.975	4.055	0	0.356	0.795
lrm (HTN01~ IL6pgml +Sex+age+FMI)	IL6pgml	0.018	0.8741	1.018	0.815	1.272	2.282	5.743	3.46	1.064	0.492	2.301	0.002	0.298	0.779
lrm (HTN01 ~ TNFαR1pgml +Sex+age+FMI)	TNFαR1pgml	0	0.1681	1	1	1	9927.125	13650.625	3723.5	1.616	0.817	3.196	0.001	0.329	0.781
lrm (HTN01 ~ MIP1αpgml+Sex+age+FMI)	MIP1αpgml	0.008	0.0648	1.008	0.999	1.017	121.75	199.75	78	1.905	0.961	3.775	0	0.378	0.809
lrm (HTN01 ~ MCP1pgml +Sex+age+FMI)	MCP1pgml	0.002	0.3134	1.002	0.998	1.007	421.75	574.5	152.75	1.458	0.7	3.036	0.001	0.314	0.768
lrm (HTN01 ~ ICAM1pgml +Sex+age+FMI)	ICAM1pgml	0	0.1406	1	1	1	445708	646911.125	201203.125	1.814	0.821	4.008	0.001	0.333	0.794
lrm (HTN01~ sCD163ngml +Sex+age+FMI)	sCD163ngml	0.002	0.1719	1.002	0.999	1.004	416.975	729.025	312.05	1.733	0.787	3.815	0.001	0.327	0.774
lrm (HTN01 ~ CD4CD38 +Sex+age+FMI)	CD4CD38	-0.134	0.0718	0.875	0.757	1.012	5.3	10.9	5.6	0.473	0.21	1.069	0.001	0.354	0.81
lrm (HTN01 ~ Eos+Sex +age+FMI)	Eos_percent	0.643	0.0299	1.902	1.065	3.398	1.1	2.7	1.6	2.797	1.106	7.078	0	0.373	0.808
lrm (HTN01 ~ IL17pgml +Sex + age+FMI)	IL17pgml	0.007	0.3334	1.007	0.993	1.02	36.25	87	50.75	1.4	0.708	2.766	0.001	0.313	0.773
lrm (HTN01 ~ VCAM1pgml +Sex+age+FMI)	VCAM1pgml	0	0.2444	1	1	1	471585.5	650258.5	178673	1.466	0.77	2.794	0.001	0.32	0.774

Formula	Variable of interest	Effect (One Unit)	P	Odds Ratio (One Unit)	OR (Lower 95%)	OR (Upper 95%)	Value (25% Quantile)	Value (75% Quantile)	Value Diff (75%-25%)	Odds Ratio (Diff: 75%-25%)	OR (Diff, Lower 95%)	OR (Diff, Upper 95%)	P	R2	C
lrm (HTN01 ~ IL5pgml)	IL5pgml	0.167	0.0128	1.182	1.036	1.348	4.645	9.448	4.803	2.23	1.186	4.194	0.007	0.131	0.638
lrm (HTN01 ~ IL6pgml)	IL6pgml	0.152	0.0904	1.164	0.976	1.388	2.282	5.743	3.46	1.692	0.92	3.11	0.074	0.06	0.656

Formula	Variable of interest	Effect (One Unit)	P	Odds Ratio (One Unit)	OR (Lower 95%)	OR (Upper 95%)	Value (25% Quantile)	Value (75% Quantile)	Value Diff (75%-25%)	Odds Ratio (Diff: 75%-25%)	OR (Diff, Lower 95%)	OR (Diff, Upper 95%)	P	R2	C
lrm (HTN01 ~ TNFαR1pgml)	TNFαR1pgml	0	0.0051	1	1	1	9927.125	13650.625	3723.5	2.447	1.308	4.577	0.001	0.187	0.715
lrm (HTN01 ~ MIP1αpgml)	MIP1αpgml	0.01	0.0255	1.01	1.001	1.018	121.75	199.75	78	2.123	1.097	4.111	0.002	0.167	0.658
lrm (HTN01 ~ MCP1pgml)	MCP1pgml	0.004	0.0935	1.004	0.999	1.008	421.75	574.5	152.75	1.767	0.908	3.436	0.083	0.056	0.64
lrm (HTN01 ~ ICAM1pgml)	ICAM1pgml	0	0.0334	1	1	1	445708	646911.125	201203.125	2.077	1.059	4.073	0.024	0.094	0.674
lrm (HTN01 ~ sCD163ngml)	sCD163ngml	0.002	0.0399	1.002	1	1.004	416.975	729.025	312.05	1.995	1.032	3.856	0.031	0.086	0.629
lrm (HTN01 ~ CD4CD38)	CD4CD38	-0.194	0.006	0.823	0.717	0.946	5.3	10.9	5.6	0.337	0.155	0.732	0.002	0.181	0.726
lrm (HTN01 ~ Eos)	Eos	0.706	0.0074	2.025	1.209	3.394	1.1	2.7	1.6	3.093	1.355	7.064	0.005	0.145	0.689
lrm (HTN01 ~ IL17pgml)	IL17pgml	0.01	0.0556	1.01	1	1.021	36.25	87	50.75	1.695	0.988	2.911	0.041	0.077	0.589
lrm (HTN01 ~ VCAM1pgml)	VCAM1pgml	0	0.0473	1	1	1	471585.5	650258.5	178673	1.735	1.007	2.99	0.028	0.089	0.61

Table S5. Demographics and Clinical Data For HIV-negative Subjects.

	Normotensive controls (n=25)	**Hypertensive** patients (n=25)	
Age median years (IQR)	40.4 (27.5, 51.2)	45.5 (38.3, 54.9)	*<0.06*
Female n (%)	20(80%)	18(72%)	ns
Race, Whites n (%)	19(76%)	17(68%)	
Systolic BP (mmHg, median (IQR)	112.0 (105.7, 121.0)	137.0 (121.0, 147.0)	<0.0002
Diastolic BP (mmHg, median (IQR)	68.00 (62.67, 74.00)	80.00 (74.00, 87.00)	<0.0001
MAP (mmHg, median (IQR)	82.67 (76.11, 88.78)	97.00 (90.44, 106.00)	<0.0001
BMI median (IQR) kg/m2	23.80 (21.12, 27.05)	32.20 (28.10, 36.00)	<0.0001

n = number of subjects in each group; x±SEM = mean ± standard error of the mean; F = female; M= male; W= white; B=black; A=Asian; N=native American; BP = blood pressure; MAP = mean arterial pressure; BMI = body mass index. P values for the unpaired t-test and chi-square comparisons between groups are in the rightmost column. ns indicates lack of statistical significance.

Table S6. Association between hypertension and demographic and clinical characteristics in the HIV-negative cohort using logistic regression adjusted for body mass Index, age, and **sex**.

				OR (75% to 25%)	**OR (75% to 25%, 95% CI)**			**Adjusted OR (75% to 25%)**	**Adjusted OR (75% to 25%, 95% CI)**		
Variable	25% Quantile	75% Quantile	Value Diff (75%-25%)	OR (Diff: 75%-25%)	OR (Diff, Lower 95%)	OR (Diff, Upper 95%)	p-value	Odds Ratio (Diff: 75%-25%)	OR (Diff, Lower 95%)	OR (Diff, Upper 95%)	p-value
Eosinophil	68.95	153.7	84.75	2.543	1.11	5.826	0.027	2.646	0.8318	8.416	0.0993
Eosinophil %	0.012	0.022	0.010	1.334	0.811	2.195	0.03	1.365	0.749	2.489	0.31
Age	36.39	54.23	17.84	1.978	0.848	4.616	0.115	1.955	0.6237	6.13	0.25
BMI	23.8	32.7	8.9	11.577	2.799	47.877	<0.001	12.43	2.684	57.58	0.0013
Sex, M:F				1.556	0.419	5.779	0.509	1.58	0.2535	9.845	0.6243

Table S7. Demographics and clinical data for both the 50 HIV-negative and 70 HIV-positive cohorts combined.

	HIV^- ***n=50***	**HIV^+** ***n=70***	**Combined** ***n=120***	**p-value**
Eos median (IQR)	1.68 (1.22, 2.20)	1.70 (1.10, 2.70)	1.70 (1.10, 2.43)	0.793
Age median years (IQR)	44.34 (36.39, 54.23)	45.00 (39.00, 50.00)	45.00 (37.24, 51.00)	0.868
Female n (%)	38(76)	30(43)	68(57)	<0.001
BMI median (IQR) kg/m2	27.60 (23.80, 32.70)	30.29 (23.98, 35.61)	28.30 (23.84, 34.60)	0.192
Hypertensive	25(50)	31(44)	56(47)	0.536
HIV	0(0)	70(100)	70(58)	<0.001

n is the number of subjects in each group.

Table S8. Association between hypertension and demographic/clinical characteristics in the both the 50 HIV-negative and 70 HIV-positive cohorts logistic regression.

Variable	OR	OR (Lower 95%)	OR (Upper 95%)	p-value
Eosinophil	1.518	8.004706e-06	0.007796146	0.0521
Age	1.063	1.015124e+00	1.113708391	0.0095
BMI Sex,	1.177	1.093814e+00	1.266014678	<0.0001
M:F HIV	2.011	9.961915e-01	2.314035505	0.1561
	0.399	1.523392e-01	1.047409729	0.0621

Table S9. Demographics and clinical data for the confirmatory HIV-negative cohort from the synthetic derivative.

	Normotensive controls (n= 71314)	**Hypertensive** patients (n= 9725)	**p value**
Age median years (IQR)	43.77 (36.02, 53.37)	51.75 (43.34, 56.95)	<*0.001*
Female n (%)	39912 (56)	3911 (40)	<0.001
Whites n (%)	63538 (89)	8010 (82)	<0.001
Eos median (IQR)	0.120 (0.065, 0.200)	0.135 (0.080, 0.220)	<0.001
BMI median (IQR) kg/m2	27.02 (23.43, 31.73)	30.98 (26.58, 36.38)	<0.0001

n = number of subjects in each group, F = female; BMI = body mass index. P values for the unpaired t-test and chi-square comparisons between groups are in the rightmost column.

Table S10. Association between hypertension and demographic and clinical characteristics in the HIV-negative cohort from the synthetic derivative using logistic regression.

Variable	25% Quantile	75% Quantile	Value Diff (75%-25%)	OR (Diff: 75%-25%)	OR (Diff, Lower 95%)	OR (Diff, Upper 95%)	p-value
Eosinophils	0.069	0.2	0.131	1.034	1.012	1.056	0.0021
Age	36.435	54.204	17.769	1.981	1.902	2.064	<0.0001
BMI	23.687	32.365	8.678	1.738	1.697	1.779	<0.0001
Sex, M:F				1.893	1.810	1.979	<0.0001

Table S11. Model Result analysis combining the HIV positive and negative cohorts to determine the interaction between elevated eosinophils and HIV.

Coef	S.E.	Wald	Z	Pr(>|Z|)
Intercept	-9.5387	1.935	-4.93	<0.0001
Sex=Male	0.6912	0.4981	1.39	0.1652
age at visit	0.0615	0.0237	2.6	0.0094
CalculatedBMI	0.1621	0.0373	4.34	<0.0001
Cohort=HIV Negative	1.6131	0.9352	1.72	0.0845
Eos_percent	0.5929	0.2961	2	0.0452
Cohort=HIV Negative*Eos_percent	-0.3612	0.4067	-0.89	0.3744

Table S12. Prediction results with one unit increase of eosinophilia in HIV positive and negative cohorts.

Sex	age	BMI	Cohort	Contrast	S.E.	Lower	Upper	Z	Pr(>|z|)
Female	45	28.3	Control	0.231701	0.284	-0.324	0.788	0.820	0.414
Female	45	28.3	HIV	0.592943	0.296	0.013	1.173	2.000	0.045

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RESEARCH NOTE

Open Access

HIV, immune activation and salt-sensitive hypertension (HISH): a research proposal

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Abstract

Objective: The objective of this study is to quantify and compare the effect of excess dietary salt on immune cell activation and blood pressure in HIV versus HIV negative individuals.

Results: Salt-sensitivity is associated with increased immune cell activation in animal studies. This concept has not been tested in people living with HIV. This study will therefore add more information in elucidating the interaction between HIV infection and/or anti-retroviral therapy (ART), immune-activation/inflammation and hypertension.

Keywords: Hypertension, Immune activation, Salt-sensitivity, HIV

Introduction

Salt-sensitive hypertension is defined as a change in blood pressure (BP) greater than 10% or >5 mmHg in response to either increased or reduced salt intake [1, 2]. This phenomenon is more common in individuals with higher BP, blacks, elderly, and is seen in certain comorbid conditions such as chronic kidney disease, diabetes mellitus or metabolic syndrome [3]. Salt has been implicated to initiate an inflammatory process that could result in hypertension [4–6]. Moreover, inflammation-driven salt accumulation is evident on sites of infection and/or inflammation [7].

Antiretroviral (ART) treated People living with HIV (PLWH) are prone to development of hypertension and elevated BP [8–10]. One of the reasons for this could be the interaction of a generalized systemic suboptimal immune activation and inflammation that persists, traditional risk factors including dietary salt intake, HIV particles and ART [11, 12]. However, the effect of salt on BP and immune activation has not been tested in PLWH and dietary assessments are never part of routine assessment in the clinical setting especially in Africa hence the need for this proposed study.

Study objectives:

1. To determine the prevalence of salt-sensitive hypertension in PLWH and compare with HIV negative population
2. To quantify and compare the effect of excess dietary salt on immune cell activation and BP in ART-treated normotensive, ART-treated hypertensive, HIV negative hypertensive and HIV negative normotensive individuals.

Main text

Methods

Study site, design and patients

We propose to conduct a three-week prospective study at Livingstone Central Hospital (LCH) involving PLWH attending routine ART and HIV negative controls from volunteer health workers who will be matched for age, sex, body mass index (BMI), and waist circumference (WC). LCH is the largest referral hospital in Southern Province of Zambia that offers ART and general medical services to the community with approximately 3776 PLWH enrolled in ART.

Eligibility criteria

Inclusion criteria The study cohort will include all adults (aged 18 and above) who will be required to verbally consent and sign a consent form and should be attending medical clinic for both general clinics and ART. Four groups will be established for comparison namely:

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1. ART treated normotensive individuals
2. ART treated hypertensive individuals
3. Hypertensive individuals who are HIV negative
4. Health controls.

Exclusion criteria Exclusion from the study will be based on existence of co-morbidities such as diabetes mellitus and cancer, and also those with existing and recent past opportunistic infections, syphilis, hepatitis C and B virus infection and tuberculosis infection; and sick patients (clients seeking healthcare due to an illness rather than routine ART clinic reviews) will be excluded. Those with recent and current alcohol consumption and smoking status will also be excluded from the study.

Study variables

Response variables Primary: Salt-sensitivity of BP

Secondary: Markers of antigen presenting cell activation CD80, CD83, CD86, CD69 and IsoLGs, pro-inflammatory markers such as interleukin 17A (IL-17A), IL-6, tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ) and intermediate monocyte subset ($CD14^{++}CD16^{+}$) which are raised in inflammation.

Explanatory variables Traditional risk factors: age, sex, WC, BMI, waist-to-hip ratio (WHR), sedentary life style, physical activity, fruit and vegetable intake, hours of sleep.

Clinical risk factors: BP, Electrocardiogram (ECG) parameters, lipid profile, Urine and blood electrolyte (sodium, potassium and chloride), Urine macro- and micro-analysis, ART regimen, duration on ART, adherence to ART, family history of hypertension and diabetes risk, duration of hypertension, medical history.

Immune related factors: Immune status (CD4 and HIV RNA viral load absolute counts), soluble CD14 (sCD14), Complete blood count, C-reactive protein (CRP).

Sample size

For aim 1, OpenEpi online software (Kelsey's method) will be used to compute a total sample size of 236 (118 hypertensive versus 118 normotensive in 1:1 ratio each between PLWH and HIV negative groups) for salt-sensitive hypertension with the following inputs: 95% significance level at 80% power; Estimated percent of salt-sensitive hypertension in the hypertensive and normotensive population was 50% and 25% respectively [13–15].

For aim 2, G*Power [16, 17] version 3.1.9.4 will be employed to calculate a total sample size of 48 (12 for each group). The input assumptions are detailed in Table 1.

Table 1 Sample size determination

F tests using MANOVA for repeated measures, within–between interaction		
Options	Pillai V, O'Brien-Shieh Algorithm	
Analysis	A priori: Compute sample Size	
Input	Effect size f(v)	1
	α err prob	0.05
	Power (1-β err prob)	0.80
	Number of groups	4
	Number of measurements	3
Output	Non-centrality parameter λ	22
	Critical F	2.8477260
	Numerator df	6.0
	Denominator df	14.0
	Sample size	11 per group
	Lost to follow (10%)	1 per group
	Total sample size	48
	Actual power	0.825
	Pillai V	1.0

Sampling

For selection of hypertensive and normotensive study participants, simple random sampling will be used assisted by an online random number generator. PLWH will be randomly selected during their routine medical visits.

In order to create equal groups in the strata, usage of randomization blocked in blocks of size four and six will be employed.

Procedure for aim 1 In order to determine the prevalence of salt-sensitive hypertension in PLWH and HIV negative population, we will conduct salt-sensitive analyses as described below:

Salt sensitivity analysis We will employ a modification of the procedure described by He et al. [18] as shown in Fig. 1. Briefly, in the 1st week (7 days), participants will be requested to avoid consuming processed foods and adding salt to their food. Participants will be required to record their daily diet. In the 2nd week, they will be given the World health organisation (WHO) recommended low salt (2.3 g), and in the 3rd week, high salt (9 g/day). The changes for low salt (2.3 g NaCl/day) on BP will be calculated as previously described [18]. BP will be monitored three times each day and on the last day of each phase a 24-h BP using an ambulatory BP monitoring device will be employed to assure that BP does not exceed 180/110 mmHg, in which case the protocol will be discontinued for safety.

Sodium excretion We will assess sodium excretion in urine samples collected over 24 h period as previously described by Rakova et al. [19]. Briefly, a 24-h urine will be collected by participants in aliquots, transported to

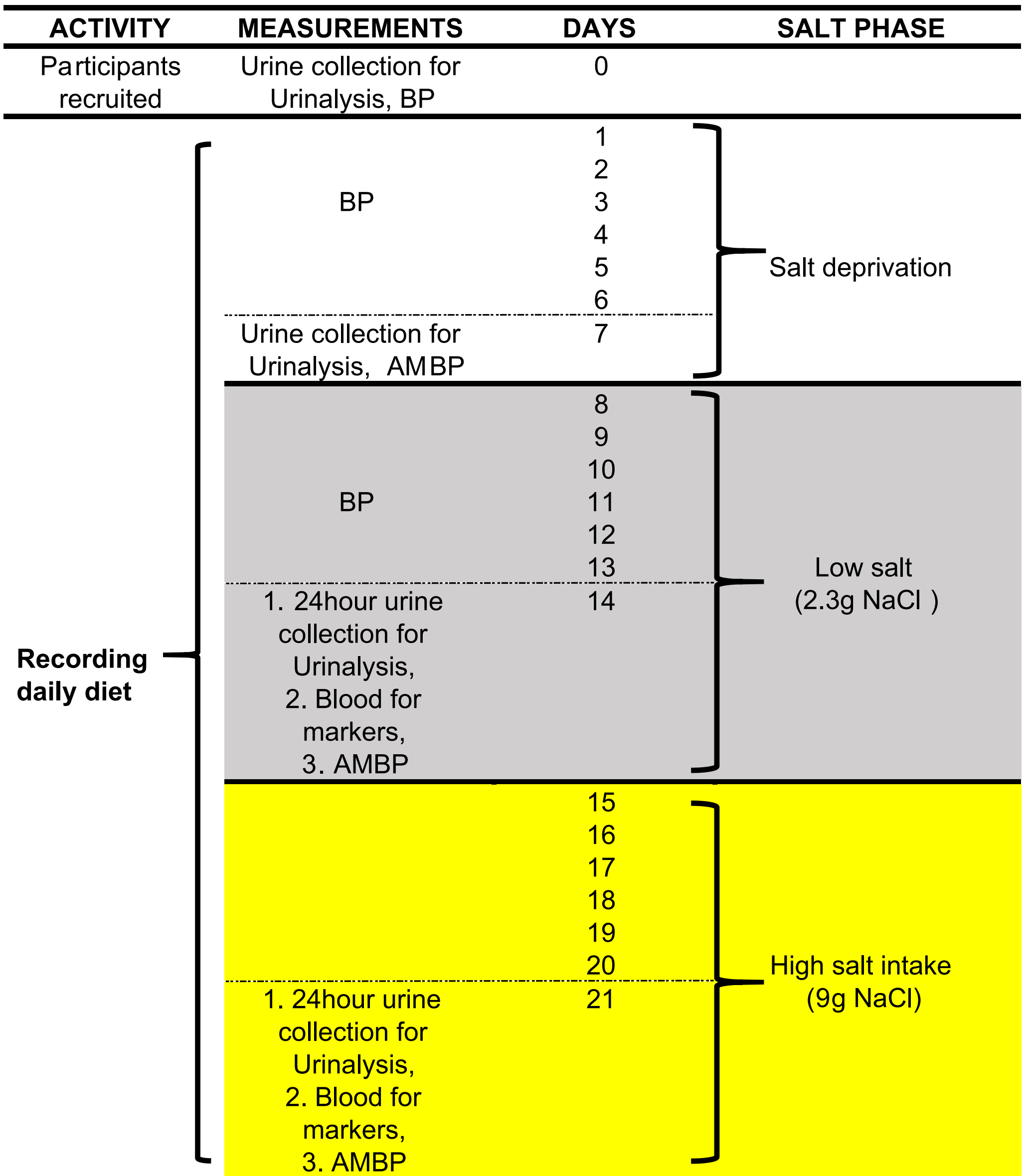

Fig. 1 In the salt deprivation phase, Participants will be recruited on day 0, urine sample collected and requested to avoid adding salt to their food or consume processed foods that contain salt for the next 7 days. In the low salt phase, participants will be provided with 2.3 g of sodium everyday apportioned in three parts to add to their meals. In the high salt phase participants will be provided with 9 g of dietary salt and split as previously described above. Blood pressure (BP) will be measured everyday (day 0 to 21) between 17:00 and 19:00 h or between 06:00 and 08:00 h. Ambulatory blood pressure (AMBP) will be measured on days 7, 14 and 21. A 24-h urine will be collected on days 14 and 21 for urinalysis

the laboratory and sodium measured. An average sodium excretion will be calculated and used as a surrogate of dietary intake.

Procedure for aim 2 Blood measurements will be conducted three times (Fig. 1) at baseline (day 7 before low salt intake is commenced), day 14 and on day 21 (last day of high salt intake). Heparinized blood specimens will be collected to compare IsoLGs, inflammatory markers (IL-17A, IL-6, TNF-α, IFN-γ) and immune activation markers (CD80, CD86, CD83) in all four groups. Daily BPs will be collected and a 24-h BP on the last day of each phase.

Laboratory procedures and flow cytometry analysis are elaborated in Additional file 1.

Statistical analysis

We will use FlowJo software (Tree Star, Inc.) for flow cytometry data analysis. STATA version 15 or SPSS version 22 and Graph pad prism version 8 will be used for statistical inferences and to determine if dietary salt is associated with inflammation and/or immune activation in HIV. Analysis of variance or the Kruskal–Wallis test to compare pro- and anti-inflammatory cytokines and cells, activation markers and hypertension status in all four groups will be employed followed by the Dunnett post hoc test for pairwise comparisons or the Tukey test to compare all the groups with the control (HIV negative normotensive) group. We will also compare the means of the markers of immune cell activation between salt-sensitive and salt-resistant subjects, dichotomized by a drop of ≥ 10 mmHg in SBP owing to salt loading, as described above. For this purpose, we will carry out unpaired t-tests for normally distributed data or Mann–Whitney tests for non-normally distributed data.

Regression models will be used to examine the relationship between outcome variables and selected determinant factors, to determine the impact of each variable on the outcomes and to control for confounding.

Discussion

Murine model and few human studies have shown that excess dietary salt induces BP elevation and activates cells of the innate and adaptive immune system [14, 20]. The interaction between dietary salt, BP and immune activation is a fairly new concept with recent evidence that salt can accumulate in skin without commensurate water retention and induce inflammation [1]. Furthermore, high consumption of dietary salt correlates positively with BP and is associated with the development or exacerbation of hypertension [21–23].

Previous studies have found that dendritic cells (DCs) accumulate isolevuglandin (IsoLG)-protein adducts during hypertension [4]. IsoLGs are highly reactive products of oxidation of fatty acids that rapidly adduct to lysines on proteins and their accumulation is associated with DC activation [4]. Recent studies have established that elevated Na+ is a potent stimulus for IsoLG-protein adduct formation in murine DCs [20]. Na+ enters DCs through amiloride sensitive transporters. Intracellular Na+ is exchanged for calcium (Ca2+) via the Na+/Ca2+ exchanger. Ca2+ activates protein kinase C (PKC) which in turn phosphorylates the NADPH oxidase subunit p47phox. This leads to activation of the NADPH oxidase, increased superoxide (O2·−) and IsoLG-protein adduct formation [20]. Adoptive transfer of salt-exposed DCs primes hypertension in response to a sub-pressor dose of angiotensin II and IsoLG-protein adduct formation is absent in mice lacking the NADPH oxidase and pharmacological scavenging of IsoLGs prevents DC activation, hypertension and end-organ damage [4, 20] (Fig. 2).

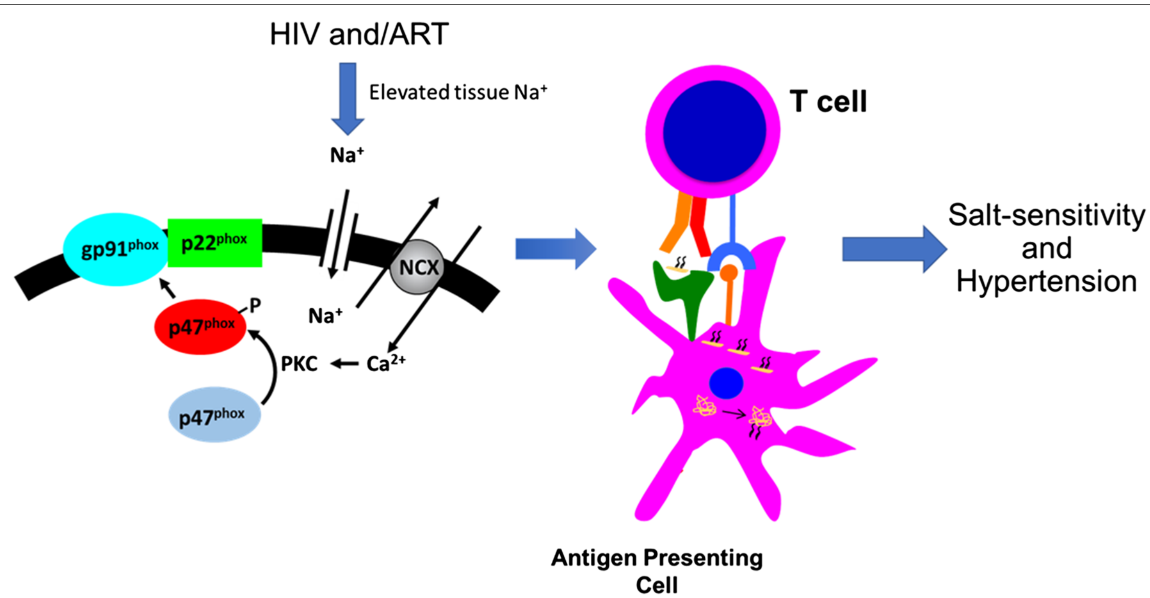

Fig. 2 HIV and Salt-sensitive hypertension working Hypothesis. We hypothesize that HIV infection and treatment can lead to increased accumulation of sodium in tissues. This can activate the immune system leading to hypertension. Sodium enters antigen presenting cells and is exchanged for calcium through the sodium hydrogen exchanger 1. Calcium activates protein kinase C (PKC), which then activates NADPH oxidase by phosphorylating its subunit $p47^{phox}$. This leads to increased production of superoxide with subsequent formation of immunogenic isolevuglandin (IsoLG)-protein adducts. IsoLGs activate DCs which in turn promote T cell proliferation and production of cytokines that contribute to salt-sensitive hypertension [4]

These studies in mice suggest that the immune system can exhibit salt-sensitivity, however the role of Na+ in activating human antigen presenting cells such as monocytes, and the interplay between elevated Na+ and salt sensitivity, has not been defined in the context of HIV. Sodium intake is positively associated with BP and accounts for much of the age-related increase in BP [24, 25]. Independent of other factors, excess intake of salt is associated with an increased risk of stroke [26, 27], cardiovascular disease and other adverse outcomes including death [28]. However, the effects of salt on BP may vary among individuals due to salt-sensitivity and salt resistance [26, 29]. This study will identify patients with or without salt-sensitive hypertension among HIV-negative and HIV-positive participants and examine levels of IsoLG-protein adducts and the activation markers of monocytes.

There are no studies known to us that have studied salt-sensitive hypertension and immune activation in PLWH. With this novel perspective, it is expected that PLWH may exhibit increased salt-sensitivity and immune activation compared to HIV negative individuals. This study has the potential to create points of intervention that will improve the management of hypertension and prevent dietary salt-related hypertension in future studies. Moreover, there is evidence that in the general population of Zambia, salt consumption exceeds that recommended by WHO [22]. This study is therefore, of clinical interest.

Strengths of the study

1. This is likely, the first study known to us that will explain or report a possible relationship between HIV, immune activation and salt-sensitive hypertension.
2. Generally, salt consumption in Africa and Zambia is higher than recommended. With increasing incidence of hypertension, knowledge about a possible interaction in this research is of clinical interest.

Limitations

- Sodium excretion exhibits circaseptan rhythm and infra-radian rhythm depending on salt intake, increases or decreases in intake affects BP, However, a 24-h urine recovers about 95% of dietary salt intake.
- Though there are no established criteria for salt-sensitivity, we employ already published methods with maximal controls to avoid possible confounders. Information generated will provide baseline data for hypotheses generation for future planned studies.
- The underlying factors associated with HIV, immune activation and salt-sensitive hypertension are multifactorial, many factors not studied are likely to confound the results of the study. However, the experimental nature of the study will minimize confounding.
- This proposed study cannot determine causality and exhaust the mechanism and possible interactions between salt, immune system and HIV. Hence, further studies are warrantable.

Additional file

Additional file 1. Laboratory procedures.

Abbreviations

AMBP: ambulatory blood pressure; ART: antiretroviral therapy; BMI: body mass index; BP: blood pressure; CRP: C-reactive protein; DC: dendritic cell; DMSO: dimethyl sulfoxide; ECG: electrocardiogram; GM-CSF: granulocyte macrophage stimulating factor; HISH: HIV, Immune-activation and salt-sensitive hypertension study; FMO: flow minus one; HEPES: 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HIV: human immunodeficiency virus; IFN-γ: interferon gamma; IL: interleukin; IsoLG: isolevuglandins; LCH: Livingstone Central Hospital; MIFlowCyt: Minimum Information about a Flow Cytometry Experiment; PBMC: peripheral blood mononuclear cells; PLWH: people living with HIV; PMA: phorbol-12-myristate 13-acetate; PKC: protein kinase C; RPMI: Roswell Park Memorial Institute; sCD14: soluble CD14; TNF-α: tumor necrosis factor alpha; UVP: University of Zambia Vanderbilt partnership; WB: whole blood; WC: waist circumference; WHO: World Health Organizations; WHR: waist-to-hip ratio.

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Authors' contributions

SKM and AK conceived the study. SKM, BMH, SN, GK, DCH, WM, JRK, AK and SMM contributed to the writing of the manuscript. SKM is the principal investigator and guarantor. All authors provided feedback. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article. For other data, these may be requested through the corresponding author.

Ethics approval and consent to participate

Ethical approval was obtained from the University of Zambia Health Sciences Research Ethics Committee (UNZAHSREC) (Assurance no. FWA00026270, IRB no. 00011000) on the 2nd April 2019. Permission to conduct the study was granted by the Livingstone Central Hospital Administration. All participants will be asked to consent by signing a consent form before being included in the study. All data collected will de-identified and used for research purposes only.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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INFLAMMATION AND CARDIOVASCULAR DISEASES (A KIRABO, SECTION EDITOR)

Patho-immune Mechanisms of Hypertension in HIV: a Systematic and Thematic Review

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Abstract

Purpose of Review To systematically review recent findings on the role of immune cell activation in the pathogenesis of hypertension in people living with HIV (PLWH) and compare studies from Sub-Saharan Africa with what is reported in the USA and European literature according to guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses.

Recent Findings PLWH have an increased risk for development of hypertension and cardiovascular disease. Chronic immune activation contributes to hypertension but the inflammatory milieu that predisposes PLWH to hypertension is poorly understood. We identified 45 relevant studies from 13 unique African countries. The prevalence of hypertension in PLWH on antiretroviral therapy (ART) and the ART-naive PLWH ranged from 6 to 50% and 2 to 41%, respectively. Interleukin (IL)-17A, interferon (IFN)-γ, and higher $CD4^+$ T cell counts were associated with hypertension in ART-treated participants.

Summary Targeting adaptive immune activation could provide improved care for hypertensive PLWH. Further research is needed to characterize the inflammatory milieu contributing to hypertension in PLWH especially in African populations where the global burden of HIV is the highest.

Keywords HIV · Hypertension · Inflammation · Patho-immune mechanisms

Introduction

The introduction of antiretroviral therapy (ART) has improved survival among people living with HIV (PLWH), but this success is accompanied by a high burden of cardiovascular disease [1–3]. Hypertension is a major risk factor to cardiovascular disease and its prevalence is higher in PLWH despite viral suppression by ART [4]. The mechanisms contributing to hypertension and cardiovascular disease in PLWH are not fully understood.

A heightened systemic inflammatory process including activation of the innate and adaptive immune systems contributes to the development of hypertension in the general population and in experimental animal studies [5–7]. However, it is

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unknown which innate and adaptive immune factors are most closely linked to hypertension in PLWH. At present, much of our understanding of the role of immune cell activation in the pathogenesis of hypertension comes from experimental animal and human studies in the HIV-negative context, but similar inflammatory biomarkers implicated in these HIV-negative studies have also been associated with hypertension in HIV infection. In this review, we present the current understanding on HIV, hypertension, and immune activation and how these may be related and highlight gaps and the need for further studies. We review studies conducted in Sub-Saharan Africa where the global HIV infection burden is the highest. We also performed a comparative analysis contrasting studies from Sub-Saharan Africa with what is reported in the USA and European literature.

The aim of our current study was to estimate the prevalence of hypertension in PLWH from African studies, identify inflammatory markers or markers of immune activation associated with hypertension in antiretroviral (ART)-treated HIV-positive individuals, and to explore the possible mechanistic interaction between immune activation, hypertension, and HIV in cohorts from Sub-Saharan Africa and contrast with European and US cohorts.

Methods

We followed guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [8, 9].

Eligibility Criteria for Studies Included in the Review We considered studies on HIV-positive human subjects with or without the HIV-negative population as controls. For exposure and outcome variables, we considered HIV infection as the exposure and our outcome measures were hypertension or blood pressure, inflammation, and/or immune activation. Only published articles in peer-reviewed journals were considered. Articles in languages other than English were not considered. We only included studies from Sub-Saharan African, USA, and European HIV-positive populations that reported on hypertension and HIV excluding studies that did not report HIV at all. We also excluded studies reporting opportunistic infections and other coinfections to reduce bias when deducting on the contribution of HIV on hypertension. In case of duplicate publications, the article with more complete data was included. Extracted information focused on study design, population, sample size, country where study was conducted, and details of findings with conclusions. The source of research articles was derived from Pubmed, Medline, google scholar, and google search. Dates of coverage were not specified to filter studies addressing hypertension and inflammation or immune activation in HIV owing to the scarcity of published studies. Other articles were identified from reference lists of related studies from the included study. Search terms that were used in databases employed the use of Boolean operators "AND," "OR," and "NOT" to refine our search by combining or limiting terms. The key terms used to search for articles were "hypertension," "blood pressure," "immune activation," "inflammation," "Africa," and "HIV."

Studies were screened based on abstract information that reported HIV or/and inflammation or immune activation and hypertension or blood pressure. A full article was then obtained and screened to ensure eligibility. The PRISMA 2009 Flow Diagrams for article identification, screening, eligibility, and inclusion in Sub-Saharan African and the Western populations are shown in Supplementary Figures 1 and 2 found at the following link: https://figshare.com/s/38ff86137d00153423ac. For data extraction, we used a data collection form that identifies the author, study, population, sample size, methods, findings, and limitations.

Risk of bias was assessed by two reviewers. For the criteria used to assess internal validity of included studies, we made use of the Cochrane Risk of Bias tool, paying attention especially to parts such as completeness of outcome data, selective outcome reporting, reporting bias, and any other bias that may affect the exposure-outcome relationship. For any disagreements between the reviewers over the risk of bias in particular studies, this was resolved by discussion, with involvement of a third review author where necessary.

Data Synthesis We did not conduct a meta-analysis. We only conducted a systematic thematic review due to the qualitative nature of our study and due to excessive heterogeneity of population, outcome, or methodology. To assist with the result and discussion write-ups, we constructed summary tables and provided a narrative synthesis (thematic or content analysis). This includes investigation of the similarities and the differences between the findings of different studies, as well as exploration of patterns in the data. Reasons for similarities and differences in the findings were also explored systematically. Studies were grouped based on reported parameters. We also considered how the results of studies might be affected by factors such as methodological differences, variable characteristics of the populations studied, or interventions investigated. Conceptual models were used to explore relationships and patterns from study findings and we related the findings to existing concepts from animal models to generate hypotheses and the need for further studies.

Results

Characteristics of the Studies We performed a systematic review of studies on HIV, hypertension, and immunity from countries in Sub-Saharan Africa, USA, and Europe. For Sub-Saharan Africa studies shown in Table 1, we included

Table 1 Characteristics of studies included in the Sub-Saharan African studies

Study characteristics	Number (*n*) or percentage (%)
Number of studies included	45
Number of African countries where the studies were conducted	13
Number of types of studies	3
Studies reporting on inflammation *plus* hypertension *plus* HIV only	5
Studies reporting on hypertension *plus* HIV only	35
Studies reporting on HIV *plus* immune activation/inflammation	5
Studies reporting on inflammation *plus* hypertension in African populations	0
Overall prevalence of HTN in PLWH on ART (% range)	6.4–50.2
Overall prevalence of HTN in ART-naive PLWH (% range)	2–41
Overall prevalence of HTN in HIV-negative population (% range)	13.7–44
HTN prevalence percentage differences within studies, *range* (│ *magnitude*│)	
PLWH on ART *minus* ART naive	− 12.3 to 23.4 (│ 35.7│)
PLWH on ART *minus* HIV negative	− 6.3 to 12.4 (│ 18.7│)
HIV negative *minus* ART naive	− 5.3 to 11 (│ 16.3│)

HTN, hypertension; HIV, human immune deficiency virus; ART, antiretroviral therapy; PLWH, people living with HIV

45 studies from 13 countries. Of the 45 studies, 27 were cross-sectional, 15 were prospective, and three were retrospective. Most of the studies (35) reported on both hypertension and HIV. The prevalence of hypertension was highest in the HIV-positive group on ART (ranging from 6.4 to 50.2%) followed by HIV-negative controls (13.7 to 44%). ART-naive patients recorded the lowest prevalence ranging from 2 to 41% by group comparisons. The prevalence differences of groups within studies were highest between ART treated and ART-naive HIV-positive groups (35.7) followed by ART treated and HIV-negative group (18.7) with the least difference in magnitude between HIV negative and ART-naive groups.

When we included USA and European studies, shown in Supplementary Table 1 found at the following link: https://figshare.com/s/38ff86137d00153423ac, the global prevalence of hypertension in PLWH ranged from 4 to 57% (van Ziest et al. 2017) [10]. Hypertension was more prevalent in HIV uninfected (71%) versus HIV infected (57%) in a large longitudinal study by Armah et al. [11]. HIV-infected veterans with HIV-1 RNA ≥ 500 copies/mL or CD4 count < 200 cells/μL had a significantly higher prevalence of elevated IL-6 and D-dimer after adjusting for comorbidities and had significantly higher prevalence of elevated sCD14 compared to uninfected veterans. Manner et al. [12] reported prevalence of 35% hypertension in HIV-infected individuals and the prevalence did not change during the follow-up time (3.4 ± 0.8 years). CD4 T cell count < 50 cells/μL and increased duration of ART were independent predictors of sustained hypertension throughout the study period. Older age, male gender, BMI > 25 kg/m^2, and baseline CD4 cell count ≥ 200 cells/μL were also independent predictors of sustained hypertension. Markers of microbial translocation predicted hypertension in HIV-infected individuals. Manner et al. found that both LPS and sCD14 independently predicted subsequent blood pressure levels after adjustment for age and gender [12]. These results suggest that ART may act as a contributing factor to inflammation and the increased prevalence of hypertension in the PLWH.

Higher Levels of IL-17A, IFN-γ, and CD4$^+$ T Cells Are Associated with Hypertension in HIV We found that among studies reporting on both inflammation, immunity, and hypertension in HIV (Table 2), higher levels of IL-17A, IFN-γ, [13••], and CD4$^+$ T cell count (Peck et al.) [4] were significantly associated with hypertension in ART treated HIV-positive individuals. The cross-section study by Chepchirchir et al. reported that females were more likely to have higher IL-17A levels than males and IL-17A was affected by BMI but not stress levels, ART, World Health Organization (WHO) stage, and CD4$^+$ count [13••]. However, inflammatory cytokines IL-2, IL-6, IL-8, tumor necrosis factor alpha (TNF-α), and anti-inflammatory cytokines IL-4 and IL-10 were not associated with hypertension. The cross-section study by Peck et al. reported higher cases of hypertension among PLWH on ART [28.7% (43/150)] compared to ART-naive participants [5.3% (8/151)]; however, the prevalence was higher in HIV-negative patients [16.3% (25/153)] compared to ART-naive HIV-positive patients. The inflammatory markers, C-reactive protein (CRP), and IL-6 in the prospective study by Fourie et al. where the three participant groups had comparable blood pressures did not differ between HIV positive on ART, ART naive, and HIV-negative groups. In another prospective study by Okello et al., blood pressure increases were reported in the first 6 months of ART initiation then plateaued. Traditional risk factors including older age, male gender, African-American, higher body mass index (BMI), central obesity, previous cardiovascular events,

Table 2 Characteristics and findings of studies reporting on inflammation, hypertension, and HIV

Author/	Type of study, country, and population	Sample size and subjects	Key findings	Limitations/notes/conclusion
Chepchirchir et al. 2018 [13••]	Cross-section study conducted in Kenya among PLHIV	126 HIV-positive with and without hypertension	• HTN prevalence was 23.2% • IL-17A was associated with hypertension in HIV {HTN 75.51 ± 84.89 versus normotensives 42.23 ± 64.40; CI 6.81–59.77, $p < 0.001$ and females were more likely to have higher IL-17A levels than males in HIV ($p < 0.001$) • IL-17A was affected by BMI $r(124) = 0.223$ $p = 0.070$ but not stress levels, ART, WHO stage and CD4+ count • IFN-γ {HTN 6.87 ± 35.66; normotensives 0.00 ± 0.00 CI − 1.96–15.71, $p = 0.003$} correlated negatively with hypertension status ($r_s = -0.217$, $p < 0.015$) • IL-4,-2,-6,-8,-10, TNFα were not associated with hypertension	• Monitoring and analysis of cytokine expression may help to predict patients' pathways in their response to cART therapy and risk of metabolic disorders • IFNγ is potentially useful in determining risk of developing hypertension in this population • Mechanism of interaction between hypertension and inflammation or immune activation not very explicit
Peck et al. 2014 [14••]	Cross-sectional study conducted in Tanzania among PLHIV	454 participants: HIV+ on ART, ART-naive HIV+ and HIV-negative controls	• Prevalence of HTN in HIV negative was 16.3% (25/153) • Prevalence of HTN in HIV+ on ART was 28.7 (43/150) was the highest ($p = 0.01$) among the groups with higher odds HTN even after adjustment (odds ratio (OR) = 2.19 (1.18 to 4.05), $p = 0.01$) • Prevalence of HTN in ART-naive HIV+ was 5.3% (8/151), and lowest among group ($p = 0.003$) with lower odds of HTN even after adjustment (OR = 0.35 (0.15 to 0.84), $p = 0.02$) • The prevalence HTN was lowest in group with the lowest average CD4+ T cell count (HIV-infected ART naive) and highest in the group in which the CD4+ T cell count had been low and had then been reconstituted in the setting of ART • In the HIV+ group, higher CD4+ T cell counts were associated with more hypertension and higher blood pressures • Age, vigorous work, current alcohol use, and BMI were all associated with hypertension • Only PI use was associated with HTN • Among the HIV-infected adults with HTN, 75% were undiagnosed, 85% were untreated, and > 95% were uncontrolled	• HIV-infected adults with hypertension were rarely aware of their diagnosis but often have evidence of kidney disease • It was unknown whether chronic inflammation accelerates HTN • High prevalence of hypertension among HIV-infected adults on ART could be related to dysregulated inflammation due to immune reconstitution • Mechanism of interaction between hypertension and inflammation or immune activation not explicit
Fourie et al. 2015 [15]	Prospective study conducted in South Africa	309 participants: 66 HIV+ on ART, 78 (ART-naive HIV+ and165 HIV-negative controls	• The inflammatory markers (CRP and IL-6) did not differ between the three groups • Endothelial activation was not accompanied by increased inflammation • BP comparable among groups	• Mechanism of interaction between hypertension and inflammation or immune activation not explicit • Several cytokines not reported
Okello et al. 2016 [16]	Prospective cohort study conducted in Uganda	536 HIV positive initiating ART	• Systolic BP increased by 9.6 mmHg/year (95% CI 7.3–11.8) in the first 6 months of ART, then plateaued • Male gender, overweight, and a CD4 count < 100 cells were associated with incident hypertension	• Blood pressure increases early after ART initiation in Ugandans. Traditional risk factors, rather than immune activation were associated with incident hypertension in this population • Only sCD14, sCD163 (immune activation markers), and IL-6 (inflammatory marker) were examined

Table 2 (continued)

Author/	Type of study, country, and population	Sample size and subjects	Key findings	Limitations/notes/conclusion
				• Mechanism of interaction between hypertension and inflammation or immune activation not explicit
Borkum et al. 2017 [17]	Cross-sectional study conducted among South African blacks	67 HIV-positive participants on ART > 5 years	• Prevalence of non-dipping BP was 65% • High levels of inflammation (hsCRP) • There was no association on multivariable analysis between dipping status and traditional risk factors for non-dipping BP, such as obesity, autonomic dysfunction, and older age	• 91% ($n = 61$) were females • Inflammation was only assessed using hsCRP • Mechanism of interaction between hypertension and inflammation or immune activation not explicit

PLHIV, people living with HIV; HTN, hypertension; HIV, human immunodeficiency virus; BP, blood pressure; CRP, C-reactive protein; ART, antiretroviral therapy; PI, protease inhibitors; IL, interleukin; BMI, body mass index; CI, confidence interval; TNFα, tumor necrosis factor alpha; IFNγ, interferon gamma; WHO, World Health Organization; cART, combinational ART; sCD14, soluble CD14; sCD163, soluble CD163; hsCRP, high-sensitivity C-reactive protein

chronic kidney disease, family history of hypertension and cardiovascular disease, diabetes, and dyslipidemia rather than immune activation were associated with incident hypertension in this study. Borkum et al. reported high levels of inflammation and non-dipping blood pressure [14••].

Prevalence of Hypertension Is Higher in ART-Treated PLWH As shown in Table 3, hypertension prevalence was mostly higher in men than women [15–17] and higher among ART treated versus ART naive (28.7% vs 5.3%; 17% vs 2%; 30 vs 21.9% and 38% vs 19%, respectively) [17, 18]. However, one study [22] reported contradictory findings (ART vs ART naive, 12.3 vs 19%). In another study, [17] reported higher prevalence of hypertension in ART treated group (28.7%) compared to the HIV-negative controls (16.3%) but two studies [22, 25] reported the opposite (41% vs 44% and 12.3% vs 13.7%, respectively). Peck et al. reported higher prevalence of hypertension in HIV negative vs ART-naive HIV-positive group (16.3% vs 5.3%) [17]. On the contrary, Ogunmola et al. reported lower prevalence of hypertension in HIV negative vs ART-naive HIV-positive group (13.7% vs 19%, respectively). Association between hypertension and traditional risk factors including specific ART regimens varied between studies.

ART Treatment in PLWH Is Associated with Higher Inflammation As shown in Table 4, we found that PLWH on ART had higher inflammatory markers including IL-6, CRP, intracellular adhesion molecule 1 (ICAM-1), and vascular adhesion molecule 1 (VCAM-1) compared with HIV-negative individuals (Fourie et al.) [55]. Intermediate monocytes ($CD14^{+}16^{+}$) increased with viremia in immune-compromised patients and microbial translocation was a major force driving chronic systemic inflammation in HIV-positive individuals on ART.

Discussion

In the current study, we found that the prevalence of hypertension in PLWH in Sub-Saharan African countries ranged from 2.0 to 50.2% with most cases among those receiving ART. Besides traditional risk factors and the effect of ART on blood pressure, IL-17A, IFN-γ, and $CD4^{+}$ T cells were among the inflammatory parameters associated with hypertension in ART treated PLWH [13••, 56]. The mechanism of interaction between immune activation or inflammation and hypertension in HIV remains elusive and warrants further studies. Our findings and conceptual hypothesis of how immune activation may contribute to hypertension in HIV is shown in Fig. 1. We propose that HIV viral protein and ART interacts with components of the immune system to synergistically induce kidney damage, vascular dysfunction, alterations in sympathetic nervous outflow and lead to hypertension. This process is exacerbated by presence of traditional risk factors exacerbates including obesity, excess dietary salt intake, smoking, and genetic predisposition.

Prevalence of Hypertension in HIV

The prevalence of hypertension in PLWH varies by population and subgroups even within the same countries. Similar to our findings, Martin-Iguacel et al. and Nguyen et al. reported prevalence ranging from 4 to 54% [57••] and 8.7 to 45.9% among PLWH in low- and middle-income countries [58]. However, in our study, we further segregated prevalence by HIV infection and ART treatment and report magnitude of differences between the groups (Table 1). Higher hypertension prevalence was reported in ART treated PLWH compared with ART-naive participants except in the study by Ogunmola et al. who reported the opposite [22]. This

Table 3 Characteristics and findings of studies reporting on hypertension and HIV

Author/	Type of study, country and population	Sample size and subjects	Key findings	Limitations/notes/conclusion
Bloomfield et al. 2011 [18]	Retrospective study conducted in Kenya among PLWH	12,194 HIV-positive participants	• HTN prevalence among men 11.2% and women 7.4%, overall 8.7% • Age, overweight/obesity, longer duration on PI were not associated with HTN • HTN more common in younger HIV vs older • Higher HTN cases associated with patients with higher CD4 in men than women but blunted HTN occurred with older age	Immune mechanisms not addressed explicitly
Julius et al. 2011 [19]	Cross-sectional study conducted in South Africa among PLWH on ART	304	Prevalence of HTN was 19.1%. 23.9% in men and 17.7% in women (95% confidence interval (CI) 14.7–23.5), $p = 0.10$)	Immune mechanism not addressed
Diouf et al. 2012 [20]	Cross-section study conducted in Senegal among PLWH	242 HIV	• 28.1% had hypertension • ART duration not associated with HTN • Longer exposure to LPV/r was associated with a reduced risk of hypertension	Higher hypertension observed for male patients with CD4 count above 200 cells/μL, but differences were not statistically significant
Ngatchou et al. 2013 [21]	Cross-sectional study conducted in Cameroon among PLWH	204 participants consisting of 108 HIV ART naive vs 96 HIV negative	Prevalence of HTN in HIV was 41% and HIV negative 44%	Immune mechanism or inflammation as it relates to hypertension was not addressed
Parikh et al. 2013 [22]	Cross-sectional study conducted in Uganda and Zimbabwe among PLWH	3316	Prevalence of systolic and diastolic hypertension was 21.3% and 19.0% for older adults; and 9.2% and 3.5% for younger adults with HIV (both, $p < 0.001$)	Immune mechanism or inflammation as it relates to hypertension was not addressed
Ekali et al. 2013 [23]	Cross-sectional study conducted in Cameroon among PLWH	143	SBP and DBP increased with duration on ART. HTN was associated with longer duration on ART	Immune mechanism or inflammation as it relates to hypertension was not addressed
Muhammad et al. 2013 [24]	Cross-sectional study conducted in Nigeria among PLWH	200 HIV+ ART and ART-naive participants	HTN prevalence was 17% in ART and 2% in ART naive ($p < 0.001$) HAART was associated with HTN	Immune mechanism or inflammation as it relates to hypertension was not addressed
Mateen et al. 2013 [25]	Cross-sectional study conducted in Uganda among PLWH initiating ART	5563	HTN prevalence was 27.9%	Immune mechanism or inflammation as it relates to hypertension was not addressed
Botha et al. 2014 [26]	Prospective study conducted in South Africa among PLWH	137 participants: 66 HIV+ on ART and 71 HIV+ ART-naive participants	HIV+ on ART had higher pulse pressure (13.3%; $p = 0.004$), systolic blood pressure (4.5%; $p = 0.029$), and CD4 cell count (9.2%; $p = 0.009$) levels over 5 years	Immune mechanism or inflammation as it relates to hypertension was not addressed
Ogunmola et al. 2014 [27]	Cross-section conducted in Nigeria among PLWH	403 participants. Groups: HIV-negative controls, HIV+ on ART, and ART-naive HIV+	• Prevalence was 13.7% in HIV negative, 19.0% in HIV+ ART naive, and 12.3% in HIV-positive ART subjects • Multivariate regression analysis showed no relationship between hypertension and HIV status ($p = 0.293$) or ART status ($p = 0.587$) but only with BMI	Immune mechanism or inflammation as it relates to hypertension was not addressed
Shaffer et al. 2014 [28]	Prospective study; randomized, open-label ART trials among	741	Over 144 weeks NVP compared to LPV/r group had greater mean	Immune mechanism or inflammation as it relates to

Table 3 (continued)

Author/	Type of study, country and population	Sample size and subjects	Key findings	Limitations/notes/conclusion
	7 African countries (South Africa; Kenya; Zimbabwe, Botswana, Zambia, Malawi, and Uganda)/population included HIV+ women only with immunocompromised CD < 200		increases in BP (diastolic BP 22.7% vs. 6.5%)	hypertension was not addressed
Sawadogo 2014 [29]	Cross-sectional study conducted in Burkina Faso among PLWH on ART	400 participants	Hypertension prevalence was 12.0%	Immune mechanism or inflammation as it relates to hypertension was not addressed
Kagaruki et al. 2014 [30]	Cross-section study conducted in Tanzania	671 participants HIV+ on ART and ART naive	• The prevalence of hypertension was 26.2% and was high among those on ART (30.0% vs 21.9%, $p = 0.010$) • Aged > 40 years (AOR = 2.52, 95% CI 1.37–4.63), abnormal waist circumference (AOR = 2.37 95% CI 1.13–5.00), overweight/obesity (AOR = 2.71, 95% CI 1.26–5.84), and male sex (AOR = 1.17, 1.02–4.20) were the predictors of hypertension among patients on ART while raised total cholesterol (AOR = 1.47 (1.01–2.21) and being aged > 40 years (AOR = 3.42, 95% CI 2.06–5.70) were predictors for hypertension among ART-naive patients	Immune mechanism or inflammation as it relates to hypertension was not addressed
Abrahams et al. 2015 [31]	Prospective study from South Africa in HIV+ women	103 participants	• Systolic and diastolic blood pressure increased significantly and the proportion of participants with hypertension increased from 3.9 to 15.5% ($p < 0.001$) • Long-term exposure to ART, increased in hypertension • Stavudine and efavirenz and nevirapine were significantly associated with hypertension	Immune mechanism or inflammation as it relates to hypertension was not addressed
Okello et al. 2015 [32]	Prospective study in Uganda	3389 participants; HIV+ initiating ART	• 13% incidence of HTN • Male gender (AHR 1.88, 95% CI 1.49–2.39), increasing age (AHR 1.36, 95% CI 1.02–1.82 for those > 40 years compared to those aged 30 years or less), nadir CD4 count (AHR 0.77, 95% CI 0.60–0.99 and AHR 0.64 95%CI 0.41–1.00 for a nadir CD4 cell count 100–350 and > 350 cells/mm^3 compared to < 100 cell/mm^3, respectively), and high baseline body mass index (AHR 2.50, 95% CI 1.56–4.01 for those with a BMI ≥ 30 kg/m^2 versus normal BMI) were independently associated with increased risk of hypertension • Neither use of TDF versus AZT, nor use of NVP versus EFV was associated with risk of hypertension in the multivariate model	• Results are the first to document high rates incident hypertension during longitudinal follow-up among initially normotensive PLHIV initiating ART • Particularly notable was the magnitude of hypertension incidence in younger groups • Potential mechanisms for increased incidence of hypertension postulated in this paper include HIV-associated chronic inflammation, immune suppression, endothelial activation, and dysfunction, as well as the direct infection of arterial vascular smooth muscle cells by HIV • Alternatively, incident hypertension in this setting

Table 3 (continued)

Author/	Type of study, country and population	Sample size and subjects	Key findings	Limitations/notes/conclusion
			• Inverse relationships between hypertension risk and CD4 count nadir. Data suggest that immunosuppression and/or viral burden play a role in promoting early vascular damage as evidenced by the association of a low CD4 cell count with subclinical atherosclerotic damage, a processor of hypertension	may reflect a "return to health" as individuals gain weight during ART, particularly with advanced pre-ART disease stages Limitation: smoking status and laboratory results of blood glucose, serum creatinine, serum electrolytes, and serum lipids were not available for most patients; therefore, the contribution of clinical factors known to be associated with hypertension such as diabetes mellitus, chronic kidney disease, and hyperlipidemia could not be assessed
Dimala et al. 2016 [33]	Cross-sectional study conducted in Cameroon among PLWH	200 HIV+ ART and ART-naive participants	• Prevalence of HTN in patients on HAART was twice (38%; 95% CI 28.5–48.3) that of the HAART-naive patients (19%; 95% CI, 11.8–28.1), $p = 0.003$ • CD4 cell count (mean ± SD, cells/μL) of ART 501 ± 225 was higher than the ART naive 197 ± 160 < 0.001 • HTN was associated with older age and male gender, in the HAART group	Immune mechanism or inflammation as it relates to hypertension was not addressed
Feigl et al. 2016 [34]	Prospective study from South Africa among PLWH	505 participants: HIV+ on ART, ART-naive HIV+ and HIV-negative controls	• DBP increased in ART naive but SBP reduced compared to the HIV-negative group • SBP in the HIV+ on ART− group showed a significant decline	They could not control for a range of potential time-variant confounders in their analysis due to lack of data
Kalyesubula et al. 2016 [35]	Retrospective study from Uganda among PLWH	1996 HIV+ on ART and ART-naive participants	• Prevalence of hypertension was 20.9% (418/1996) rising from 16.9% in 2009 to 32.3% in 2013 • Patients > 50 years of age had 3.12 times the odds of hypertension compared with patients aged 20–29 years (95% CI 2.00 to 4.85) • Men had 1.65 times the odds of hypertension compared with women (95% CI 1.34 to 2.03) and patients with a BMI of 35–39 kg/m^2 had 3.93 times the odds of hypertension compared with patients with a BMI < 25 kg/m^2 • Patients with a WHO disease staging of 3 or 4 had 0.60 times the odds of hypertension compared with patients with stage 1 or 2 (95% CI 0.46 to 0.76)	Analysis did not distinguish ART and ART-naive patients as this can confound results
Kwarisiima et al. 2016 [36]	Cross-sectional study from Uganda	94,274 participants HIV+ and HIV negative	• Hypertension prevalence was 11% among HIV-positive individuals • 79% of patients were previously undiagnosed, 85% were not taking medication, and 50% of patients on	Immune mechanism or inflammation as it relates to hypertension was not explicitly addressed

Table 3 (continued)

Author/	Type of study, country and population	Sample size and subjects	Key findings	Limitations/notes/conclusion
			medication had uncontrolled blood pressure • Multivariate predictors of hypertension included older age, male gender, higher BMI, lack of education, alcohol use, and residence in Eastern Uganda • HIV-negative status was independently associated with higher odds of hypertension (OR 1.2, 95% CI 1.1–1.4). Viral suppression of HIV did not significantly predict hypertension among HIV positives • The prevalence of HTN was greater among HIV-negative adults (14%) than among HIV-positive adults (11%) • Among HIV-positive adults with HTN, 20% reported prior knowledge of HTN, and 14% reported taking medication • Similar to the overall population, 46% of HIV-positive adults with HTN achieved BP control with antihypertensive medications	
Njelekela et al. 2016 [37]	Cross-sectional study from Tanzania among PLWH	34,111 HIV-positive ART naive only	• Prevalence of hypertension was found to be 12.5% • Risk of hypertension was 10% more in male than female patients • Patients aged ≥ 50 years had more than 2-fold increased risk for hypertension compared to 30–39-year-old patients • Overweight and obesity were associated with 51% and 94% increased risk for hypertension compared to normal weight patients • Low $CD4^+$ T cell count, advanced WHO clinical disease stage, and history of TB were associated with 10%, 42%, and 14% decreased risk for hypertension	Immune suppression and history of TB were associated with lower risk for hypertension
Osegbe et al. 2016 [38]	Cross-section study from Nigeria	283 HIV+ on ART, ART naive and HIV-negative participants	• SBP in HIV+ ART was higher (124.9 ± 20.7 mmHg) compared to HIV+ ART naive which was higher than (121.5 ± 20.7) HIV negative (114.8 ± 11.7 $p = 0.001$) • hsCRP = 2.9 (1.4–11.6), $p = 0.002$ were higher among the HIV-naive subjects • Higher prevalences of the risk factors were noted among the HIV-treated subjects except low HDL-C ($p < 0.001$) and hsCRP ($p = 0.03$) which were higher in the HIV-naive group	Immune mechanism or inflammation as it relates to hypertension was not addressed; however, hsCRP was higher among ART-naive patients
Divala et al. 2016 [39]	Cross-section study from Malawi	952	• Prevalence was 23.7% (95%-confidence interval	Immune mechanism or inflammation as it relates to

Table 3 (continued)

Author/	Type of study, country and population	Sample size and subjects	Key findings	Limitations/notes/conclusion
		HIV+ (95% were on ART and 5% ART naive)	21.1–26.6; rural 21.0% vs. urban 26.5%; $p = 0.047$) • Hypertension diagnosis was associated with increasing age, higher body mass index, presence of proteinuria, being on regimen zidovudine/lamivudine/nevirapine and inversely with World Health Organization clinical stage at ART initiation	hypertension was not addressed
Magodoro et al. 2016 [40]	Cross-section study conducted in Zimbabwe among PLWH	1033 participants	• Hypertension prevalence was 10.2%	Studies had other substantial burden of comorbid non-communicable diseases among HIV-infected patients in a high HIV and low-income setting
Nduka et al. 2016 [41]	Prospective study from Nigeria	303 participants—229 on ART and 74 ART naive	In this propensity score-matched sample, the estimated average treatment effects on the treated (ATT) for the effects of antiretroviral therapy on systolic (7.85 mmHg, 95% CI 3.72 to 15.68) and diastolic blood pressure (7.45 mmHg, 95% CI 4.99 to 13.61) were statistically significant ($p < 0.001$ for each)	There is a high probability that the epidemiological association between antiretroviral therapy and increased blood pressure be causal in nature among people living with HIV in Sub-Saharan African settings
Bauer et al. 2017 [42]	Prospective study conducted in Lusaka, Zambia among PLWH	896 cohort HIV+ on ART participants	• 98 (10.9%) had HBP, and 57 (6.4%) had HTN • Increasing age (10-year increase in age: adjusted odds ratio [AOR] = 1.50; 95% confidence interval [CI] 1.20–1.93), male sex (AOR = 2.33, 95% CI 1.43–3.80), and overweight/obesity (AOR = 4.07; 95% CI 1.94–8.53) were associated with HBP • Among 66 patients with HBP, 35 (53.0%) reported awareness of the condition • Pre-ART $CD4^+$ count was not associated with HBP HBP tends to become more common after ART initiation and may cause HTN	Coinfection with HBV, HBP was defined as a single elevated measurement, and BP was measured only once at each time point. This may have inflated the number of patients truly needing treatment, as only 58% of patients with HBP were confirmed to have HTN These data demonstrate that although integration of BP screening and management in HIV care settings was feasible in Zambia, virtually no patient had optimal management of HBP
Kazooba et al. 2017 [43]	Cross-section study from Uganda	1024 HIV+ ART	• Hypertension prevalence was 22.6% • Protease inhibitor (PI) containing regimens were significantly associated with, hypertension • Men had significantly higher mean SBP ($p = 0.004$) • Increasing age was significantly associated with higher means of SBP, DBP • Increasing intensity of physical activity was significantly associated with lower; SBP, DBP	Immune mechanism or inflammation as it relates to hypertension was not addressed
Mayanja et al. 2017 [44]	Prospective study from Uganda	1095 HIV+ on ART individuals	• Patients on non-PI regimens had higher mean diastolic hypertension than patients on PI regimens, $p < 0.001$	Immune mechanism or inflammation as it relates to hypertension was not addressed

Table 3 (continued)

Author/	Type of study, country and population	Sample size and subjects	Key findings	Limitations/notes/conclusion
			• Prevalence of hypertension was 14.5%	
Okpa et al. 2017 [45]	Cross-section study from Nigeria	112 HIV+ and 309 HIV-negative participants	• The prevalence of HTN was 19.5% among HIV-infected persons • The prevalence of pre-HTN in HIV-positive and HIV-negative participants was 34.5% and 38.9%, respectively • The higher prevalence of HTN in HIV patients has been shown to be associated with the use of ART • In our study, the risk factors that were associated with HTN in HIV-infected patients and the HIV-N controls were older age, increased weight and BMI, and presence of obesity. Male sex was associated with HTN in the HIV-infected population, but this was, however, not so in the general population • Male sex and duration of exposure to ART and CD4 count levels > 200 cells/mm^3 were associated with HTN in HIV-infected patients, whereas the absence of family history of HTN was significantly associated with HTN in both groups • The mean age, weight, and BMI were significantly higher in HIV patients with HTN as compared to those without HTN, $p < 0.05$ • A proportion of HIV patients with a family history of HTN and obesity had HTN, and these were statistically significant ($p < 0.05$) • Also, the mean waist-hip ratio, duration of illness, exposure to ART, and CD4 count levels were higher in HIV patients with HTN as compared to those without HTN, but these were not statistically significant	Diet and sodium intake of the participants and renal function of the participants which have been shown in several studies to have effect on BP were not looked at in our study Immune mechanism or inflammation as it relates to hypertension was not addressed
RodrõÂguez-Arbolõ et al. 2017 [46]	Prospective study from Tanzania	955 HIV+ initiating ART	• 111 (11.6%) were hypertensive at recruitment • 80 (9.6%) of them developed hypertension during a median follow-up of 144 days from time of enrolment into the cohort [incidence rate 120.0 cases/1000 person-years, 95% confidence interval (CI) 97.2 ± 150.0]	Traditional cardiovascular risk factors predicted incident hypertension, but no association was observed with immunological or ART status. These data support the implementation of routine hypertension screening and integrated management into HIV programs in rural Sub-Saharan Africa
Isa et al. 2017 [47]	Prospective study from Nigeria/	Follow-up from initiating HIV+ ART and naive	• Prevalence of hypertension at enrolment was 19.3% (95% CI 17.6–20.9%) • Age ($p < 0.001$), male sex ($p = 0.004$), and body mass index (BMI) ($p < 0.001$) were	Hypertension is common in HIV-infected individuals attending the HIV clinic. Patients initiating ART have a high risk of developing

Table 3 (continued)

Author/	Type of study, country and population	Sample size and subjects	Key findings	Limitations/notes/conclusion
			independent risk factors for hypertension • 12 months after initiating ART, a further 31% (95% CI 17.6–20.9%) had developed hypertension. Total prevalence at that point was 50.2% • Hypertension among those on ART was associated with age ($p = 0.009$) and BMI ($p = 0.008$), but not with sex • There were no independently significant associations between hypertension and $CD4^+$ counts, viral load, or type of ART	hypertension in the first year of ART
Magande et al. 2016 [48]	Case control study from Zimbabwe among HIV+ on ART	300 (152 controlled HTN, 152 uncontrolled HTN)	• Adding salt to dishes regularly AOR = 5.69 (3.19–10.16), body mass index (BMI) above 25 kg/m^2 AOR = 2.81 (1.60–4.91) and history of elevated blood pressure in previous year AOR = 2.34 (1.33–4.13) were independent risk factors for uncontrolled hypertension • Independent protective factors were duration more than 2 years since HIV diagnosis AOR = 0.58 (0.35–0.95), duration less than 5 years since hypertension diagnosis aOR = 0.50 (0.30–0.83) and walking or cycling as a means of transport AOR = 0.27 (0.16–0.48)	Salt HTN interaction attributed to water retention resulting in high intravascular volumes. Immune mechanism or inflammation as it relates to hypertension was not addressed
Chepchirchir et al. 2018 [13••]	Cross-section study from Kenya among HIV+	297	The prevalence of hypertension was found to be 23.2%	Hypertension is a highly prevalent comorbidity in HIV patients. The risk factors include prolonged use of ART as well as increased body mass index. The effects of hypertension on HIV progression include low $CD4^+$ T cell counts which complicate the underlying immunosuppression
Okello et al. 2017 [49]	Cross-section study from Uganda among PLWH	577 HIV-infected and 538 matched HIV-uninfected participants	• HIV infection was associated with 3.3 mmHg lower systolic BP (1.2–5.3 mmHg), 1.5 mmHg lower diastolic BP (0.2–2.9 mmHg), 0.3 m/s lower pulse wave velocity (0.1–0.4 mmHg), and 30% lower odds of hypertension (10%–50%) • Body mass index mediated 25% of the association between HIV and systolic BP	HIV infection was inversely associated with systolic BP, diastolic BP, and pulse wave velocity Immune mechanism or inflammation as it relates to hypertension was not addressed
Mukeba-Tshialala et al. 2017 [50]	Cross-section study from the Republic of Congo	592 HIV-uninfected and 445 HIV-infected patients	• 11.5% of HIV-infected patients had an average blood pressure suggesting hypertension versus 10.6% of HIV uninfected ($p = 0.751$) • But in absolute value, HIV-infected patients had a median of diastolic	Immune mechanism or inflammation as it relates to hypertension was not addressed

Table 3 (continued)

Author/	Type of study, country and population	Sample size and subjects	Key findings	Limitations/notes/conclusion
			blood pressure of 90 mmHg versus 85 mmHg of HIV uninfected ($p < 0.001$)	
Mathabire Rücker et al. 2018 [51]	Cross-section from Malawi among PLWH	379 HIV-infected patients on ART and 356 controls participated	• Among HIV patients, the prevalence of hypertension was 19.5% (95% CI 15.6–23.6), of which 60.3% (n = 44) was previously undiagnosed • Among controls, 25.8% (95% CI 21.6–30.7) prevalence of HTN and 37.0% of controls with elevated blood pressure	Immune mechanism or inflammation as it relates to hypertension was not addressed

DBP, diastolic blood pressure; SBP, systolic blood pressure; PLWH, people living with HIV; HTN, hypertension; HIV, human immunodeficiency virus; BP, blood pressure; ART, antiretroviral therapy; PI, protease inhibitors; BMI, body mass index; CI, confidence interval; cART, combinational ART; TDF, Tenofovir; AZT, Zidovudine; NVP, Nevirapine; EFV, Efavirenz; HAART, highly active antiretroviral therapy; LPV/r, lopinavir/ritonavir; TC, total cholesterol; HDL-c, high-density lipoprotein cholesterol

contradictory finding is likely due to differential effects of specific ART regimens and other traditional risk factors differing between study populations. The effect of specific regimens on blood pressure has not yet been well established except for the low to moderate increase attributed to non-nucleoside reverse transcriptase inhibitors (NNRTI's) and protease inhibitors (PIs) [59, 60]. Prior studies have shown that patients become hypertensive in most cases at least after 2 years of ART and systolic pressure increases further after 5 years of ART [60], and that there is no association between HIV status, ART, and hypertension following short-term follow-up of less than 2 years [22, 61].

Markers of Immune Activation or Inflammation Associated with Hypertension in HIV

Traditional risk factors associated with hypertension in HIV such as older age, male gender, African-American, higher BMI, central obesity, previous CV events, chronic kidney disease, family history of hypertension and CVD, diabetes, and dyslipidemia have been well studied [57••, 58]. It is also well established that HIV infection and exposure to ART (more than 2 years), through metabolic disturbances and endothelial dysfunction, might have an additional role in the development of hypertension in HIV patients [57••, 58]. However, little is known about the contribution of the innate and adaptive immune system in the development or propagation of hypertension in HIV.

In our systematic review, we found that an improved immune status as determined by higher CD4 T cells was associated with higher hypertension prevalence [17]. However, one study be Okello et al. found that CD4 T cell count of less than 100 was associated with incident hypertension [61]. Martin-Iguacel et al. and Nguyen et al. also reported similar findings [57••, 58]. We found that T cell-derived cytokines IL-17A and IFN-γ were associated with hypertension in ART treated PLWH as reported by Chepchirchir et al. [13••]. These cytokines have been reported to contribute to the genesis of hypertension in the HIV-negative general population and experimental animal studies [6]. Further studies are needed to ascertain the contribution of CD4 T cells and their cytokines IL-17 and IFN-γ in concert with existing traditional risk factors on blood pressure elevation in HIV.

Immune Activation, Inflammation, and Hypertension

The role of innate and adaptive immunity including specific cell types and cytokines in the development and maintenance of hypertension has been extensively studied in humans and animal models elsewhere [6, 7, 62•, 63–65]. Using multiple experimental animal models, studies have shown that hypertensive stimuli including angiotensin II, aldosterone, endothelin-1, and salt induce activation of immune cells, which infiltrate the vasculature and the kidneys, and release cytokines that induce increased salt and water retention leading to hypertension [6]. This process is mediated in part by increases oxidative stress leading to oxidation of fatty acids and formation of isolevuglandins (IsoLGs) in antigen-presenting cells. IsoLGs activate these cells to produce pro-inflammatory cytokines IL-6, IL-1β, and IL-23, express costimulatory proteins CD80 and CD86, and activate T cells to produce pro-hypertensive cytokines IFN-γ and IL-17A [7]. In humans, plasma isoprostanes, which are produced in concert with IsoLGs, are elevated in hypertension and markedly elevated in patients with resistant hypertension, and IsoLGs are markedly elevated in circulating monocytes of hypertensive patients [7]. IL-17A and IFN-γ have been implicated in the genesis and maintenance of hypertension due in part to

Table 4 Studies on HIV and immune activation/inflammation

Author/	Type of study/populations/country/study period	Sample size/methods	Key findings	Limitations/notes
Fourie et al. 2011 [52]	Sub-study nested in the larger longitudinal, multinational PURE study in South Africa among PLWH/HIV+ and HIV−	600 HIV+ and HIV-negative controls	• HIV+ showed higher IL-6, CRP, ICAM-1, and VCAM-1 levels compared to HIV negative • Accelerated vascular aging and probable early atherosclerosis in the older HIV-infected participants • HIV-1 Tat protein (therefore HIV itself) induced the expression of ICAM-1 and VCAM-1	Immune mechanism or inflammation as it relates to hypertension was not addressed
Cassol et al. 2010 [53]	Cross-section study conducted from South Africa	90 participants 10 HIV-negative controls, 20 HIV+ on ART, and 60 ART-naive HIV+	• $CD14^{+}16^{+}$ positively correlated with HIV-1 viremia • IL-6 increased in presence of OPIs • sCD14 and TNF correlated with plasma LPS levels and remained elevated in ART • Microbial translocation is a major force driving chronic inflammation in HIV-infected Africans receiving cART	All participants were immunocompromised with CD4 counts of < 200 cells/μL Immune mechanism or inflammation as it relates to hypertension was not addressed Prevention of monocyte activation may be especially effective at enhancing therapeutic outcomes
Fourie et al. 2011 [52]	Cross-section study from South Africa	300 ART-naive HIV+ vs 300 HIV negative	• Suggestion of inflammatory injury of the endothelium • Indication of accelerated vascular aging and probable early atherosclerosis in the older HIV-infected participants	Contribution of innate and adaptive immune system were not included or addressed
Canipe et al. 2014 [54]	Zambia/pilot study 12 months prospective	33 HIV+ on ART for 12 months	• Biomarkers of increased microbial translocation (lipopolysaccharide binding protein, endotoxin core IgG and IgM, and soluble CD14) were associated with high levels of systemic inflammation before and after initiation of ART, suggesting that impaired gut immune defenses contribute to innate immune activation in this population	All the HIV-infected adults in the study were undernourished
Siedner et al. 2016 [55]	Prospective study from Uganda	105 HIV positive initiating ART	• Persistent immune activation (sCD14 level and kynurenine-tryptophan ratio) and inflammation (IL-6) despite ART-mediated viral suppression predicted future atherosclerotic burden among HIV-infected Ugandans	Immune mechanism or inflammation as it relates to hypertension was not addressed

PLWH, people living with HIV; HIV, human immunodeficiency virus; CRP, C-reactive protein; ART, antiretroviral therapy; IL, interleukin; TNFα, tumor necrosis factor alpha; cART, combinational ART; sCD14, soluble CD14; sCD163, soluble CD163; VCAM-1, vascular cell adhesion molecule 1; ICAM-1, intercellular adhesion molecule 1

their direct effect in causing endothelial dysfunction and renal damage [6, 66]. Thus, similar immuno-pathophysiological mechanisms underlying hypertension in the general population may also contribute to elevated blood pressure in PLWH.

Potential Mechanisms Leading to Inflammation in HIV

The pathophysiology of HIV-related hypertension seems to emerge from three factors: HIV-related inflammation, HIV-related proteins, and genetic predisposition [59]. It is believed that the HIV viral proteins (negative factor (Nef), transcription proteins (Tat) and glycoprotein 120 (gp-120)) induce hypertension through vascular oxidative stress, smooth myocyte proliferation and migration, and endothelial dysfunction especially in patients with high HIV viral load [59]. Tat, a transactivator protein for HIV replication, is known to be secreted extracellularly by infected cells and has been shown to activate endothelial cells by increasing expression of endothelial-leukocyte adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin which induce initial binding of leukocytes to the blood vessel wall [67]. The levels of soluble ICAM-1 concentration correlate with HIV

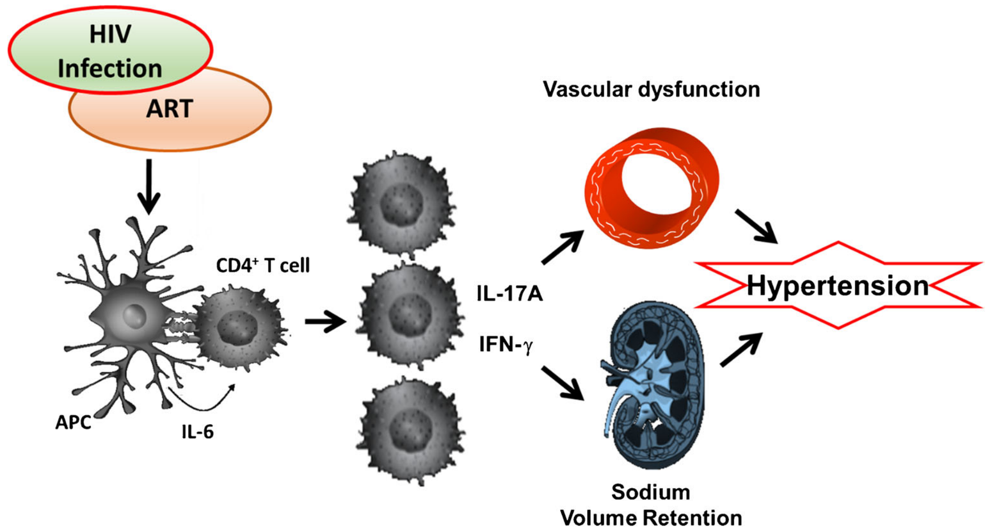

Fig. 1 Conceptual schematic of the effect of HIV infection and treatment can activate the immune system leading to hypertension. Viral proteins and/or antiretroviral therapy (ART) activates antigen presenting and T cells which infiltrate the vasculature and the kidneys and release cytokines IL-6, IL-17A, and IFN-γ which promote vascular dysfunction, retention of sodium, and water, leading to hypertension

disease as well as reduction in CD4 count. Tat also induces IL-6 production which increases endothelial permeability [67]. Tat can also suppress the bone morphogenic protein receptor 2 (BMPR-2) responsible for regulating endothelial cell proliferation and survival. This results in increased vascular smooth muscle proliferation and activation of endothelial cells leading to pulmonary arterial hypertension (PAH) [68]. In HIV-positive patients, Tat has been shown to increase the transcription of IL-17 and secretion by T cells causing a pro-inflammatory milieu and has been associated with a devastating immune reconstitution inflammatory syndrome (IRIS) in the brain [69]. The HIV Gp120 is found on the surface of the HIV envelope and can also be found in circulation from viral turn over [70]. Gp120 stimulates monocytes and macrophages to release pro-inflammatory cytokines, promotes an increase in markers of apoptosis, and stimulates the secretion of endothelin-1, a potent vasoconstrictor [68]. It induces endothelial apoptosis by interacting with CXCR4 also expressed on endothelial cells [70]. Gp120 and TNF-α synergistically decrease eNOS and NO in human coronary artery endothelial cells [70].

DCs, the most potent antigen-presenting cells of the innate immune system, have been shown to interact with components of HIV-1 both intrinsically (following fusion of cellular and viral membrane) and extrinsically (prior to being infected) through pathogen recognition receptors (PRRs) resulting in activation of the adaptive immune system [71]. Through their CD28/B7 ligands CD80 and CD86 (also expressed on monocytes and B cells), DCs provide a costimulatory signal necessary for T cell activation and survival. In hypertension, there is an increase in B7 ligand expression leading to T cell activation [5, 72]. An important element in the activation of T cells by DCs and other cells in hypertension is antigen presentation. It is unclear what antigens are presented to T cells in HIV and it is not known if IsoLG-adducted peptides might play a role in hypertension associated with HIV.

Monocyte activation has an important role in HIV-infected persons on ART. Monocytes are chronically activated during HIV infection and it is now evident that inflammatory mediators produced by monocytes (especially IL-6), independent of T cell activation, also predict serious non-AIDS events (SNAEs) in virologically suppressed HIV-infected persons treated with ART [73]. IL-6 production was higher in monocytes than other cells and was associated with increased odds of SNAE and mortality but not the percentage of activated CD4 and CD8 T cells (those expressing CD38 or CD38 and HLA-DR) [73]. Infected monocytes, especially intermediate monocytes expressing $CD14^+$ and $CD16^+$, produce more pro-inflammatory cytokines as compared to their counterpart. They adhere to endothelial cells and transmigrate into the sub endothelial area where their pro-inflammatory activity is increased. It is evident that in HIV, monocytes and macrophages have a reduced phagocytic activity and they age prematurely [70]. Monocytes/macrophages have been implicated in the pathogenesis of hypertension in the general population and experimental animal models [5].

Several mechanisms of immune cell activation have been reported in HIV. First, there is direct binding of the HIV envelope protein gp120/160 to CD4 and/or C-C chemokine receptor type 5 (CCR5) which down-modulates the expression of $CD3^+$ T cell receptor in infected cells resulting in cell death; second, there is an interaction between HIV viral particles and plasmacytoid dendritic cells (DCs) through Toll like receptor (TLR) stimulation which activates $CD8^+$ cytotoxic cells; third, there is microbial translocation from a leaky gut into the circulation leading to a generalized systemic inflammatory process [74]. Also important is the dysfunction of T regulatory cells and immune-senescence resulting in an uncontrolled over activation of immune cells in HIV [74]. The resultant low-grade chronic inflammation which persists even after initiation of ART is associated with hypertension and cardiovascular disease [75, 76].

IL-6 production by monocytes and macrophages is increased following HIV infection and is a valuable prognostic marker for disease progression [77, 78]. Soluble CD14 (sCD14) produced from monocytes and macrophages is also elevated in HIV-infected persons regardless of treatment status and is an independent predictor of mortality [73]. Anzinger et al. reported that increased microbial translocation from the gut resulting from depletion of $CD4^+$ T cells in acute HIV infection results in monocyte activation and higher sCD14 [73]. The expression of programmed death-1 (PD-1), a member of the B7:28 inhibitory molecules, is rapidly increased on $CD4^+$ and $CD8^+$ T cells, B cells, natural killer cells, and monocytes as they interact with PD-L1, its ligand on antigen-presenting cells such that engagement on virus-infected cells leads to impaired generation of effector T cell responses [74]. Other markers which have been associated with immune activation in HIV include increased expression of $CD28^-$, $CD57^+$ and reduced expression of CD127 on $CD4^+$ and $CD8^+$ T cells [79, 80].

In our analysis, we found HIV infection alone is associated with an inflammatory milieu involving higher levels of IL-6, CRP, VCAM-1 ICAM-1, and intermediate monocytes ($CD14^+16^+$) among studies conducted in Sub-Saharan African cohorts [17, 55]. There is a generalized systemic immune activation following HIV infection which subsides but still persists at low-grade or subclinical following initiation of ART involving activation of both $CD4^+$ and $CD8^+$ T cells expressing high levels of CD38 [74]. Other T cell activation markers such as human leukocyte antigen–antigen D related (HLA-DR) as well as increases in pro-inflammatory cytokines tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6), and interleukin 1 beta (IL-1β) have been reported in PLWH in the western countries [74]. Increased expression of CD38 alone or in concert with $HLA\text{-}DR^+$ in T cells is a marker of disease progression and mortality in some cases correlating directly with HIV viral load and indirectly with $CD4^+$ count [74]. This continuous immune activation and inflammation has been proposed to contribute to endothelial dysfunction, hypertension, and cardiovascular disease [81].

Limitations, Conclusion, and Future Perspectives

Almost all studies except one [13••] were not designed to compare blood pressure or hypertension and inflammation in PLWH as the primary outcomes. There is need for additional prospective and mechanistic studies to establish the relationship between inflammation and hypertension in HIV infection, and ART. Most studies addressing the mechanistic role of inflammation in the genesis and progression of hypertension have been conducted in murine models and humans in the HIV-negative context. The inflammatory milieu in HIV infection is obviously complex and very different from the general population and further studies are needed to determine the specific contribution of the various attributes in HIV including the viral proteins and ART on inducing inflammation associated with hypertension.

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Compliance with Ethical Standards

Conflict of Interest The authors declare no conflicts of interest relevant to this manuscript.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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