Investigating platelet and endothelial activation in ART-treated women living with HIV and obesity

By

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PREFACE

This study described in this thesis was carried out by Ms Snenhlanhla A. Mfusi and has not been submitted in any other form to another University. This study was carried out in the Discipline of Human Physiology, School of Laboratory Medicine and Medical Sciences, College of Health Sciences, University of KwaZulu-Natal, Durban, South Africa, under the supervision of Prof. B. B Nkambule.

Snenhlanhla Mfusi  Date _____

(216015242)

Prof. B. B. Nkambule _  Date _____

Dr Sherika Hanley  Date _____

DECLARATION

I, Ms Snenhlanhla A. Mfusi declares that:

1. The work described in this study has not been submitted to UKZN or any other tertiary institution for purposes of obtaining an academic qualification, whether by myself or any other party.

2. The thesis does not contain another person's writing, data, pictures, or other information unless specifically acknowledged as being sourced from other persons or researchers. Where other written sources have been quoted then:

Their words have been re-written, but the general information attributed to them has been referenced.

Where their exact words have been used, then it has been properly referenced in the reference section.

3. Sig  … Date……..

DEDICATION

I would love to dedicate this to:

God, the Almighty, who provides.

My dearest mother – You have been nothing but supportive to me throughout. Your prayers, encouragements and believing in me have kept me going. I hope this made you proud.

My family and friends – Thank you for the continuous support and providing strength for me to carry on

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- My parents for believing in me and constant reminder of my dreams. I am who I am because of your love and support
- My friends and colleagues for their positive attitude, love and pleasant company.

ABSTRACT

Background: Antiretroviral therapy (ART) has reduced morbidity and mortality in people living with Human immunodeficiency virus (PLWH). However, metabolic and thrombotic complications have now become prevalent in the aging population of PLWH. The spectrum of cardiovascular disease in patients with HIV is broad and the mechanisms underlying the risk of cardiovascular disease (CVD) in PLWH remains complex and multifactorial. This includes an interplay between traditional risk factors such as obesity which in the general population is more prevalent in women. This study aimed to assess the association between platelet activation, endothelial activation and CVD-risk in women living with HIV.

Methods: In this study we included 66 female participants living with HIV (n=33 normal weight and n=33 overweight/obese) enrolled in the prospective multi-country PEPFAR PROMise Ongoing Treatment Evaluation (PROMOTE) study from the Umlazi clinical research site. The time of blood draws ranges from December 2018- November 2019. We measured the levels of high sensitivity c-reactive protein (hsCRP), lipid profiles, platelet activation (P-selectin, CD36 and platelet factor-4) and markers of endothelial activation (endothelin-1, von Willebrand factor).

Results: Women living with HIV(WLHIV) and obesity showed significantly elevated levels of soluble CD36 4.36[2.71-9.53] when compared to the control group 2.79[2.24-3.55], p=0.0064. Furthermore, the levels of (vWF) were elevated in WLHIV and obesity 8.83[1.59-9.78] when compared to controls 5.34[0.65-7.7] p=0.0009. However, the levels of soluble P-selectin, platelet factor-4 (PF4) and endothelin-1 (ET-1) were comparable between two study groups (p>0.05). Lastly, the levels of hsCRP levels were significantly higher in WLHIV and obesity (7.71±9.95) when compared to controls (3.68±5.89) p= 0.0005.

Conclusion: The levels of platelet and endothelial activation are elevated in WLHIV and obesity despite successful ART. Moreover, the levels of inflammation remain persistently high even during ART. Therefore, WLHIV and obesity are at an increased risk of developing CVD.

Keywords: Cardiovascular disease, obesity, platelet activation, endothelial activation, antiretroviral therapy

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LIST OF ABBREVIATIONS

AIDS	Acquired immunodeficiency syndrome
ACC/AHA	American College of Cardiology/American Heart Association
ART	Antiretroviral therapy
ASCVD	Atherosclerotic Cardiovascular Disease Risk Score
BMI	Body Mass Index
BREC	Biomedical Research Ethics Committee
cART	combined antiretroviral therapy
CHD	Coronary heart disease
CS	Cross sectional
CVD	Cardiovascular diseases
CVE	Cardiovascular Event
D:A:D	Data Collection on Adverse Events of Anti-HIV Drugs
ELISA	Enzyme-linked immunoassay
ET-1	Endothelin-1
FRS	Framingham risk score
HAART	Highly active antiretroviral therapy
HCV	Hepatitis C virus
HDL-c	High-density lipoprotein cholesterol
HIV	Human immunodeficiency virus
hs-CRP	High-sensitivity C-reactive protein
ICAM-1	Intercellular adhesion molecule-1
IL-6	Interleukin-6
IScHeMiA	Integration of cardiovascular disease SCreening and prevention in the HIV Management plan for women of reproductive age
LDL-c	Low-density lipoprotein cholesterol
LDMS	Laboratory Data Management System
MI	Myocardial infarction
MCP-1	Monocyte chemoattractant protein-1
MIP-1	Macrophage inflammatory protein-1
MetS	Metabolic syndrome
NCD	Non-communicable disease
NNRTI	Non-nucleoside reverse transcriptase inhibitors
PAF	Platelet-activating factor
PCO	Prospective cohort

PI	Protease inhibitors
PICOTS	Population, Index prognostic factors, Comparator, Outcome, Timing and Setting
PLWH	People living with HIV
PRISMA	Preferred Reporting Items for Systematic review and Meta-analyses
PROCAM	Prospective Cardiovascular Münster study
PROMOTE	PROMise Ongoing Treatment Evaluation
QUIPS	Quality in Prognostic Studies
RCO	Retrospective cohort
SANAS	South African National Accreditation System
sCD40L	soluble CD40 ligand
SCORE	Systematic Coronary Risk Evaluation
SMART	Strategies for Management of Anti-Retroviral Therapy
TC	Total cholesterol
TG	Triglycerides
TNF	Tumor necrosis factor
V-CAM	Vascular cell adhesion molecule
vWF	von Willebrand factor
WHO	World Health Organization
WLHIV	Women living with HIV

THESIS STRUCTURE

The thesis is structured in the following manner:

Chapter 1: Introduction

Chapter 2: A systematic review and meta-analysis, "**Cardiovascular risk factors in antiretroviral therapy-treated patients living with HIV and obesity: A systematic review and meta-analysis of prognostic factors**"

Chapter 3: An experimental paper, "**Platelet activation and cardiovascular-risk in antiretroviral therapy-treated women living with HIV and obesity**"

Chapter 4: Synthesis

CHAPTER 1: INTRODUCTION

The incidence of human immunodeficiency virus (HIV) infections remains a significant challenge in developing countries (1). In the past decade, considerable efforts have been made towards increasing the roll-out and access to antiretroviral therapy (ART) have yielded a significant reduction in acquired immunodeficiency syndrome (AIDS)-related mortality and an overall improvement in the quality of life in people living with HIV (PLWH) (2,3). However, an increasing incidence of noncommunicable disease (NCD) has emerged in the ageing population of PLWH on ART (4,5). Data from the Strategies for Management of Antiretroviral Therapy (SMART) study has demonstrated an association between markers of inflammation and adverse outcome i.e. cardiovascular diseases (CVD) in ART-treated patients living with HIV (6). This implies that an increased level of inflammation is associated with HIV and platelets are reported to be key role players in the development of inflammation (7).

Platelets are essential effector cells in haemostasis that play a pivotal role in pathological thrombosis, coagulation, inflammation and orchestrate innate and adaptive immune responses (8,9). Activated platelets express surface P-selectin (CD62P) and are found at the sites of inflammation. Activated platelets release pro-inflammatory cytokines that increase the inflammatory response (10,11). Several studies have previously demonstrated that PLWH show increased platelet activation (12–17). In addition, increased plasma levels of inflammatory biomarkers such as C-reactive protein (CRP), and interleukin-6 (IL-6) and D-dimer are elevated in patients with CVD-related conditions (i.e. atherosclerosis) and may have a role in predicting the CVD risk profile (18).

A previous study reported that the platelet-activation factor (PAF) is associated with an increased cardiovascular risk (19). Platelet-activating factor is a potent lipid mediator of inflammation that has immunomodulatory effects and a pivotal role in the pathogenesis of inflammatory disorders and cardiovascular disease. Limited scientific evidence suggests that the platelet-activating factor pathway may be a mechanistic link between HIV-1 infection, systemic inflammation, and immune activation that contribute to pathogenesis of chronic HIV-related complications such as CVD (19). The role of platelets in the inflammatory and coagulation process has been reported (20). However, the relevance of this association remains unclear in the context of HIV. There are few studies that have assessed *ex vivo* platelet function in HIV-infected patients on ART (21,22).

Problem statement

A previous meta-analysis comprised of 800 000 PLWH, including studies with 3-4 years of follow-up period reported on CVD incidence of 62 events per 10,000 person-years, with a risk ratio of 2.16 when compared to uninfected controls (23,24). In Africa, the fraction of CVD attributable to HIV infection ranges from 0.36 to 0.92 percent (25). When national estimates of prevalence and cardiovascular burden were observed in 154 countries, the highest population attributable fraction was observed in countries

within sub-Saharan Africa, with HIV accounting for more than 15% of the cardiovascular burden in Swaziland, Botswana, Lesotho and South Africa (25). A previous study reported on an increased incidence of cardiovascular diseases; including acute myocardial infarction (MI), stroke, and atherosclerosis, in patients with HIV compared with the uninfected individuals (25). The relative risk for CVD increased in patients with HIV over the age of 45 years (25). Other studies have shown that the risk of MI alone is elevated in patients with HIV across a wide range of ages. Moreover, a linear association between age, duration of ART and CVD mortality has been reported (26). Furthermore, previous studies from Kenya have reported a nearly 8-fold increase in the prevalence of obesity in women compared to men (27–30). Although platelet function and endothelial dysfunction are implicated in the development of CVD in HIV-infected patients on ART, the precise role of activated platelets in CVD-risk of PLWH and obesity remains unclear.

Aim of this study

I) To evaluate the association between platelet activation, and endothelial activation and cardiovascular risk in ART-treated women living with HIV and obesity.

Objectives

i) To determine the levels of soluble P- selectin and soluble CD36 in ART-treated women living with HIV and obesity.

ii) To determine the levels of von Willebrand Factor (vWF) and Endothelin-1 (ET-1) in ART-treated women living with HIV and obesity.

iii) To determine CVD risk in women living with HIV and obesity using the FRS and D:A:D risk scores.

References

1. UNAIDS epidemiological estimates. 2020;
2. Forsythe SS, McGreevey W, Whiteside A, Shah M, Cohen J, Hecht R, et al. Twenty years of antiretroviral therapy for people living with hiv: Global costs, health achievements, economic benefits. Health Aff. 2019;38(7):1163–72.
3. Cohort CAT. Life expectancy of individuals on combination antiretroviral therapy in high-income countries: a collaborative analysis of 14 cohort studies. Lancet. 2008;372(9635):293–9.
4. Patel P, Rose C, Collins P, Nuche-Berenguer B, Al E. HHS Public Access Author manuscript AIDS. Author manuscript; available in PMC 2019 February 19. Noncommunicable diseases among HIV-infected persons in low- income and middle-income countries: a systematic review and meta-analysis. Aids. 2018;32(32):S5–20.
5. Jespersen NA, Axelsen F, Dollerup J, Nørgaard M, Larsen CS. The burden of non-communicable diseases and mortality in people living with HIV (PLHIV) in the pre-, early- and late-HAART era. HIV Med. 2021;1–13.
6. El-Sadr WM, Lundgren JD, Neaton JD, Gordin F, Abrams D, Arduino RC, et al. CD4+ count-guided interruption of antiretroviral treatment. N Engl J Med. 2006 Nov;355(22):2283–96.
7. Arman M, Payne H, Ponomaryov T, Brill A. Role of Platelets in Inflammation.
8. Semple JW, Italiano JEJ, Freedman J. Platelets and the immune continuum. Nat Rev Immunol. 2011 Apr;11(4):264–74.
9. Vieira-de-Abreu A, Campbell RA, Weyrich AS, Zimmerman GA. Platelets: versatile effector cells in hemostasis, inflammation, and the immune continuum. Semin Immunopathol. 2012 Jan;34(1):5–30.
10. Woollard KJ, Suhartoyo A, Harris EE, Eisenhardt SU, Jackson SP, Peter K, et al. Pathophysiological levels of soluble P-selectin mediate adhesion of leukocytes to the endothelium through mac-1 activation. Circ Res. 2008;103(10):1128–38.
11. Hottz ED, Medeiros-de-Moraes IM, Vieira-de-Abreu A, de Assis EF, Vals-de-Souza R, Castro-Faria-Neto HC, et al. Platelet activation and apoptosis modulate monocyte inflammatory responses in dengue. J Immunol. 2014 Aug;193(4):1864–72.
12. Nkambule BB, Davison G, Ipp H. The value of flow cytometry in the measurement of platelet activation and aggregation in human immunodeficiency virus infection. Platelets. 2015;26(3):250–7.
13. Nkambule BB, Davison GM, Ipp H. The evaluation of platelet function in HIV infected, asymptomatic treatment-naïve individuals using flow cytometry. Thromb Res [Internet]. 2015;135(6):1131–9. Available from: http://europepmc.org/abstract/MED/25900311
14. Damien P, Cognasse F, Lucht F, Suy F, Pozzetto B, Garraud O, et al. Highly Active Antiretroviral Therapy Alters Inflammation Linked to Platelet Cytokines in HIV-1–Infected Patients. J Infect Dis. 2013;208(5):868–70.

15. Francisci D, Giannini S, Baldelli F, Leone M, Belfiori B, Guglielmini G, et al. HIV type 1 infection, and not short-term HAART, induces endothelial dysfunction. Aids. 2009;23(5):589–96.
16. O'Halloran J, Dunne E, Gurwith M, Lambert J, Sheehan G, Feeney E, et al. The effect of initiation of antiretroviral therapy on monocyte, endothelial and platelet function in HIV-1 infection. HIV Med. 2015 Jun 25;16.
17. Landrø L, Ueland T, Otterdal K, Frøland SS, Aukrust P. Persistently raised plasma levels of platelet-derived inflammatory mediators in HIV-infected patients during highly active anti-retroviral therapy. Vol. 9, Journal of thrombosis and haemostasis : JTH. England; 2011. p. 1075–7.
18. Zakynthinos E, Pappa N. Inflammatory biomarkers in coronary artery disease. J Cardiol. 2009 Jun;53(3):317–33.
19. Kelesidis T, Papakonstantinou V, Detopoulou P, Fragopoulou E, Chini M, Lazanas MC, et al. The Role of Platelet-Activating Factor in Chronic Inflammation, Immune Activation, and Comorbidities Associated with HIV Infection. AIDS Rev [Internet]. 2015;17(4):191–201. Available from: https://pubmed.ncbi.nlm.nih.gov/26616844
20. Weber M, Hamm C. Novel biomarkers - The long march from bench to bedside. Eur Heart J. 2008;29(9):1079–81.
21. Satchell CS, Cotter AG, O'Connor EF, Peace AJ, Tedesco AF, Clare A, et al. Platelet function and HIV: a case-control study. AIDS. 2010 Mar;24(5):649–57.
22. Falcinelli E, Francisci D, Belfiori B, Petito E, Guglielmini G, Malincarne L, et al. In vivo platelet activation and platelet hyperreactivity in abacavir-treated HIV-infected patients. Thromb Haemost. 2013 Aug;110(2):349–57.
23. Klein D, Hurley LB, Quesenberry CPJ, Sidney S. Do protease inhibitors increase the risk for coronary heart disease in patients with HIV-1 infection? J Acquir Immune Defic Syndr. 2002 Aug;30(5):471–7.
24. Triant VA, Lee H, Hadigan C, Grinspoon SK. Increased acute myocardial infarction rates and cardiovascular risk factors among patients with human immunodeficiency virus disease. J Clin Endocrinol Metab. 2007 Jul;92(7):2506–12.
25. Shah, Anoop S VStelzle, Dominik Lee, Kuan Ken, Beck, Eduard J, Alam, Shirjel, Clifford, Sarah, Longenecker, Chris T, Strachan, Fiona, Bagchi, Shashwatee, Whiteley, William Rajagopalan, Sanjay,Kottilil, Shyamasundaran,Nair, Harish,Newby, David E,McAllister NL. Global Burden of Atherosclerotic Cardiovascular Disease in People Living With HIV: Systematic Review and Meta-Analysis. Circulation. 2018 Sep;138(11):1100–12.
26. Todowede OO, Sartorius B, Magula N, Schutte AE. Association of predicted 10 years cardiovascular mortality risk with duration of HIV infection and antiretroviral therapy among HIV-infected individuals in Durban, South Africa. Diabetol Metab Syndr. 2019;11:105.

27. Norris SA, Wrottesley S, Mohamed RS, Micklesfield LK. Africa in transition: growth trends in children and implications for nutrition. Ann Nutr Metab. 2014;64 Suppl 2:8–13.
28. Ziraba AK, Fotso JC, Ochako R. Overweight and obesity in urban Africa: A problem of the rich or the poor? BMC Public Health. 2009;9:1–9.
29. Mkuu R, Barry A, Yonga G, Nafukho F, Wernz C, Gilreath T, et al. Prevalence and factors associated with overweight and obesity in Kenya. Prev Med Reports [Internet]. 2021;22:101340. Available from: https://www.sciencedirect.com/science/article/pii/S2211335521000310
30. Mbochi RW, Kuria E, Kimiywe J, Ochola S, Steyn NP. Predictors of overweight and obesity in adult women in Nairobi Province, Kenya. BMC Public Health. 2012;12(1):1–9.

Chapter prologue

Following is the systematic review and meta-analysis addressing the prognostic factors associated with poor clinical outcomes in PLWH and obesity.

CHAPTER 2: A SYTEMATIC REVIEW AND META-ANALYSIS

Cardiovascular risk factors in antiretroviral therapy-treated patients living with HIV and obesity: A systematic review and meta-analysis of prognostic factors

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This is an article that has been submitted to AIDS reviews.

Abstract

Objectives: To review and provide a synthesis of prognostic factors of metabolic complications in people living with HIV (PLWH) and obesity on antiretroviral therapy (ART). In addition, to assess the modulatory effect of ART and obesity on traditional cardiovascular risk factors.

Methods*:* We searched the MEDLINE, Academic Search Complete, Health Source: Nursing/Academic Edition databases for eligible studies from inception until August 2021. The certainty of evidence in the included studies were determined using the Quality in Prognostic Studies (QUIPS) tool. A random-effects model was used to pool the reported effect estimates.

Results: We retrieved a total of 51 citations, and after full-text screening, only 14 studies met the inclusion criteria. A total of 14 potential predictors of metabolic complications and cardiovascular risk were identified in PLWH and obesity. The pooled estimates showed that gender [OR: 1.61 (95%CI 0.66, 2.57, p<0.001)], body mass index [OR: 1.34 (95%CI 0.47, 2.20, p<0.001)], CD4 counts [OR: 1.61 (95%CI 0.90, 1.42, p<0.001)] and IL-6 levels [OR: 2.57 (95%CI 2.05, 3.10, p=0.230)] were associated with increased CVD-risk in PLWH. Notably, only CD4 T cell counts, and IL-6 levels were confirmed prognostic factors, that retained their predictive value in PLWH and obesity after adjusting for covariates.

Conclusion*:* In this systematic review and meta-analysis of prognostic factors, we identified nadir CD4 counts (100-199 mm^3) and basal IL-6 levels as prognostic factors for cardiometabolic disease in PLWH and obesity.

Other: **Systematic review PROSPERO registration:** CRD42021234560

Keywords: Body mass index, cardiovascular disease, prognostic factors, antiretroviral therapy, systematic review

Introduction

Antiretroviral therapy (ART) has significantly reduced the mortality rates in people living with HIV (PLWH) (1,2). However, there is a gradual increase in the prevalence of metabolic and cardiovascular disease (CVD) in the ageing population of PLWH on ART (3–6). An interplay between traditional risk factors such as the body mass index (BMI), dyslipidaemia (4,7–10) and the use of certain ART drugs (9,10) modifies the CVD risk of PLWH. In fact, HIV-related risk factors such as immune dysfunction and chronic inflammation are independent risk factors for CVD and are as reliable as the traditional CVD-risk factors (7,11–13). Interestingly, obesity is a major risk factor for CVD, and it is associated with chronic inflammation, immune dysfunction and metabolic dysfunction among PLWH (14,15). Despite the well-described link between obesity and incidence of CVD, prognostic factors associated with poor cardiovascular-related outcomes in ART-treated PLWH remain controversial (16,17).

The predictive value of conventional CVD risk factors in comparison to HIV-specific factors in PLWH on successful ART remains controversial (18,19). HIV-specific factors such as low nadir CD4 T lymphocyte counts, HIV-1 RNA levels, and chronic inflammation are independently associated with an increased CVD risk in PLWH (18,20). In addition, increased interleukin-6 (IL-6) levels, intercellular adhesion molecule-1 (ICAM-1), soluble tumour-necrosis factor-α receptors 1 and 2 (TNFR-1 and 2), and monocyte activation were associated with increased prevalence of coronary stenosis in PLWH (21,22).

Several studies have assessed CVD-risk in PLWH using the Framingham risk score (23–25) or the Prospective Cardiovascular Munster Study (PROCAM) (26–28) score, in patients with HIV(28). In the last decade the predictive value of several CVD-risk scores have been evaluated (28–31), and inconsistent findings have been reported in PLWH using various prediction models. The prognostic value of traditional CVD-risk scores remains unclear in PLWH on ART (32). Moreover, the additive effect of comorbidities such as obesity on the CVD risk profile of PLWH remains elusive.

The Data Collection on Adverse Events on Anti-HIV Drugs Cohorts (D:A:D) score is one of the CVD-risk scores developed for PLWH on ART (33). Unlike the Framingham and PROCAM score, the D:A:D score considers the use of antiretroviral drugs and CD4 counts as candidate risk factors for CVD (8,33). To date, only a few studies have assessed the external validity of these predictive models in ethnically diverse populations and PLWH with comorbidities such as obesity. The addition of independent risk factors such as inflammation and immune activation as predictive factors for CVD could potentially enhance the predictive value of CVD-risk algorithms used in PLWH (20,34). This systematic review and meta-analysis aimed to synthesize and assess the predictive value of traditional and novel prognostic factors in ART-treated PLWH.

Methods

Eligibility criteria

We included studies based on an eligibility criterion developed using the population, index prognostic factors, outcome, Timing and Setting (PICOTS) guidelines (35). We included both randomized and non-randomized controlled trials reporting on ART-treated adults (18 years or older) living with HIV and obesity. We defined predictive models for CVD in PLWH and obesity as multivariable models used in predicting cardiovascular outcomes in the included studies. Eligible studies reported on the index prognostic factors included in the Framingham risk score (36). In addition, predictors reported before the initiation of ART and post-treatment were considered. We excluded case series studies and reviews.

Search strategy and study selection

A search strategy was developed using medical subject headings (MeSH) for MEDLINE, which were adapted for the EBSCOhost search engine. We also searched the Academic Search Complete, Health Source: Nursing/Academic Edition electronic databases from inception to the 31st of August 2021. The search terms included obesity, cardiovascular disease, antiretroviral therapy and prognosis (Supplementary file 2). Two independent reviewers (SAM and ZAM) screened the retrieved abstracts and full texts. Moreover, the bibliography of the included studies was screened to augment the database search. A third reviewer (BBN) was consulted for arbitration in instances of disagreements.

Data collection process

Two reviewers independently extracted the data items using a predefined data extraction sheet, designed using the CHARMS-PF checklist (37). The extracted data items included the author's name, year of publication, country, source of data, sample size, aim of the study, candidate predictors, model development, and main findings of the study.

Risk of bias and quality assessment

Two independent reviewers assessed the certainty of evidence in the included studies using the Quality in Prognostic Studies (QUIPS) tool (38). The QUIPS tool assesses the quality of prognostic studies based on six domains. These include study participation, study attrition, measurement of prognostic factors, measurement of outcomes, measurement of confounders, and statistical analysis and reporting. Two reviewers independently evaluated the overall quality of evidence using the Grading of Recommendation Assessment Development and Evaluation (GRADE) approach. The Cohen’s kappa

score was used to measure the interrater reliability (39) and in cases of disagreements, a third reviewer (BBN) was consulted for arbitration.

Statistical analysis

Data for potential prognostic factors were expressed as odds ratio (OR) or hazards ratio (HR). The Cohen's kappa scores were used to measure interrater reliability. For assessing variance due to heterogeneity across studies the Higgins I^2 statistic was used. We used a random-effect model to pool the reported effect estimates. . P-value < 0.05 was considered significant. In addition, we performed a subgroup analysis based on the reported candidate predictors.

Results

Study selection

We retrieved a total of 51 citations from databases search, and only 34 studies were selected for full-text screening after removing duplicates. Of these, 20 studies were excluded due to, irrelevance (n=18), language (n=1) and a study not reporting on CVD-risk (n=1). Only 14 studies met the inclusion criteria and were included in the qualitative and quantitative synthesis (Figure 1).

Characteristics of included studies

The included studies were published between 2011 and 2020 (Table 1). The included studies comprised of 60% cross-sectional studies (n= 8), a retrospective cohort study (n=1), a prospective cohort (n=1), a retrospective study (n=1), a prospective study (n=1) and two observational studies (n=2). The overall sample size ranged from 158 to 5839 patients with HIV. Only one (7.1%) was a multi-centre study (Australia, Europe, and USA) and 13 (92.9%) were single centre studies. In the included studies, the measured predictors of CVD varied and only ten (71,4%) of the included studies made use of logistic regression models and reported on adjusted effect measures. Also, only one study (7.1%) reported on the sensitivity and 13 (92.9%) did not report on any sensitivity and specificity of the reported risk prediction models.

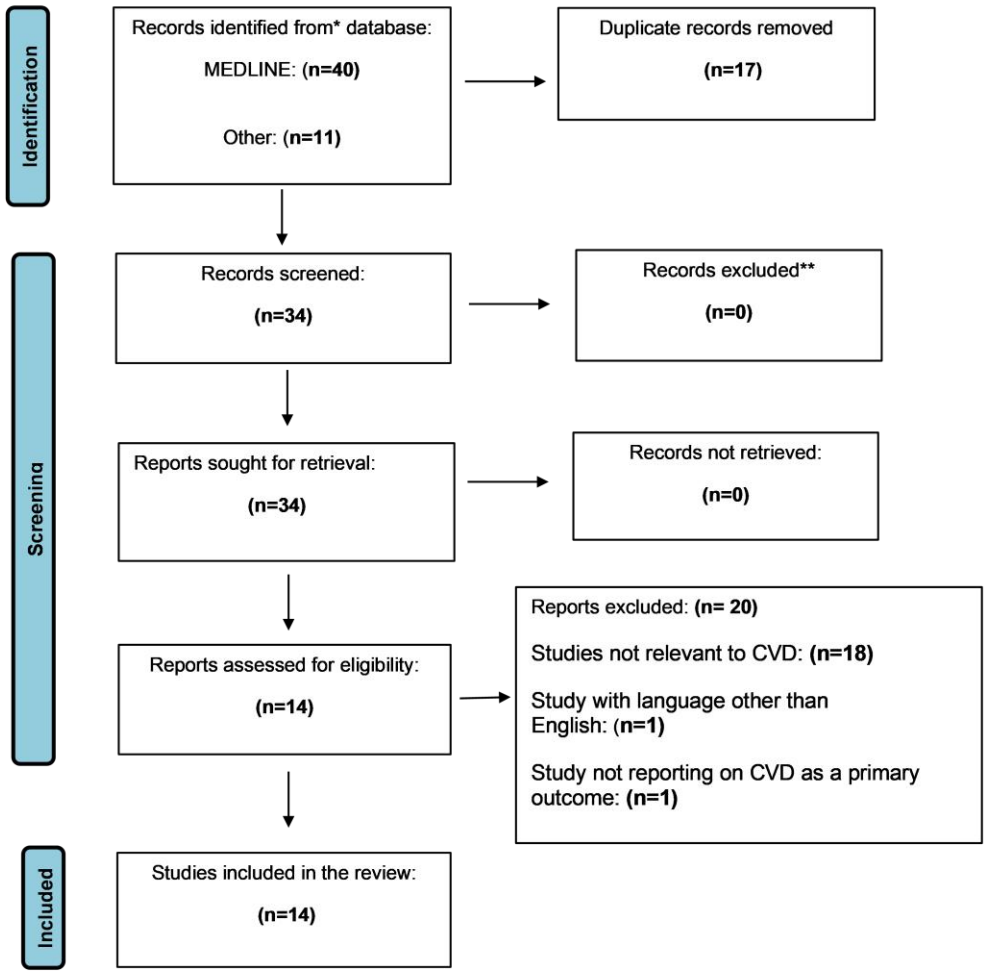

Figure 1: Preferred Reporting Items for Systematic review and Meta-analyses diagram detailing the screening and selection of the included studies.

Risk of bias assessment

The risk of bias for each study was assessed using the QUIPS tool (38). Two studies were scored as good (25-28 points) (40,41) while seven were scored as fair (21-24 points) (42–48) and the other five were scored as poor (15-20 points) (9,49–52). The included studies had high risk of bias in the domains of study participants with a median score of 3 (1-6) out of possible score of 6 (overall agreement 66.6%, k=0.33) and study attrition with a median score of 1 (0-4) out of possible score of 4 (overall agreement: 80.7%, k=0.63). Only one study (48) was scored as moderate in the study attrition domain. The risk of bias was relatively low for the prognostic factor measurements with a median score of 4 out of possible score of 6 (overall agreement: 100%, k=1.00), outcome measurements with a median score of 4 (2-4) out of possible score of 3.6 (overall agreement: 92.3%, k=0.85), confounding measures and account with median score of 3.8 (3-4) out of possible score of 4 (overall agreement: 84.6%, k=0.70) and

statistical analysis with a median score of 5 (4-6) out of possible score of 6 (overall agreement: 89.7%, k=0.79) domains, except for two studies (44,48) that were scored as moderate in the outcome measurement domain (Figure 2).

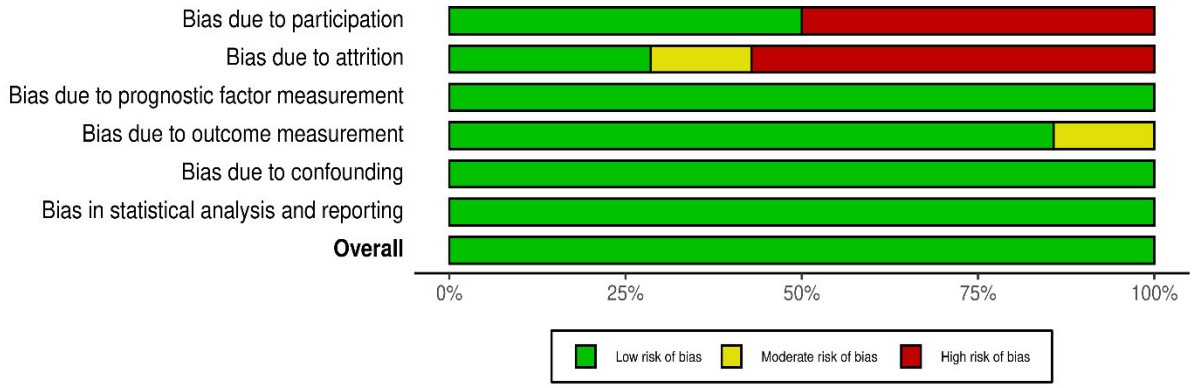

Figure 2: Risk of bias assessment of the included studies (n=14).

Confirming prognostic factors

Among the seven predictors for CVD identified from the included studies, multivariate results were not available for high-sensitivity C-reactive protein (hsCRP) and ethnicity. Only age, sex, BMI (≥30 kg/m^2), IL-6 and CD4 counts (100-199 cells/mm^3) were all available on multivariate analyses and therefore were considered as potential prognostic factors based on the chosen criteria for prognostic factors.

Table 1: Characteristics of included studies (n=14).

Authors	Country	Study design	Sample size	Mean±SD or Median(IQR) Age (years)	CVD Risk prediction models	Main findings
Julius et al. (2011) (42)	South Africa	CS	HIV infected (n= 304) (Females=237; Males=67)	35.8±5.3	None	Low HDL levels and obesity were more prevalent in WLHIV. However, WLHIV were at a lower risk of hypertriglyceridemia. The duration of HAART was not associated with dyslipidaemia.
De Socio et al. (2013) (44)	Italy	CS	N=1182 (Females=340; Males=842)	46.97±9.4	None	Advanced disease stage was common in hypertensive patients. A low Nadir CD4 T cell count (<200 cells/µl) and duration of ART were independently associated with hypertension in patients living with HIV.
Koethe et al.(2013) (43)	United States	RCO	N=158 (Females =47; Males=111)	45(41-50)	None	The association between BMI and increasing levels of hsCRP and TNF-α receptor 1 were attenuated at BMI levels above 30 kg/m^2. Whereas IL-6 and MIP-1 α levels were elevated in patients with obesity. Moreover, higher pre-ART IL-6 levels were associated with increased risk of fatal and nonfatal CVEs when compared to hsCRP levels.

Nery et al. (2013) (9)	Brazil	CS	N= 294 (Females=70, Males=224)	36.8±10.3	FRS, PROCAM, D:A:D	The most prevalent dyslipidaemia was low HDL-c and obesity was associated with an increased risk for CVD. Majority of PLWH were classified as low risk for future CVE.
Conley et al. (2015) (45)	United States	PCO	N= 452 (Female=99, Males=353)	41(36-48)	None	Obesity was independently associated with elevated levels of hsCRP, IL-6, sCD163 and pro-inflammatory monocytes. Leptin levels and insulin resistance modified the associations between obesity and inflammatory cytokines.
Achhra et al. (2016) (46)	Europe, Australia, and USA	Multi-CO	N=9321 (Females=2312, Males=7009)	39.6±10.1	D:A:D	The association between short-term gain in BMI following ART and long-term risk of CVD differed based on pre-ART BMI. Patients in the normal or mid quartile category experienced a 18-20% increased risk for CVD per unit gain of BMI. The use of PIs was also associated with a marked increase in BMI when compared to NNRTIs.
Hirigo and Tesfaye (2016) (40)	Ethiopia	CS	N=185 (Females=117, Males=68)	32(26.5-38)	None	WLHIV were at a significantly higher risk of developing the MetS when compared to men. In addition, a greater proportion of WLHIV had low HDL-c levels. Interestingly, males had elevated triglyceride levels in comparison to females.

Muyanja et al. (2016) (49)	Uganda	CS	N= 250 (Females=169, Males=81)	36(30-43)	FRS	WLHIV had an increased prevalence of MetS when compared to men. However, females had relatively low CVD-risk scores. There were no independent risk factors associated with a moderate to high risk 10-year Framingham risk score.
Ilouze et al. (2016) (50)	Northeast England	RCO	N= 560 (Females=126, Males=385)	45 (38-52)	None	Black ethnicity, type 2 diabetes and a higher CD4 count were associated with being overweight. While only Ethnicity and type 2 diabetes were independently associated with obesity in PLWH.
Guo et al. (2017) (51)	China	CS	N= 973 (Females=251, Males=722)	36±10.2	FRS,D:A:D, ACC/AHA ASVCD Risk Score	Age and smoking status were directly associated with an increased CVD risk. However, HIV-specific factors such as Nadir CD4 count, baseline viral load were not associated with CVD-risk. WLHIV had a favourable risk profile when compared to men.
Kintu et al. (2018) (47)	Tanzania	PCO	N=79 074 (Females=52 980, Males=26 094)	37(31-44)	None	WLHIV were at a 2-fold increased risk of developing obesity when compared to men. Age was not associated with obesity, but socioeconomic status was associated with obesity in PLWH. Lastly lower CD4 count, and recent initiation of ART were associated with an increased risk of obesity.

Obry-Roguet et al. (2018) (48)	France	CS	N=862 (Females=, 277, Males=585)	52(47-58)	None	Age, sex, alcohol consumption, absence of HCV coinfection, and HIV transmission risk group but not cART regimen were associated with being overweight or obesity in PLWH
Sears et al. (2019) (52)	United States	CS	N=1235 (Females=284, Males=953)	Not Available	None	A third of PLWH had MetS and women were at 2-fold increased risk of having MetS. Age, sex and current smoking were associated with the MetS.
Touloumni et al. (2020) (41)	Greece	CS	N= 10659 (Females=1556, Males=9103)	44(34-56)	FRS, SCORE	PLWH were more likely to be current smokers and had dyslipidaemia and hypertension. In addition, PLWH had a higher risk of fatal CVD, despite being less likely to be obese.

ACC/AHA: American College of Cardiology/American Heart Association; **ASCVD:** Atherosclerotic Cardiovascular Disease Risk Score; **cART:** combined antiretroviral therapy; **CS:** Cross-sectional; **CVE:** Cardiovascular events; **D:A:D:** Data Collection on Adverse events of Anti-HIV Drugs**; FRS:** Framingham CVD Risk Score; **HAART:** Highly active antiretroviral therapy; **hsCRP:** High-sensitivity C-reactive protein; **HCV:** Hepatitis C virus; **MetS:** Metabolic syndrome **;MIP-1α:** Macrophage inflammatory protein-1**; NNRTI:** Non-nucleoside reverse transcriptase inhibitors; **RCO:** Retrospective cohort; **SCORE:** Systematic Coronary Risk Evaluation; **TNF-α:** Tumor necrosis factor-α

Traditional risk factors for cardiovascular disease in people living with HIV and obesity

Three (21%) of the included studies reported age as a risk factor for CVD (48,49,52). Two of these studies reported on age as a predictor for developing cardiometabolic disease in ART-treated patients living with HIV and obesity (49,52) with the pooled OR was 0.74 (95%CI: 0.00, 1.48). The substantial level of heterogeneity was (I^2=97.92%, p<0.001) (Figure 3). Notably, females were reported to have a two-fold risk of obesity and metabolic disorders compared to males (47,51) [OR: 1.61 (95%CI 0.66, 2.57)] with the evidence of substantial level of heterogeneity (I^2=97.01%, p<0.001) (Figure 3). Moreover, women were more likely to have a favourable CVD-risk profile compared to men (51). In addition, elevated total cholesterol to HDL ratio was significantly associated with fatal CVD in males (53).

Four (29%) of the included studies reported on BMI as a predictor for CVD (40,44,46,48). Of these, three reported a higher BMI in ART-treated patients compared to ART-naïve patients (40,44,48) . However, one study(41) reported that ART-treated patients living with HIV were less likely to be obese. Furthermore, then reported that PLWH have relatively high risk of developing CVD according to a 5-year CVD-risk estimation Systematic Coronary Risk Evaluation (SCORE). These differences may be due to the lack of accounting for differences in the use of antidiabetic or antihypertensive drugs and the fact that the BMI was often not measured, or the measurements were not recorded and the analysis relied on imputed data. In addition, one (46) study reported that patients living with HIV and obesity had a higher incidence rate ratio of CVD after ART-initiation. Two studies reported that higher BMI ($\geq$30 kg/m^2) is significantly associated with high prevalence of hypertension (44) and metabolic syndrome (40) in PLWH [OR: 1.34 (95%CI 0.47,2.20)] with the presence of substantial level of heterogeneity (I^2=99.56%, p<0.001) (Figure 3).

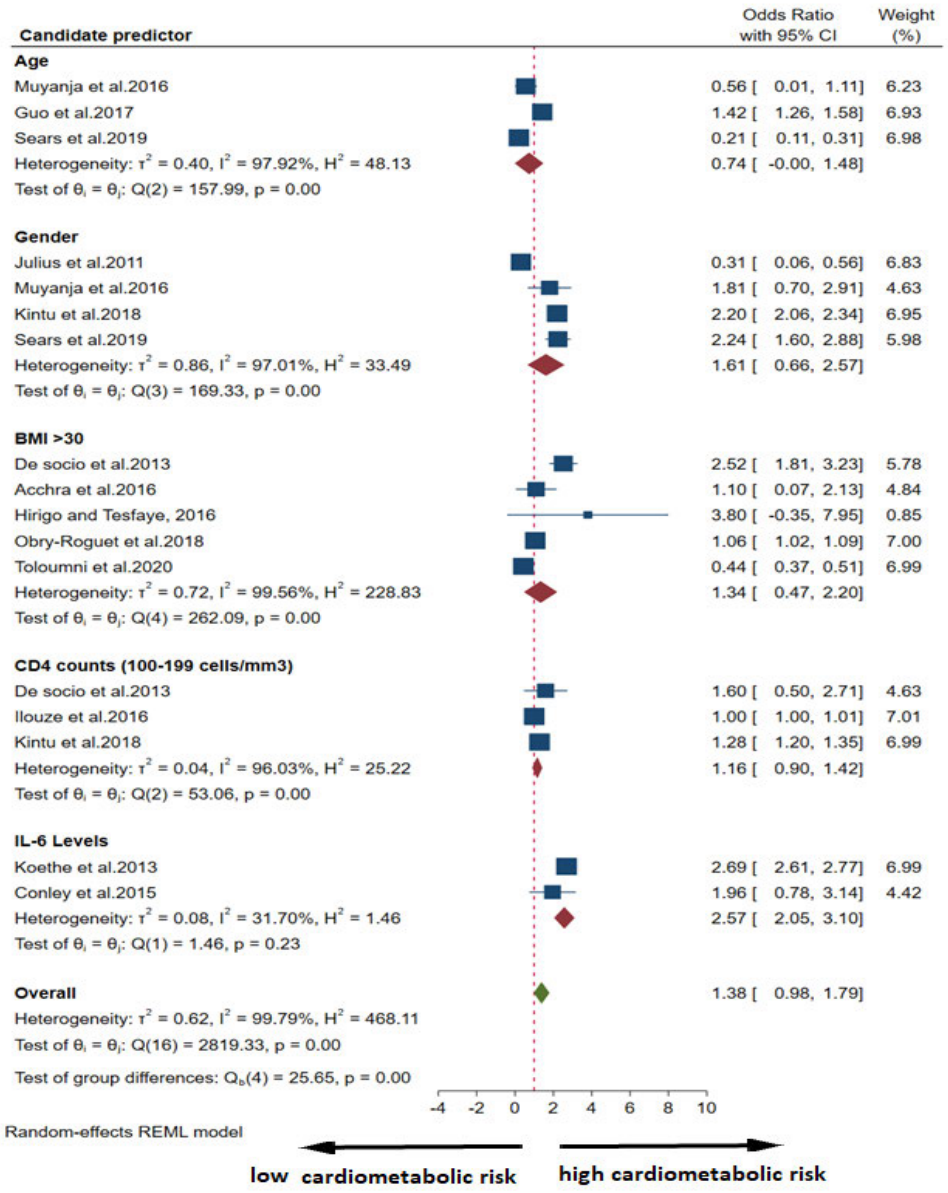

Figure 3: Prognostic factors of cardiovascular risk in people on antiretroviral therapy living with human immunodeficiency virus and obesity.

HIV-Specific factors in people living with HIV and Obesity

Four studies (29%) reported on an additive effect of CD4 count and obesity as a CVD predictor (44,46,47,50), and one study (50) showed that a higher CD4 count in obese patients with HIV was independently associated with CVD-risk in ART-treated patients [OR:1.16 (95%CI 0.90, 1.42) (Figure 3). However, there was a substantial level of heterogeneity between these studies (T^2=0.04, I^2= 96.03%, p< 0.001.) Conversely, a single study reported on an inverse association between the BMI and CD4 counts (46). Lastly, inflammatory biomarkers as predictors of CVD-risk were reported in only three of the included studies (9,43,45). One study reported elevated levels of IL-6, hsCRP and sCD163 to be significantly associated with obesity in ART-treated patients living with HIV (45). Consistent with the results of the study (43) that reported on elevated IL-6, hsCRP, monocyte chemoattractant protein-1 (MCP-1), and TNFR-1 and TNFR-2 to be strongly associated with an increased BMI in ART-treated patients living with HIV. Notably, another study reported that hsCRP was the most frequent aggravating factor >3.0 mg/L found in 32.1% of participants (9) and the pooled OR was 2.57 (95%CI 2.05, 3.10) without statistical significance and substantial level of heterogeneity (T^2=0.08, I^2=31.70%%, p= 0.23) (Figure 3).

Discussion

The aim of this systematic review and meta-analysis was to provide a synthesis of prognostic factors for cardiometabolic disease in adult PLWH. To our knowledge, this is the first systematic review and meta-analysis focused on risk factors for cardiometabolic disease in PLWH and obesity. The reported candidate predictors in both univariate and multivariate analyses, estimated the risk of cardiometabolic disease such as dyslipidemia(42), hypertension (44), obesity (9,43,45,47,48) and metabolic syndrome (40). The spectrum of CVD in the aging population with HIV is broad and includes peripheral artery disease (54), ischemia heart disease and cerebrovascular disease (55). Although several traditional and HIV-specific risk factors for cardiometabolic disease have been reported in ART-treated PLWH, the relevance of these factors in PLWH and obesity remains uncertain, with only a few studies that have evaluated the associations between these factors and established CVD risk scores (9,41,46,49,51). In these studies, women were at higher risk of metabolic disease (40,46), however had lower CVD risk scores in comparison to men (49).

In the univariate analyses PLWH and obesity, age (48,51), smoking (51,52), low levels of HDL (9,42) were associated with increased CVD-risk. Notably, incongruent findings on HIV-specific factors have been reported and a trend towards a significant association with the CVD risk profile has been reported (42), while others have shown no association between nadir CD4 counts and baseline viral loads with the CVD-risk profile of PLWH on ART (42,48,51). The confirmation of prognostic factors was based on statistically significant findings in multivariate analysis and the direction of effect estimates in the included studies. Amongst the five prognostic factors included the meta-analysis, sex, IL-6 levels and CD4 counts (100-199 cells/mm^3) were the only factors that met the criteria for confirmation of prognostic factors (figure 3). Despite a high prevalence of MetS in WLHIV, the CVD risk scores were lower in females in comparison to males living with HIV on ART (49,51).

There is a lack of multi-ethnic studies reporting on CVD risk models in cohorts comprised of PLWH, due to many studies reporting on populations predominantly derived from the North America, and Europe. In this systematic review, only a few of the included studies reported on CVD risk scores, these included the FRS (9,41,49,51), D:A:D (9,46,51) and SCORE equation (41). The D:A:D equation is the most accurate score for the prediction of CVD in PLWH (33) and the risk assessment is modified by incorporation of CD4 counts (56). While the inclusion of CRP levels in the FRS enhances the global coronary risk in the intermediate risk group (57). Despite the reported association between ART initiation and platelet activation (58,59) and endothelial dysfunction (60), the current CVD risk prediction models do not account for how basal levels of endothelial or platelet activation may potentiate CVD risk in PLWH on ART. The incorporation of basal levels of platelet and endothelial activation prior to the initiation of ART, may enhance the precision of prediction models and improve the prognostication of PLWH and obesity.

The strengths of the current meta-analysis include the quality of the included studies and the methodological approach used to provide pooled effect estimates derived from multivariable analysis. Majority of the included studies were graded as either good or fair based on the QUIPS tool. There are a few caveats that should be considered in the interpretation and generalizability of the findings of this systematic review and meta-analysis. Firstly, there were methodological limitations due to limited number of studies reporting on similar prognostic factors in PLWH and obesity. Therefore, our random effects meta-analysis and subgroup analysis was limited to the reported prognostic factor and further exploration of sources of heterogeneity such geographical and clinical differences were not determined. Lastly, caution should be taken when interpreting the confirmed prognostic factors as these were restricted to populations predominantly derived from the North America, and Europe. Therefore, future multi-ethnic prospective cohort studies are required to determine the predictive value and relevance of these reported prognostic factors in PLWH and obesity. In addition, the incorporation of markers of platelet activation and endothelial function in prognostication of PLWH on ART should be considered and validated in large cohort studies.

Conclusion

In this systematic review and meta-analysis, we identified and confirmed nadir CD4 counts between 100-199 cells/mm^3, and IL-6 levels prior to initiation of ART as prognostic factors strongly associated with cardiometabolic risk in PLWH and obesity. Future studies incorporating platelet activation and endothelial function to these confirmed prognostic factors, in the risk modelling of PLWH and obesity on ART, could further enhance the precision of prediction models and improve the prognostication of PLWH and obesity.

Other information

This systematic review and meta-analysis were prepared following the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) 2020 statement. This systematic review and meta-analysis were registered under the PROSPERO registration: CRD42021234560.

Authors' contribution

SAM and BBN conceptualized, designed the study, and drafted the manuscript. SAM and ZAM performed the screening of articles, additionally ZAM helped draft the manuscript. All authors wrote and approved the final manuscript. BBN is the guarantor of the systematic review and meta-analysis.

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Patients' approval for publication

Not required.

Competing interest

None declared

References

1. Mocroft A, Ledergerber B, Katlama C, Kirk O, Reiss P, d'Arminio Monforte A, et al. Decline in the AIDS and death rates in the EuroSIDA study: an observational study. Lancet (London, England). 2003 Jul;362(9377):22–9.

2. Quinn TC. HIV epidemiology and the effects of antiviral therapy on long-term consequences. AIDS. 2008 Sep;22 Suppl 3(Suppl 3):S7-12.

3. Triant VA, Lee H, Hadigan C, Grinspoon SK. Increased acute myocardial infarction rates and cardiovascular risk factors among patients with human immunodeficiency virus disease. J Clin Endocrinol Metab. 2007 Jul;92(7):2506–12.

4. Freiberg MS, Chang C-CH, Kuller LH, Skanderson M, Lowy E, Kraemer KL, et al. HIV infection and the risk of acute myocardial infarction. JAMA Intern Med. 2013 Apr;173(8):614–22.

5. Althoff KN, McGinnis KA, Wyatt CM, Freiberg MS, Gilbert C, Oursler KK, et al. Comparison of risk and age at diagnosis of myocardial infarction, end-stage renal disease, and non-AIDS-defining cancer in HIV-infected versus uninfected adults. Clin Infect Dis an Off Publ Infect Dis Soc Am. 2015 Feb;60(4):627–38.

6. Schouten J, Wit FW, Stolte IG, Kootstra NA, van der Valk M, Geerlings SE, et al. Cross-sectional comparison of the prevalence of age-associated comorbidities and their risk factors between HIV-infected and uninfected individuals: the AGEhIV cohort study. Clin Infect Dis an Off Publ Infect Dis Soc Am. 2014 Dec;59(12):1787–97.

7. Thompson-paul AM, Lichtenstein KA, Armon C, Jr FJP, Skarbinski J, Chmiel JS, et al. HHS Public Access. 2017;63(11):1508–16.

8. Law MG, Friis-Møller N, El-Sadr WM, Weber R, Reiss P, D'Arminio Monforte A, et al. The use of the Framingham equation to predict myocardial infarctions in HIV-infected patients: comparison with observed events in the D:A:D Study. HIV Med. 2006 May;7(4):218–30.

9. Nery MW, Martelli CMT, Aparecida Silveira E, Sousa CA De, Falco MDO, Castro ADCO De, et al. Cardiovascular risk assessment: A comparison of the Framingham, PROCAM, and D:A:D equations in HIV-infected persons. Sci World J. 2013;2013.

10. Dourado I, Veras MA de SM, Barreira D, de Brito AM. [AIDS epidemic trends after the introduction of antiretroviral therapy in Brazil]. Rev Saude Publica. 2006 Apr;40 Suppl:9–17.

11. Silverberg MJ, Leyden WA, Xu L, Horberg MA, Chao CR, Towner WJ, et al. Immunodeficiency and risk of myocardial infarction among HIV-positive individuals with

access to care. J Acquir Immune Defic Syndr. 2014 Feb;65(2):160–6.

12. Islam FM, Wu J, Jansson J, Wilson DP. Relative risk of cardiovascular disease among people living with HIV: a systematic review and meta-analysis. HIV Med. 2012 Sep;13(8):453–68.

13. Holmberg SD, Moorman AC, Williamson JM, Tong TC, Ward DJ, Wood KC, et al. Protease inhibitors and cardiovascular outcomes in patients with HIV-1. Lancet (London, England). 2002 Nov;360(9347):1747–8.

14. Godfrey C, Bremer A, Alba D, Apovian C, Koethe JR, Koliwad S, et al. Obesity and Fat Metabolism in Human Immunodeficiency Virus-Infected Individuals: Immunopathogenic Mechanisms and Clinical Implications. J Infect Dis. 2019 Jul;220(3):420–31.

15. Ritchie SA, Connell JMC. The link between abdominal obesity, metabolic syndrome and cardiovascular disease. Nutr Metab Cardiovasc Dis. 2007 May;17(4):319–26.

16. Anne-Lise P, Chang C-CH, So-Armah KA, Butt AA, Leaf DA, Budoff M, et al. Human immunodeficiency virus infection, cardiovascular risk factor profile and risk for acute myocardial infarction. J Acquir Immune Defic Syndr. 2015;68(2):209.

17. Pereira B, Mazzitelli M, Milinkovic A, Moyle G, Mandalia S, Al-Hussaini A, et al. Predictive value of HIV-related versus traditional risk factors for coronary atherosclerosis in people aging with HIV. AIDS Res Hum Retroviruses. 2021 Oct;

18. Diaz CM, Segura ER, Luz PM, Clark JL, Ribeiro SR, De Boni R, et al. Traditional and HIV-specific risk factors for cardiovascular morbidity and mortality among HIV-infected adults in Brazil: a retrospective cohort study. BMC Infect Dis. 2016;16(1):1–13.

19. Thomas GP, Li X, Post WS, Jacobson LP, Witt MD, Brown TT, et al. Associations between antiretroviral use and subclinical coronary atherosclerosis. AIDS. 2016 Oct;30(16):2477–86.

20. Libby P. Interleukin-1 Beta as a Target for Atherosclerosis Therapy: Biological Basis of CANTOS and Beyond. J Am Coll Cardiol. 2017 Oct;70(18):2278–89.

21. Bahrami H, Budoff M, Haberlen SA, Rezaeian P, Ketlogetswe K, Tracy R, et al. Inflammatory Markers Associated With Subclinical Coronary Artery Disease: The Multicenter AIDS Cohort Study. J Am Heart Assoc. 2016 Jun;5(6).

22. Subramanian S, Tawakol A, Burdo TH, Abbara S, Wei J, Vijayakumar J, et al. Arterial inflammation in patients with HIV. JAMA. 2012 Jul;308(4):379–86.

23. Giacomelli A, Conti F, Pezzati L, Oreni L, Ridolfo AL, Morena V, et al. Impact of switching to TAF/FTC/RPV, TAF/FTC/EVG/cobi and ABC/3TC/DTG on cardiovascular risk and lipid profile in people living with HIV: a retrospective cohort study. BMC Infect Dis. 2021

Jun;21(1):595.

24. Lu W-L, Lee Y-T, Sheu G-T. Metabolic Syndrome Prevalence and Cardiovascular Risk Assessment in HIV-Positive Men with and without Antiretroviral Therapy. Medicina (Kaunas). 2021 Jun;57(6).

25. Schulz C-A, Mavarani L, Reinsch N, Albayrak-Rena S, Potthoff A, Brockmeyer N, et al. Prediction of future cardiovascular events by Framingham, SCORE and asCVD risk scores is less accurate in HIV-positive individuals from the HIV-HEART Study compared with the general population. HIV Med. 2021 Sep;22(8):732–41.

26. Lister-Del Pino P, León-Amenero G, Leiva-Montejo A, Segura ER. [Concordance between Procam and Framingham cardiovascular risk scores among men receiving HIV treatment at a National Hospital in Lima, Peru 2013]. Rev Peru Med Exp Salud Publica. 2015 Oct;32(4):731–8.

27. Pirš M, Jug B, Eržen B, Šabović M, Karner P, Poljak M, et al. Cardiovascular risk assessment in HIV-infected male patients: a comparison of Framingham, SCORE, PROCAM and D:A:D risk equations. Acta dermatovenerologica Alpina, Pannonica, Adriat. 2014;23(3):43–7.

28. Knobel H, Jericó C, Montero M, Sorli ML, Velat M, Guelar A, et al. Global cardiovascular risk in patients with HIV infection: concordance and differences in estimates according to three risk equations (Framingham, SCORE, and PROCAM). AIDS Patient Care STDS. 2007 Jul;21(7):452–7.

29. Lundgren JD, Battegay M, Behrens G, De Wit S, Guaraldi G, Katlama C, et al. European AIDS Clinical Society (EACS) guidelines on the prevention and management of metabolic diseases in HIV*. HIV Med [Internet]. 2008 Feb 1;9(2):72–81. Available from: https://doi.org/10.1111/j.1468-1293.2007.00534.x

30. Cahn P, Leite O, Rosales A, Cabello R, Alvarez CA, Seas C, et al. Metabolic profile and cardiovascular risk factors among Latin American HIV-infected patients receiving HAART . Vol. 14, Brazilian Journal of Infectious Diseases . scielo ; 2010. p. 158–66.

31. Barros ZM, de Alencar Ximenes RA, Miranda-Filho DB, de Albuquerque M de FPM, Melo HRL, Carvalho EH, et al. Comparison between the Framingham and prospective cardiovascular of Münster scores for risk assessment of coronary heart disease in human immunodeficiency virus-positive patients in Pernambuco, Brazil. Metab Syndr Relat Disord. 2010 Dec;8(6):489–97.

32. D'Agostino Sr RB. Cardiovascular Risk Estimation in 2012: Lessons Learned and Applicability to the HIV Population. J Infect Dis [Internet]. 2012 Jun 1;205(suppl_3):S362–7.

Available from: https://doi.org/10.1093/infdis/jis196

33. Friis-Møller N, Thiébaut R, Reiss P, Weber R, Monforte AD, De Wit S, et al. Predicting the risk of cardiovascular disease in HIV-infected patients: the data collection on adverse effects of anti-HIV drugs study. Eur J Cardiovasc Prev Rehabil Off J Eur Soc Cardiol Work Groups Epidemiol Prev Card Rehabil Exerc Physiol. 2010 Oct;17(5):491–501.

34. Libby P, Hansson GK. Inflammation and immunity in diseases of the arterial tree: players and layers. Circ Res. 2015 Jan;116(2):307–11.

35. Riley RD, Moons KGM, Snell KIE, Ensor J, Hooft L, Altman DG, et al. A guide to systematic review and meta-analysis of prognostic factor studies. BMJ. 2019;364.

36. Wannamethee SG, Shaper AG, Lennon L, Morris RW. Metabolic syndrome vs Framingham Risk Score for prediction of coronary heart disease, stroke, and type 2 diabetes mellitus. Arch Intern Med. 2005;165(22):2644–50.

37. Moons KGM, de Groot JAH, Bouwmeester W, Vergouwe Y, Mallett S, Altman DG, et al. Critical Appraisal and Data Extraction for Systematic Reviews of Prediction Modelling Studies: The CHARMS Checklist. PLoS Med. 2014;11(10).

38. Hayden JA, Co P. Annals of Internal Medicine Academia and Clinic Evaluation of the Quality of Prognosis Studies in Systematic Reviews. 2006;427–38.

39. Landis JR, Koch GG. The Measurement of Observer Agreement for Categorical Data. Biometrics [Internet]. 1977 May 21;33(1):159–74. Available from: http://www.jstor.org/stable/2529310

40. Hirigo AT, Tesfaye DY. Influences of gender in metabolic syndrome and its components among people living with HIV virus using antiretroviral treatment in Hawassa, southern Ethiopia. BMC Res Notes. 2016;9(1):1–8.

41. Touloumi G, Kalpourtzi N, Papastamopoulos V, Paparizos V, Adamis G, Antoniadou A, et al. Cardiovascular risk factors in HIV infected individuals: Comparison with general adult control population in Greece. PLoS One. 2020;15(3):1–17.

42. Julius H, Basu D, Ricci E, Wing J, Kusari Basu J, Pocaterra D, et al. The Burden of Metabolic Diseases Amongst HIV Positive Patients on HAART Attending the Johannesburg Hospital. Curr HIV Res. 2012;9(4):247–52.

43. Koethe JR, Dee K, Bian A, Shintani A, Turner M, Bebawy S, et al. Circulating interleukin-6, soluble CD14, and other inflammation biomarker levels differ between obese and nonobese HIV-infected adults on antiretroviral therapy. AIDS Res Hum Retroviruses. 2013;29(7):1019–

277 25.

278 44. De Socio GV, Ricci E, Maggi P, Parruti G, Pucci G, Di Biagio A, et al. Prevalence, awareness,
279 treatment, and control rate of hypertension in HIV-infected patients: The HIV-HY study. Am J
280 Hypertens. 2014;27(2):222–8.

281 45. Conley LJ, Bush TJ, Rupert AW, Sereti I, Patel P, Brooks JT, et al. Obesity is associated with
282 greater inflammation and monocyte activation among HIV-infected adults receiving
283 antiretroviral therapy. Aids. 2015;29(16):2201–7.

284 46. Achhra AC, Mocroft A, Reiss P, Sabin C, Ryom L, de Wit S, et al. Short-term weight gain
285 after antiretroviral therapy initiation and subsequent risk of cardiovascular disease and
286 diabetes: The D: A: D study. HIV Med. 2016;17(4):255–68.

287 47. Kintu A, Liu E, Hertzmark E, Spiegelman D, Zack RM, Muya A, et al. Incidence and Risk
288 Factors for Overweight and Obesity after Initiation of Antiretroviral Therapy in Dar es Salaam,
289 Tanzania. J Int Assoc Provid AIDS Care. 2018;17:1–10.

290 48. Obry-Roguet V, Brégigeon S, Cano CE, Lions C, Zaegel-Faucher O, Laroche H, et al. Risk
291 factors associated with overweight and obesity in HIV-infected people. Medicine (Baltimore).
292 2018;97(23):e10956.

293 49. Muyanja D, Muzoora C, Muyingo A, Muyindike W, Siedner MJ. High Prevalence of
294 Metabolic Syndrome and Cardiovascular Disease Risk among People with HIV on Stable ART
295 in Southwestern Uganda. AIDS Patient Care STDS. 2016;30(1):4–10.

296 50. Ilozue C, Howe B, Shaw S, Haigh K, Hussey J, Price DA, et al. Obesity in the HIV-infected
297 population in Northeast England: a particular issue in Black-African women. Int J STD AIDS.
298 2017;28(3):284–9.

299 51. Guo F, Hsieh E, Lv W, Han Y, Xie J, Li Y, et al. Cardiovascular disease risk among Chinese
300 antiretroviral-naïve adults with advanced HIV disease. BMC Infect Dis. 2017;17(1):1–11.

301 52. Sears S, Buendia JR, Odem S, Qobadi M, Wortley P, Mgbere O, et al. Metabolic Syndrome
302 Among People Living with HIV Receiving Medical Care in Southern United States:
303 Prevalence and Risk Factors. AIDS Behav. 2019;23(11):2916–25.

304 53. Amberbir A, Banda V, Singano V, Matengeni A, Pfaff C, Ismail Z, et al. Effect of cardio-
305 metabolic risk factors on all-cause mortality among HIV patients on antiretroviral therapy in
306 Malawi: A prospective cohort study. PLoS One. 2019;14(1):1–11.

307 54. Beckman JA, Duncan MS, Alcorn CW, So-Armah K, Butt AA, Goetz MB, et al. Association
308 of Human Immunodeficiency Virus Infection and Risk of Peripheral Artery Disease.

Circulation. 2018 Jul;138(3):255–65.

55. Alonso A, Barnes AE, Guest JL, Shah A, Shao IY, Marconi V. HIV Infection and Incidence of Cardiovascular Diseases: An Analysis of a Large Healthcare Database. J Am Heart Assoc. 2019 Jul;8(14):e012241.

56. Serrano-Villar S, Estrada V, Gómez-Garre D, Ávila M, Fuentes-Ferrer M, San RJ, et al. Diagnosis of subclinical atherosclerosis in HIV-infected patients: higher accuracy of the D:A:D risk equation over Framingham and SCORE algorithms. Eur J Prev Cardiol. 2014 Jun;21(6):739–48.

57. Koenig W, Löwel H, Baumert J, Meisinger C. C-reactive protein modulates risk prediction based on the Framingham Score: implications for future risk assessment: results from a large cohort study in southern Germany. Circulation. 2004 Mar;109(11):1349–53.

58. Nkambule BB, Mxinwa V, Mkandla Z, Mutize T, Mokgalaboni K, Nyambuya TM, et al. Platelet activation in adult HIV-infected patients on antiretroviral therapy: a systematic review and meta-analysis. BMC Med [Internet]. 2020 Nov 18;18(1):357. Available from: https://pubmed.ncbi.nlm.nih.gov/33203400

59. Taylor KA, Smyth E, Rauzi F, Cerrone M, Khawaja AA, Gazzard B, et al. Pharmacological impact of antiretroviral therapy on platelet function to investigate human immunodeficiency virus-associated cardiovascular risk. Br J Pharmacol. 2019;176(7):879–89.

60. Khawaja AA, Taylor KA, Lovell AO, Nelson M, Gazzard B, Boffito M, et al. HIV Antivirals Affect Endothelial Activation and Endothelial-Platelet Crosstalk. Circ Res. 2020 Nov;127(11):1365–80

Chapter Prologue

The next chapter is presented as an original manuscript addressing the association between platelet activation, endothelial activation and cardiovascular risk in WLHIV and obesity.

CHAPTER 3: EXPERIMENTAL PAPER

Platelet activation and cardiovascular-risk in antiretroviral therapy-treated women living with HIV and obesity

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Abstract

Background: Women living with HIV (WLHIV) in South Africa have the highest prevalence of obesity than to men. Obesity is an independent risk factor for metabolic disease and is associated with increased risk of cardiovascular disease (CVD). CVD-related complications. Therefore, this study aimed to assess the association between platelet activation, endothelial activation, and cardiovascular risk in WLHIV and obesity in South Africa.

Methods: In this study, 33 normal weight (18.50-24.99 kg/m^2) and 33 overweight/obese (≥25 kg/m^2) female participants living with HIV were co-enrolled in the Integration of cardiovascular disease SCreening and prevention in the HIV Management plan for women of reproductive age (ISCHeMia) study which is a sub-study of a prospective multi-country PEPFAR PROMise Ongoing Treatment Evaluation (PROMOTE) study from the Umlazi clinical research site. In this sub-study, the time of blood draws ranged from December 2018- November 2019. We measured the levels of high sensitivity c-reactive protein (hsCRP), lipid profiles (HDL, LDL, TC and TG), platelet activation (sP-selectin, sCD36 and PF-4) and markers of endothelial activation (ET-1 and vWF).

Results: Woman living with HIV and obesity displayed significantly elevated levels of soluble CD36 4.36[2.71-9.53] when compared to the control group 2.79[2.24-3.55] , p=0.0064. In addition, the levels of von Willebrand Factor (vWF) were elevated in WLHIV and obesity 8.83[1.59-9.78] when compared to controls 5.34[0.65-7.7] p=0.0009. However, the levels of soluble P-selectin, PF4 and endothelin-1 were comparable between two study groups (p>0.05). Lastly, the levels of hsCRP levels were significantly higher in WLHIV and obesity (7.71±9.95) when compared to controls (3.68±5.89) p= 0.0005.

Conclusion: WLHIV and obesity are at an increased risk of developing CVD-related complications despite the success of ART. Therefore, measurements of platelet and endothelial activation may be of value in predicting CVD-risk in WLHIV and obesity thus preventing CVD-related complications.

Keywords: Cardiovascular disease, platelet activation, endothelial activation, obesity, HIV

Introduction

People living with HIV (PLWH) on antiretroviral therapy (ART) are at an increased risk of developing cardiovascular disease (CVD) compared to their ART-naïve or HIV-negative counterparts with similar CVD-risk profile (1,2). The mechanism underlying CVD-risk in PLWH on ART is not entirely understood but has been attributed to a higher prevalence of modifiable risk factors for CVD prior ART initiation. These include smoking, obesity, hypertension, and dyslipidaemia (3). Several antiretroviral drugs are associated with weight gain and dyslipidaemia (4), with a disproportionately greater incremental effect of each unit increase in BMI on risk of diabetes in PLWH (5,6). In large epidemiologic studies, body mass index (BMI) has been reported as a strong predictor for cardiovascular events in PLWH (7–9).

The prevalence of modifiable risk factors such as obesity and dyslipidaemia and HIV-related risk factors including chronic immune activation and inflammation have been reported in PLWH on successful ART (8). However, the mechanisms that link immune activation, inflammation, endothelial dysfunction, and an increased risk of CVD in HIV infection remain elusive (10,11). Platelet activation has been implicated as a possible link (8,12) and the off target effects of some antiretroviral drugs(13),(14) . In our previous work, we have reported on increased platelet activation and platelet hyperreactivity in PLWH, which persists despite successful ART (15–17).

Previous studies have reported high prevalence and incidence of obesity in PLWH, with women having a disproportionate burden of obesity compared to men (18,19). Also, a relatively high rate of metabolic syndrome (a risk factor for CVD) has been reported in women compare to men in Sub-Sahara (20–22). There is growing evidence suggesting that platelets, through complex interaction with intact endothelial cells plays a major role in the initiation of the atherosclerosis(23,24). Platelets play an important role in thrombotic and inflammatory processes (10). The circulation of activated platelets has been shown to be one of the initial causes of atherosclerosis development. This is mediated by the interaction between the glycoprotein IIb-IIIa (GPIIb-IIIa) of activated platelets, ICAM-1 in endothelial cells(25) and P-selectin (CD62P) expressed in activated platelets which bind to the endothelium (26).Although a link between platelet function and CVD risk in PLWH has been investigated (27), the association between platelet activation, endothelial activation and CVD-risk in PLWH and obesity remains unclear. Therefore, this study aimed to assess the association between platelet activation, endothelial activation, and cardiovascular risk in WLHIV and obesity on ART.

Methods

Study design and Participants

The study included adult WLHIV co-enrolled in the Integration of cardiovascular disease SCreening and prevention in the HIV MAnagement plan for women of reproductive age (ISCHeMia) study which is the sub-study of the prospective multi-country PEPFAR PROMise Ongoing Treatment Evaluation (PROMOTE) study from the Umlazi clinical research site, Durban, South Africa. The ISCHeMia study is a quasi-experimental study comparing a primary health care intervention plan guided by the WHO PEN (Package of Essential Non-Communicable (PEN) Disease Interventions) in poor resource settings, with usual care in adult WLHIV. Eligibility criteria for the ISCHeMiA study included adult women who were younger than 50 years of age and on ART for at least 1 year. A full description of the ISCHeMiA study design and participant recruitment process, inclusion, and exclusion criteria have been provided in Appendix F. A complete description of the PROMOTE study is described elsewhere (28). In this additional sub-study analysis of stored blood specimens, 66 WLHIV were stratified into two major subgroups based on the World Health Organization (WHO) classification of body mass index (BMI). Our study sample comprised of 30 normal weight (18.50-24.99 kg/m^2) and 27 overweight/obese (≥25 kg/m^2) WLHIV and obesity. The time of blood draws ranged from December 2018- November 2019. This sub study was approved by the University of KwaZulu-Natal Biomedical Research Ethics Committee (BREC) study approval number (BFC220/18).

Anthropometric measurements

The measurements were conducted according to a standard protocol, and these included standing height, waist circumference and weight. Waist circumference was measured using a measuring tape, placed horizontally around the abdomen immediately above the iliac crest at the level of the umbilicus. BMI was calculated using the formula: weight(kg)/height(m^2). We categorized BMI of 18.50-24.99 kg/m^2 as normal and BMI of (≥25 kg/m^2) as overweight or obese. Patients with a measured systolic blood pressure (SBP) ≥140 mm-Hg and/or diastolic blood pressure (DBP) ≥90 mm-Hg were considered hypertensive. Smoking status and history of CVD were obtained through questionnaires or recorded from medical records.

Blood collection and plasma preparation

Whole blood was collected using acid-citrate dextrose (ACD) 1ml tubes and centrifuged (Hettich, Fohrenstrabe, Tuttlingen, Germany) at 130 g for 15 minutes to obtain platelet-rich plasma (PRP). Plasma was transferred into 1 ml tubes (BD Vacutainer, San Jose, CA) and sent to Neuberg Global laboratory, Amanzimtoti, South Africa for biochemical assays. Neuberg Global Laboratory is South African National Accreditation System (SANAS) accredited clinical laboratory.

Blood glucose and lipid measurements

The serum levels of glucose were measured using the enzymatic hexokinase method (Beckman Coulter, CA, USA). High-density lipoprotein cholesterol (HDL-CA, USA) levels were measured via enzymatic immunoinhibition – End Point (Beckman Coulter, USA). Low-density lipoprotein cholesterol (LDL-C) (mmol/L) were measured by enzymatic selective protection – End Point (Beckman Coulter, CA, USA). Triglycerides (TG) (mmol/L) were estimated using Beckman Coulter AU analyser (Beckman AU, Beckman Coulter, USA).

High sensitivity CRP measurements

High sensitivity CRP was measured using the Beckman Coulter AU analyser (Beckman Coulter, CA, USA) with a detection limit of 0.2–160 mg/L.

HIV-1 RNA and CD4 T cell count measurements

HIV-1 RNA (cp/mL) was measured using COBAS AmpliPrep/COBAS TaqMan HIV-1 test (Roche Diagnostics, Indianapolis, USA). CD4 count was determined using the Becton Dickinson Facscalibur flow cytometer (BD Bioscience,NJ USA). Both HIV-1 RNA and CD4 count testing were performed by the SANAS-accredited CAPRISA Research Laboratory.

CVD-risk assessments

To assess the CVD risk profile of WLHIV and obesity, we made use of the Framingham 5- and 10-year CVD risk equation (28). We also made use of the Data collection on Adverse Events of Anti-HIV Drugs Study (D:A:D) coronary heart disease (CHD) equation (29).

Measures of platelet activation

To determine the levels of platelet activation in WLHIV and obesity, the levels of soluble P-selectin (sCD62P), platelet glycoprotein IV (CD36) and Platelet Factor 4 (PF-4) were measured using the enzyme-linked immunosorbent assay (ELISA) (Thermofisher Scientific, Waltham, MA, USA), according to the manufacturer's instructions.

Endothelial activation

To determine the levels of endothelial activation in ART-treated WLHIV and obesity, we measured the levels of von Willebrand Factor (vWF) and Endothelin-1 (ET-1) using an ELISA method (Thermofisher Scientific, Waltham, MA, USA).

Statistical analysis

The primary outcomes of the sub-study were the levels of platelet activation (CD36 and sP-selectin) and endothelial activation (ET-1 and vWF) markers. We estimated that the sample size of 33 normal weight and 33 overweight/obese female participants would provide 81.6% power to detect a difference of 14 ng/ml of sP-selectin between the two independent groups. The sample size estimation assumed a medium to large effect size (*d*) of 0.7 and the alpha (α) set at 0.05. The pooled standard deviation of 21.11 ng/ml was used to compute the Cohen's effect size *(d)*.Independent T-test was used to compare differences between markers of platelet and endothelial activation in WLHIV and obesity and lean WLHIV(sP-selectin). Normality was assessed using Shapiro-Wilk test and one sample Kolmogorov-Smirnov test. For nonparametric data we performed a Mann-Whitney U test. All statistical analyses were performed using Prism 5 (GraphPad software, San Diego, CA). Significance was considered when p was <0.05. Data were reported as mean ± standard deviation (SD) or median interquartile range [IQR].

Results

This study consisted of a total of 66 WLHIV (33 normal weight, and 33 patients with obesity). The characteristics of the study participants are tabulated in Table 1. There were no differences in the age of the included participants across the study groups (p>0.05). Patients with obesity had a larger waist circumference (92.58±13.22) when compared to controls (75.80±5.41), p<0.0001) (shown in Table 1). High sensitivity CRP levels were significantly higher in WLHIV and obesity (7.71±9.95) when compared to controls (3.68±5.89), p= 0.0005. The study groups had a similar mean duration of ART (4 years) with most participants on EFV/FTC/TDF ART regimen and 86% of our participants had a clinically undetectable viral load (HIV-1 RNA ≤20 cp/mL). There were no patients who reported previous history of co-morbidities and/ or on any medication.

Baseline characteristics of haematological parameters

Patients with obesity had a significantly higher red blood cells count (RBC) (4.06±0.38) vs controls (3.87±0.35) p=0.0431, haemoglobin (HGB) (12.43±1.21) vs control group (11.52±1.71), p=0.0296, haematocrit (HCT) (0.38±0.04) vs (0.35±0.04), p=0.0053, and mean corpuscular haemoglobin (MCH) (30.76±2.63 vs 29.68±3.21) p<0.0001 as shown in Table 1.

Elevated LDL-c levels and decreased HDL-c levels in ART-treated WLHIV and obesity

The obese group had significantly higher LDL-c levels (2.44±0.93) compared to the control group (2.02±0.64 (p=0.0383). However, the levels of HDL-c were significantly lower in WLHIV and obesity (1.20±0.30), when compared to lean controls (1.46±0.38, p=0.0.05). The levels of total cholesterol and triglycerides were similar between the two groups (p>0.05).

Elevated levels of CD36 and vWF levels in ART-treated WLHIV and obesity

The levels of platelet activation were determined by measuring the levels of sP-selectin (sCD62P) and CD36 (platelet glycoprotein IV). WLHIV and obesity had significantly higher levels of CD36 4.36[2.71-9.53] compared to the lean controls 2.79[2.24-3.55] p=0.0064 (shown in Figure 1A). However, the levels of sP-selectin (4.43±1.88 vs 5.31±2.74) and levels of PF-4 (19803[19234-2029] vs 20085[19629-20335]) were comparable between the two groups ((Figure 1B and 1C). Interestingly, vWF levels were significantly elevated in WLHIV and obesity 8.83[1.59-9.78] compared to lean controls 5.34[0.65-7.7] p=0.0009. Whereas the levels of ET-1 were comparable between the study groups(4.36[3.58-5.04] vs 3.9[3.14-4.96)]) (p>0.05) (Figure 1D and 1E). When we performed a sensitivity analysis omitting viraemic participants (RNA viral load>20 cp/mL) and smoking, the results obtained were similar. CD36 and vWF levels were elevated in ART treated WLHIV and obesity. In addition, there were no correlation between the markers of platelet and endothelial activation with age and CD4 count. Please see supplementary file 1.

CVD- risk assessment

The median Framingham Risk Score (FRS) for assessing 5-year CVD-risk for WLHIV and obesity was 0.15[0.10-0.43] and 0.10[0.00-0.20] for the lean controls. For long-term 10-year CHD assessment by FRS, WLHIV and obesity had a median 10-year CVD risk of 0.50[0.20-1.10] in comparison to 0.30[0.15-0.50] of lean WLHIV. Lastly the median CVD-risk by D:A:D was 0.20[0.10-0.30] for WLHIV and obesity and 0.10[0.10-0.20] for lean WLHIV.

527 Table 1: Baseline characteristics of included participants (n=66).

	Control group (n=33)	Obese group (n=27)	P-value
Age (years)	32.00±4.51	33.03±6.46	0.4553
BMI (kg/m^2)	22.11±1.94	33.39±6.33	**<0.0001**
Waist circumference(cm)	75.80±5.41	92.58±13.22	**<0.0001**
SBP (mm-Hg)	111.20±11.53	116.6±13.08	0.0814
DBP (mm-Hg)	69.88±12.59	71.91±11.63	0.5028
hsCRP (mg/mL)	3.68±5.89	7.71±9.95	**0.0005**
Blood glucose (mmol/L)	4.39±0.48	4.51±0.32	0.4103
Smoking (n, %)	6 (18.18)	1 (3.03)	-
Alcohol consumption (n, %)	13 (39.43)	8 (24.24)	-
CD4 T cell count (cell/mm^3)	939.80±221.5	875.2±252.80	0.2737
HIV-1 RNA (cp/mL)			-
<20 (n, %)	30 (90.90)	27(90.00)	
>20 (n, %)	3 (9.09)	6(18.18)	
Haematological parameters			
RBC	3.87±0.35	4.06±0.38	**0.0431**
HGB (g/dL)	11.52±1.71	12.43±1.21	**0.0296**
HCT (L/L)	0.35±0.04	0.38±0.04	**0.0053**
MCV (x10^{-15} L)	90.99±7.63	94.52±6.98	0.0713
MCH (x10^{-12} g)	29.68±3.21	30.76±2.63	**<0.0001**
MCHC (g/dL)	32.55±1.19	32.53±1.05	**<0.0001**
WCC	6.19±2.55	6.13±1.42	0.3946
Neutrophil (%)	51.13±9.45	50.82±9.17	0.9708
Lymphocytes (%)	37.68±8.02	37.65±7.09	0.5024
Monocytes (%)	8.08±2.44	7.36±1.80	0.1911
Eosinophils (%)	2.47±2.53	3.61±3.17	0.1394
Basophils (%)	0.63±0.31	0.55±0.31	0.3331
PLT (x10^{-9}/L)	298.90±71.64	291.80±70.96	0.8808
Lipid profiles			
TC (mmol/L)	4.06±0.77	4.06±0.79	0.9980
LDL (mmol/L)	2.02±0.64	2.44±0.93	**0.0383**
HDL (mmol/L)	1.46±0.38	1.20±0.30	**0.0030**
TG (mmol/L)	1.34±3.66	0.90±0.55	0.5412
CVD-risk profile			
5-yr CVD FRS risk (median, IQR)	0.10[0.00-0.20]	0.15[0.10-0.43]	-
10-year CHD risk (median, IQR)	0.30[0.15-0.50]	0.50[0.20-1.10]	-
D:A:D CVD risk (median, IQR)	0.10[0.10-0.20]	0.20[0.10-0.30]	-
Duration of ART (years)	4.12±0.7	4.18±0.73	-

528 Significance ($p<0.05$) shown in boldface. **BMI:** Body mass index, **CHD:** Coronary Heart
529 Disease(FRS), **D:A:D:** Data collection on Adverse Events of Anti-HIV Drugs Study, **DBP**: Diastolic
530 Blood pressure, **FRS**: Framingham risk, **HCT**: Haematocrit, **HDL**: High-density lipoprotein, **HGB:**
531 Haemoglobin, **hsCRP**: High sensitivity C-reactive protein, **LDL**: Low-density lipoprotein, **MCH:**
532 Mean corpuscular haemoglobin, **MCHC:** Mean corpuscular haemoglobin concentration, **MCV:** Mean

corpuscular volume, **PLT:** Platelet count, **RBC**: Red blood cell, **SBP**: Systolic Blood Pressure, **TC:** Total cholesterol, **WCC:** White cell count

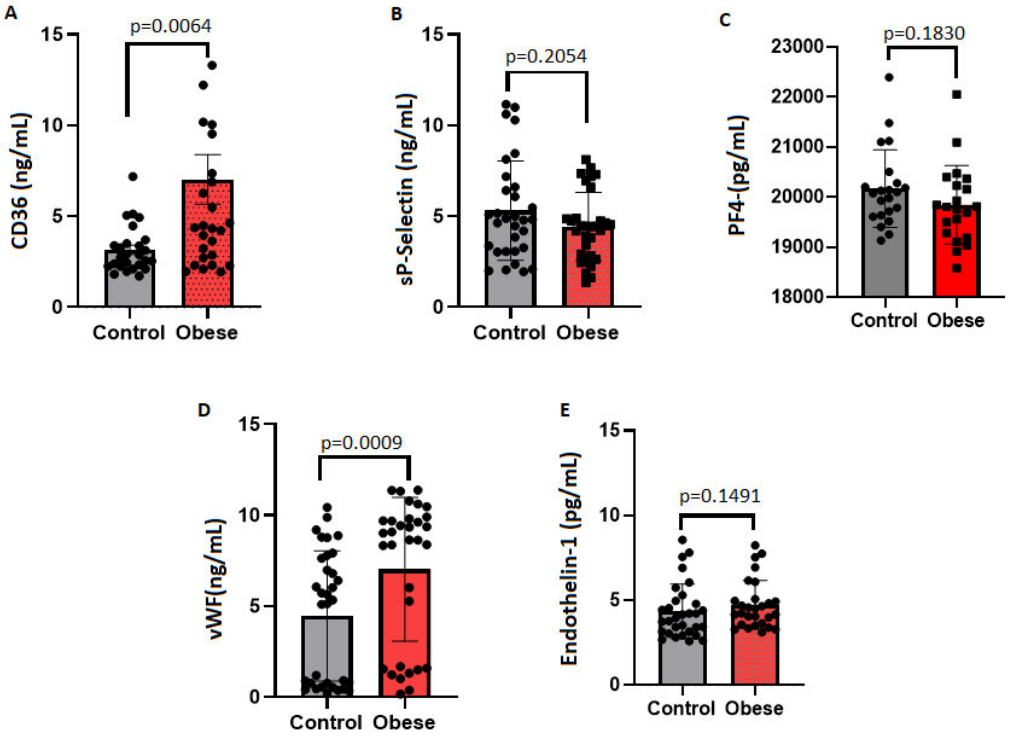

Figure 1: Platelet and endothelial activation in women living with HIV and obesity vs lean women living with HIV (control). The levels of platelet activation are shown in figure A-C. Figure (**A**) demonstrates the levels of sCD36 (sGPIV), while figure (**B**) shows sP-selectin levels and figure (**C**) illustrates the levels of platelet factor-4 (PF4) in woman living with HIV and obesity compared to lean women living with HIV. Figure D-E show the levels of endothelial activation, with figure (**D**) showing von Willebrand Factor (vWF) levels. and figure (**E**) illustrating endothelin-1 (ET-1) levels Data is presented as mean±SD or median (IQR), with a Bonferroni-corrected critical significance threshold set at p-value <0.01.

Discussion

In this study, we evaluated the association between platelet activation, endothelial activation, and cardiovascular risk in WLHIV and obesity. In the general population, high levels of hsCRP are a well-established risk marker for CVD events. Several studies have demonstrated that hsCRP and IL-6 are associated with HIV viral replication and CVD-related risk (30–32). Furthermore, elevated levels of hsCRP have been observed in ART-treated patients when compared to ART-naïve patients (33). Obesity is a pro-inflammatory condition and is associated with increased circulating inflammatory biomarkers (such as IL-6 and hsCRP) (18,34). Notably, in our study the obese group had elevated levels

of hsCRP compared to the lean group. The results of our study significantly extend these findings by demonstrating that obesity, independent of traditional factors is associated with an increased risk of CVD in WLHIV and obesity. In addition, inflammation is reported to be a key process underlying cardiovascular disorders that is accompanied and amplified by activation of platelets and consequent binding of such platelets to the endothelium (35).

Epidemiological studies have reported that high plasma levels of HDL-c protect against the development of atherosclerosis(36). Notably, HDL-c has antithrombotic and anti-inflammatory properties (37,38). In our study, WLHIV and obesity had lower HDL-c levels compared to lean WLHIV. This was expected, due to obesity being a pro-inflammatory condition.

This is the first study to report on the association between platelet activation, endothelial activation and CVD risk in WLHIV and obesity. However, previous studies have reported on elevated levels of platelet activation in ART-treated PLWH compared to uninfected individuals (17,39). Notably, our results demonstrated that a platelet activation marker CD36 was significantly higher in WLHIV and obesity. These findings were not affected by the inclusion of viraemic participants or those who were current smokers (Supplementary File 1). In our study the levels of sP-selectin and PF-4 were comparable between lean women and those living with obesity. Notably in a previous study by Gori et al., which included ART-naïve and ART-treated patients, reported elevated levels of sP-selectin and PF-4 in PLWH (40).

Markers of endothelial activation such as soluble intercellular and vascular cell adhesion molecules are associated with CVD-risk in the general population and are elevated in PLWH compared to their negative counterparts (41–43). These markers indicate chronic endothelial activation and subsequent endothelial dysfunction, which results in inflammation (41). In addition, CRP is thought to induce the secretion of markers of endothelial activation (44). The CRP-induced secretion of endothelial activation markers is inhibited by HDL-c (45). Our study showed lower HDL-c and higher levels of hsCRP and vWF in WLHIV and obesity compared to lean WLHIV. Therefore, an inflammatory response was thereby clearly activated, resulting in endothelial injury. However, unexpected results were that, although there has been evidence of chronic inflammation and endothelial damage, the levels of ET-1 were comparable between WLHIV and obesity and lean WLHIV.

Markers of endothelial activation such as soluble intercellular and vascular cell adhesion molecules are associated with CVD-risk in the general population and are elevated in PLWH compared to their negative counterparts (41–43). These markers indicate chronic endothelial activation and subsequent endothelial dysfunction, which results in inflammation (41). In addition, CRP is thought to induce the secretion of markers of endothelial activation (44). The CRP-induced secretion of endothelial activation markers is inhibited by HDL-c (45). Our study showed lower HDL-c and higher levels of hsCRP and

vWF in WLHIV and obesity compared to lean WLHIV. Therefore, an inflammatory response was thereby clearly activated, resulting in endothelial injury. However, unexpected results were that, although there has been evidence of chronic inflammation and endothelial damage, the levels of ET-1 were comparable between WLHIV and obesity and lean WLHIV.

To date there are limited studies on platelet activation, endothelial activation CVD in WLHIV and obesity. Although this the first study to report on the associations between platelet activation in WLHIV and obesity, this study had several limitations which include the lack of in-depth platelet phenotyping. Our measurements of platelet activation were restricted to soluble markers which do not infer platelet dysfunction. Future longitudinal studies aimed at assessing platelet function and platelet phenotypes in WLHIV and obesity are needed to ascertain the clinical relevance of elevated platelet activation and endothelial activation in this distinct population of WLHIV.

Conclusion

The levels of platelet and endothelial activation are elevated in WLHIV and obesity despite successful ART Moreover, the levels of inflammation were high in obese WLHIV on ART. Therefore, WLHIV and obesity are at an increased risk of developing CVD.

Author's Contribution

S Mfusi, B Nkambule and S Hanley conceptualized, designed the study, and drafted the manuscript. All authors wrote and approved the final manuscript. BBN is the guarantor of the experimental paper.

Contribution to the larger study

Mfusi SA prepared the first draft; Mfusi performed the laboratory analysis (platelet and endothelial activation measurements); Mfusi S and Nkambule B performed the statistical analyses of the data obtained. Anthropometry measurements, medical history, blood collection and CVD risk scoring were completed by Hanley S and the PROMOTE study team. The CAPRISA laboratory performed plasma preparation while Neuberg Global Laboratories performed blood glucose, lipid and High-sensitivity CRP measurements.

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Competing interest

None declared

620 **References**

621 1. Islam FM, Wu J, Jansson J, Wilson DP. Relative risk of cardiovascular disease among people
622 living with HIV: a systematic review and meta-analysis. HIV Med. 2012 Sep;13(8):453–68.

623 2. World Health Organization. Prevention of Cardiovascular Disease Pocket Guidelines for
624 Assessment and Management of Cardiovascular Risk Predicting [Internet]. World Health
625 Organization. Geneva PP - Geneva: World Health Organization; 2007. p. 1–30. Available
626 from: https://apps.who.int/iris/handle/10665/43685

627 3. Negin J, Martiniuk A, Cumming RG, Naidoo N, Phaswana-Mafuya N, Madurai L, et al.
628 Prevalence of HIV and chronic comorbidities among older adults. AIDS. 2012;26(0 1):S55.

629 4. Bakal DR, Coelho LE, Luz PM, Clark JL, De Boni RB, Cardoso SW, et al. Obesity following
630 ART initiation is common and influenced by both traditional and HIV-/ART-specific risk
631 factors. J Antimicrob Chemother. 2018;73(8):2177–85.

632 5. De Wit S, Sabin CA, Weber R, Worm SW, Reiss P, Cazanave C, et al. Incidence and risk
633 factors for new-onset diabetes in HIV-infected patients: the Data Collection on Adverse
634 Events of Anti-HIV Drugs (D:A:D) study. Diabetes Care. 2008 Jun;31(6):1224–9.

635 6. Capeau J, Bouteloup V, Katlama C, Bastard J-P, Guiyedi V, Salmon-Ceron D, et al. Ten-year
636 diabetes incidence in 1046 HIV-infected patients started on a combination antiretroviral
637 treatment. AIDS. 2012 Jan;26(3):303–14.

638 7. Womack JA, Chang CCH, So-Armah KA, Alcorn C, Baker J V., Brown ST, et al. HIV
639 infection and cardiovascular disease in women. J Am Heart Assoc. 2014;3(5):1–8.

640 8. Freiberg MS, Chang C-CH, Kuller LH, Skanderson M, Lowy E, Kraemer KL, et al. HIV
641 infection and the risk of acute myocardial infarction. JAMA Intern Med. 2013
642 Apr;173(8):614–22.

643 9. Friis-Møller N, Reiss P, Sabin CA, Weber R, Monforte A d'Arminio, El-Sadr W, et al. Class
644 of antiretroviral drugs and the risk of myocardial infarction. N Engl J Med. 2007
645 Apr;356(17):1723–35.

646 10. UNAIDS epidemiological estimates. 2020;

647 11. GBD 2016 Occupational Risk Factors Collaborators. Global and regional burden of disease
648 and injury in 2016 arising from occupational exposures: a systematic analysis for the Global
649 Burden of Disease Study 2016. Occup Environ Med [Internet]. 2020 Mar 1;77(3):133 LP –

141. Available from: http://oem.bmj.com/content/77/3/133.abstract

12. Furie B, Furie BC. Mechanisms of thrombus formation. N Engl J Med. 2008 Aug;359(9):938–49.

13. Khawaja AA, Taylor KA, Lovell AO, Nelson M, Gazzard B, Boffito M, et al. HIV Antivirals Affect Endothelial Activation and Endothelial-Platelet Crosstalk. Circ Res. 2020 Nov;127(11):1365–80.

14. Taylor KA, Smyth E, Rauzi F, Cerrone M, Khawaja AA, Gazzard B, et al. Pharmacological impact of antiretroviral therapy on platelet function to investigate human immunodeficiency virus-associated cardiovascular risk. Br J Pharmacol. 2019;176(7):879–89.

15. Nkambule BB, Davison G, Ipp H. The value of flow cytometry in the measurement of platelet activation and aggregation in human immunodeficiency virus infection. Platelets. 2015;26(3):250–7.

16. Nkambule BB, Davison GM, Ipp H. The evaluation of platelet function in HIV infected, asymptomatic treatment-naïve individuals using flow cytometry. Thromb Res [Internet]. 2015;135(6):1131–9. Available from: http://europepmc.org/abstract/MED/25900311

17. Nkambule BB, Mxinwa V, Mkandla Z, Mutize T, Mokgalaboni K, Nyambuya TM, et al. Platelet activation in adult HIV-infected patients on antiretroviral therapy: a systematic review and meta-analysis. BMC Med [Internet]. 2020 Nov 18;18(1):357. Available from: https://pubmed.ncbi.nlm.nih.gov/33203400

18. Koethe JR, Grome H, Jenkins CA, Kalams SA, Sterling TR. The metabolic and cardiovascular consequences of obesity in persons with HIV on long-term antiretroviral therapy. AIDS [Internet]. 2016 Jan 2;30(1):83–91. Available from: https://pubmed.ncbi.nlm.nih.gov/26418084

19. Senekal M, Steyn NP, Nel JH. Factors associated with overweight/obesity in economically active South African populations. Ethn Dis. 2003;13(1):109–16.

20. McCormick CL, Francis AM, Iliffe K, Webb H, Douch CJ, Pakianathan M, et al. Increasing Obesity in Treated Female HIV Patients from Sub-Saharan Africa: Potential Causes and Possible Targets for Intervention. Front Immunol [Internet]. 2014 Nov 13;5:507. Available from: https://pubmed.ncbi.nlm.nih.gov/25431572

21. Alencastro PR, Fuchs SC, Wolff FH, Ikeda ML, Brandão ABM, Barcellos NT. Independent

predictors of metabolic syndrome in HIV-infected patients. AIDS Patient Care STDS. 2011;25(11):627–34.

22. Muyanja D, Muzoora C, Muyingo A, Muyindike W, Siedner MJ. High Prevalence of Metabolic Syndrome and Cardiovascular Disease Risk among People with HIV on Stable ART in Southwestern Uganda. AIDS Patient Care STDS. 2016;30(1):4–10.

23. von Hundelshausen P, Weber C. Platelets as immune cells: bridging inflammation and cardiovascular disease. Circ Res. 2007 Jan;100(1):27–40.

24. Smyth SS, McEver RP, Weyrich AS, Morrell CN, Hoffman MR, Arepally GM, et al. Platelet functions beyond hemostasis. J Thromb Haemost. 2009 Nov;7(11):1759–66.

25. Icam- AM, Bombeli BT, Schwartz BR, Harlan JM. Adhesion of Activated Platelets to Endothelial Cells : Evidence for a GPIIbIIIa-dependent Bridging Mechanism and Novel Roles for Endothelial Intercellular. 1998;187(3).

26. Huo Y, Schober A, Forlow SB, Smith DF, Hyman MC, Jung S, et al. Circulating activated platelets exacerbate atherosclerosis in mice deficient in apolipoprotein E. Nat Med. 2003 Jan;9(1):61–7.

27. Triant VA. HIV infection and coronary heart disease: an intersection of epidemics. J Infect Dis. 2012 Jun;205 Suppl(Suppl 3):S355-61.

28. Taha TE, Yende-Zuma N, Aizire J, Chipato T, Ogwang LW, Makanani B, et al. The multi-country promote HIV antiretroviral treatment observational cohort in sub-Saharan Africa: Objectives, design, and baseline findings. PLoS One. 2018;13(12):1–14.

29. D'Agostino RBS, Vasan RS, Pencina MJ, Wolf PA, Cobain M, Massaro JM, et al. General cardiovascular risk profile for use in primary care: the Framingham Heart Study. Circulation. 2008 Feb;117(6):743–53.

30. Friis-Møller N, Thiébaut R, Reiss P, Weber R, Monforte AD, De Wit S, et al. Predicting the risk of cardiovascular disease in HIV-infected patients: the data collection on adverse effects of anti-HIV drugs study. Eur J Cardiovasc Prev Rehabil Off J Eur Soc Cardiol Work Groups Epidemiol Prev Card Rehabil Exerc Physiol. 2010 Oct;17(5):491–501.

31. Neuhaus J, Jacobs DRJ, Baker J V, Calmy A, Duprez D, La Rosa A, et al. Markers of inflammation, coagulation, and renal function are elevated in adults with HIV infection. J Infect Dis. 2010 Jun;201(12):1788–95.

32. Kuller LH, Tracy R, Belloso W, De Wit S, Drummond F, Lane HC, et al. Inflammatory and coagulation biomarkers and mortality in patients with HIV infection. PLoS Med. 2008 Oct;5(10):e203.

33. Baker J V, Duprez D. Biomarkers and HIV-associated cardiovascular disease. Curr Opin HIV AIDS [Internet]. 2010 Nov;5(6):511–6. Available from: https://pubmed.ncbi.nlm.nih.gov/20978394

34. Guimarães MMM, Greco DB, Figueiredo SM de, Fóscolo RB, Oliveira AR de J, Machado LJ de C. High-sensitivity C-reactive protein levels in HIV-infected patients treated or not with antiretroviral drugs and their correlation with factors related to cardiovascular risk and HIV infection. Atherosclerosis. 2008 Dec;201(2):434–9.

35. Kern PA, Ranganathan S, Li C, Wood L, Ranganathan G. Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance. Am J Physiol Endocrinol Metab. 2001 May;280(5):E745-51.

36. van Gils JM, Zwaginga JJ, Hordijk PL. Molecular and functional interactions among monocytes, platelets, and endothelial cells and their relevance for cardiovascular diseases. J Leukoc Biol. 2009 Feb;85(2):195–204.

37. Gordon T, Castelli WP, Hjortland MC, Kannel WB, Dawber TR. High density lipoprotein as a protective factor against coronary heart disease. The Framingham Study. Am J Med. 1977 May;62(5):707–14.

38. Patel S, Puranik R, Nakhla S, Lundman P, Stocker R, Wang XS, et al. Acute hypertriglyceridaemia in humans increases the triglyceride content and decreases the anti-inflammatory capacity of high density lipoproteins. Atherosclerosis. 2009 Jun;204(2):424–8.

39. Negre-Salvayre A, Dousset N, Ferretti G, Bacchetti T, Curatola G, Salvayre R. Antioxidant and cytoprotective properties of high-density lipoproteins in vascular cells. Free Radic Biol Med. 2006 Oct;41(7):1031–40.

40. Mesquita EC, Hottz ED, Amancio RT, Carneiro AB, Palhinha L, Coelho LE, et al. Persistent platelet activation and apoptosis in virologically suppressed HIV-infected individuals. Sci Rep [Internet]. 2018 Oct 9;8(1):14999. Available from: https://pubmed.ncbi.nlm.nih.gov/30301959

41. Gori, E, Jani, B, Quaye, I, Nyagura, M Mduluza, T Gomo Z. Platelet activation and inflammation markers as emerging risk. Cent Afr J Med [Internet]. 2017;63(1–3):15–23. Available from: https://www.ajol.info/index.php/cajm/article/view/160416

741 42. Melendez MM, McNurlan MA, Mynarcik DC, Khan S, Gelato MC. Endothelial adhesion
742 molecules are associated with inflammation in subjects with HIV disease. Clin Infect Dis an
743 Off Publ Infect Dis Soc Am. 2008 Mar;46(5):775–80.

744 43. de Gaetano Donati K, Rabagliati R, Iacoviello L, Cauda R. HIV infection, HAART, and
745 endothelial adhesion molecules: current perspectives. Lancet Infect Dis. 2004 Apr;4(4):213–
746 22.

747 44. Baker J, Ayenew W, Quick H, Hullsiek KH, Tracy R, Henry K, et al. High-density lipoprotein
748 particles and markers of inflammation and thrombotic activity in patients with untreated HIV
749 infection. J Infect Dis. 2010 Jan;201(2):285–92.

750 45. Liang Y-J, Shyu K-G, Wang B-W, Lai L-P. C-reactive protein activates the nuclear factor-
751 kappaB pathway and induces vascular cell adhesion molecule-1 expression through CD32 in
752 human umbilical vein endothelial cells and aortic endothelial cells. J Mol Cell Cardiol. 2006
753 Mar;40(3):412–20.

754 46. Wadham C, Albanese N, Roberts J, Wang L, Bagley CJ, Gamble JR, et al. High-density
755 lipoproteins neutralize C-reactive protein proinflammatory activity. Circulation. 2004
756 May;109(17):2116–22.

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CHAPTER 4: SYNTHESIS

The global prevalence of HIV-associated CVD has disproportionally increased over the past two decades, with Sub-Saharan Africa being the worst affected region (1,2). People living with HIV present with a 1.5- to 2-fold higher risk of CVD compared with uninfected individuals (3). Endothelial activation has been reported in several conditions associated with enhanced risk of CVD and atherosclerosis (4). In PLWH endothelial activation has been reported shortly after the diagnosis. Notably, increased levels of endothelial activation markers (ICAM, VCAM) (5,6) and a marker of platelet activation (sP-selectin and PF-4) has been reported in PLWH (7). This study aimed to assess the prognostic factors associated with CVD in ART-treated PLWH and obesity and the association between platelet activation, endothelial activation and CVD-risk in WLHIV.

In our systematic review we reported on BMI, age, sex and IL-6 levels as prognostic factors strongly associated with cardiometabolic risk in PLWH and obesity. However, only CD4 counts, and IL-6 were confirmed prognostic factors of CVD-risk. Since IL-6 was the only non-HIV specific confirmed prognostic factor, this may suggest that persistent inflammation plays a major role in the development of CVD in PLWH and obesity on ART.

Although the prevalence of metabolic syndrome is more prevalent in females in our systematic review and meta-analysis, sex was not confirmed as a prognostic factor. Previous studies have reported on a higher prevalence of obesity in women; hence we evaluated the association between platelet and endothelial activation and CVD-risk in ART-treated South African WLHIV and obesity. In our findings, WLHIV and obesity had elevated levels of endothelial activation and platelet activation compared to the lean WLHIV. However, more noticeable finding was that levels of sP-selectin, a common marker of both platelet and endothelial activation were comparable between the control group and the obese group. This provides a new insight, highlighting persistent platelet and endothelial activation in WLHIV which may account for increased CVD-risk despite lower risk scores (8).

The main strength of our study is that our meta-analysis included the quality of the included studies and the methodological approach used to provide pooled effect estimates derived from multivariate analysis and to our knowledge, this was the first study to report on the associations between platelet activation, endothelial activation and CVD-risk in WLHIV and obesity. There are a few caveats that should be considered in the interpretation and generalizability of the findings our study Firstly, our systematic review and meta-analysis had methodological limitations due to limited number of studies reporting on similar prognostic factors in PLWH and obesity. Therefore, our random effects meta-analysis and subgroup analysis was limited to the reported prognostic factor and further exploration of sources of heterogeneity such geographical and clinical differences were not determined. Lastly, caution should

be taken when interpreting the confirmed prognostic factors as these were restricted to populations predominantly derived from the North America, and Europe. Therefore, future multi-ethnic prospective cohort studies are required to determine the predictive value and relevance of these reported prognostic factors in PLWH and obesity.

In addition, the experimental study had several limitations which include the lack of in-depth platelet phenotyping. Our measurements of platelet activation were restricted to soluble markers which do not infer platelet dysfunction. To date there are limited studies on platelet activation, endothelial activation CVD in WLHIV and obesity. Therefore, future longitudinal studies aimed at assessing platelet function and platelet phenotypes in WLHIV and obesity are needed to ascertain the clinical relevance of elevated platelet activation and endothelial activation in this distinct population of WLHIV.

Our results suggest that women faced with a dual epidemic of HIV and obesity, may be at a high risk of developing CVD related complications. Therefore, these findings add to the evidence to linking increased CVD-risk and obesity in WLHIV and obesity despite successful ART. Notably, the measurement of platelet and endothelial activation levels of inflammatory biomarkers may be of value in the risk stratification and modelling of CVD-risk in PLWH and obesity.

References

1. Mensah GA, Roth GA, Sampson UKA, Moran AE, Feigin VL, Forouzanfar MH, et al. Mortality from cardiovascular diseases in sub-Saharan Africa, 1990-2013: a systematic analysis of data from the Global Burden of Disease Study 2013. Cardiovasc J Afr. 2015;26(2 Suppl 1):S6-10.
2. Roth, Gregory A, Huffman, Mark D, Moran, Andrew E, Feigin, Valery, Mensah, George A, Naghavi, Mohsen Murray CJL. Global and regional patterns in cardiovascular mortality from 1990 to 2013. Circulation. 2015 Oct;132(17):1667–78.
3. Bloomfield GS, Alenezi F, Barasa FA, Lumsden R, Mayosi BM, Velazquez EJ. Human Immunodeficiency Virus and Heart Failure in Low- and Middle-Income Countries. JACC Heart Fail. 2015 Aug;3(8):579–90.
4. Gresele P, Falcinelli E, Sebastiano M, Baldelli F. Endothelial and platelet function alterations in HIV-infected patients. Thromb Res. 2011 Dec 20;129:301–8.
5. Melendez MM, McNurlan MA, Mynarcik DC, Khan S, Gelato MC. Endothelial adhesion molecules are associated with inflammation in subjects with HIV disease. Clin Infect Dis an Off Publ Infect Dis Soc Am. 2008 Mar;46(5):775–80.
6. Calza L, Pocaterra D, Pavoni M, Colangeli V, Manfredi R, Verucchi G, et al. Plasma levels of VCAM-1, ICAM-1, E-Selectin, and P-Selectin in 99 HIV-positive patients versus 51 HIV-negative healthy controls. Vol. 50, Journal of acquired immune deficiency syndromes (1999). United States; 2009. p. 430–2.
7. Gori, E, Jani, B, Quaye, I, Nyagura, M Mduluza, T Gomo Z. Platelet activation and inflammation markers as emerging risk. Cent Afr J Med [Internet]. 2017;63(1–3):15–23. Available from: https://www.ajol.info/index.php/cajm/article/view/160416
8. Muyanja D, Muzoora C, Muyingo A, Muyindike W, Siedner MJ. High Prevalence of Metabolic Syndrome and Cardiovascular Disease Risk among People with HIV on Stable ART in Southwestern Uganda. AIDS Patient Care STDS. 2016;30(1):4–10

APPENDIX A: A PROTOCOL FOR A SYSTEMATIC REVIEW AND META-ANALYSIS

Cardiovascular-risk in antiretroviral therapy-treated patients living with HIV and obesity: A protocol for a systematic review and meta-analysis of prognostic factor studies

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Abstract

Introduction: The incidence of cardiovascular disease (CVD) is now at least three-fold higher in antiretroviral therapy (ART)-treated patients living with HIV and obesity compared with the general population. Therefore, this systematic review and meta-analysis will provide a comprehensive synthesis of prognostic factors in patients living with HIV and obesity.

Method: A comprehensive search will be conducted using medical subject headings for MEDLINE, adapted to the EBSCO host database. Two reviewers (SAM and ZAM) will independently screen studies. Data items will be extracted using a predefined data extraction sheet. Moreover, the risk of bias and quality of the included studies will be assessed using the Quality in Prognostic Studies (QUIPS) tool. While the quality and strengths of evidence across the selected studies will be evaluated using the Grading of Recommendations Assessment Development and Evaluation (GRADE) approach. The Cochran's Q statistic and the I^2 statistics will be used to analyze statistical heterogeneity across studies. If the included studies show substantial level of statistical heterogeneity (I^2>25%), a random-effects meta-analysis will be performed.

Ethics and dissemination: This systematic review and meta-analysis will not require ethical approval, and the findings will be published in peer-reviewed journals.

Systematic review registration: PROSERO number: CRD42021234560.

Keywords: Platelets, cardiovascular disease, obesity, antiretroviral therapy

Introduction

The incidence of human immunodeficiency virus (HIV) infections remains a significant challenge in developing countries [1]. In the past decade, considerable efforts made towards increasing the roll-out and access to antiretroviral therapy (ART) have yielded a significant reduction in acquired immunodeficiency syndrome (AIDS)-related mortality and an overall improvement in the quality of life in people living with HIV (PLWH)[2,3]. However, an increasing incidence of noncommunicable disease (NCD) has emerged in the ageing population of PLWH on ART [4,5]. This incidence is partly driven by a higher prevalence of modifiable risk factors for cardiovascular disease (CVD) before ART initiation, which includes smoking, obesity, hypertension and dyslipidaemia in PLWH[6]. Several antiretroviral drugs are associated with weight gain and dyslipidaemia [7]. Notably, the body mass index (BMI) is associated with an exacerbated inflammatory response, particularly in PLWH who are on ART [10,13]. Despite CVD and obesity being common in the PLWH population, the risk factors associated with poor clinical outcomes in ART-treated patients remain unclear.

Obesity and dyslipidaemia in PLWH are common in both treatment-naïve and treated patients[7,8]. In PLWH, altered lipid profiles are characterized by hypertriglyceridemia and decreased high-density cholesterol levels[9]. This atherogenic lipid phenotype is independently associated with poor patient outcomes following statin-based lipid-lowering therapy[10]. Recent studies have reported on the changes in risk factors of CVD in ART-treated patients[11,12]. Notably, contradictory findings on the synergy between HIV infection and noncommunicable disease on the traditional risk factors of CVD in PLWH exist[13–15]. Although the evidence on the association of ART and CVD risk has been synthesized[16,17] , the predictive value of these risk factors in PLWH and obesity remains unclear. Therefore, this systematic review and meta-analysis will provide a timely comprehensive synthesis of the prognostic factors of CVD in PLWH and obesity. Moreover, in this systematic review and meta-analysis, we will assess the predictive value of the traditional risk factors in both treatment-naïve and treated patients.

Research Question

i) What are the prognostic factors strongly associated with poor clinical outcomes in PLWH and obesity?

ii) Are synergistic effects of ART and obesity on traditional risk factors for CVD?

Objective

i) To assess the predictive value of prognostic factors associated with CVD in ART-treated patients living with obesity

Methods

This protocol for systematic review was prepared following the Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) 2015 guideline[18]. Systematic review PROSERO registration number: CRD42021234560.

Eligibility criteria

Types of studies

Both randomized and non-randomized controlled trials will be included. In addition, retrospective and prospective cohort studies will also be included.

Exclusion criteria

Reviews and case studies will be excluded.

Participants

This review will include ART-treated adults (defined as 18 years and older) living with HIV and obesity

Index prognostic factor

The predictive factors included in the Framingham risk score[19] and the World Health Organisation (WHO) risk prediction charts[20]

Comparators

We will consider the following comparators,

1. Uninfected adults with normal body weights
2. Treatment naïve and treated PLWH and obesity

Outcome

Primary outcome

1. Fatal or non-fatal CVD, reported as odds ratio (OR) or hazards ratio (HR)

Timing and setting

Predictive markers at baseline measurements before the initiation of ART and after treatment will be considered. In addition, both inpatient and outpatient cohorts will be included.

Search strategy and study selection

A comprehensive search strategy will be developed using medical subject headings (MeSH) for MEDLINE, and this will be adapted for the EBSCOhost search engine. We will search the databases from inception to the 30th of April 2021. The search strategy will consist of search terms that include obesity, cardiovascular diseases, HIV-infection, Platelet P-selectin, antiretroviral therapy (Supplementary file 2). Two independent reviewers (SAM and ZAM) will search and select the relevant studies. In cases of disagreement, a third reviewer (BBN) will be consulted for arbitration.

Data management

Data items

The reviewers (SAM and ZAM) will develop a data extraction that will include the following data items; first author's name, year of publication, country, study design, aim of the study, primary outcome, and main findings study. The reviewers will also independently carry out data extraction and check for the correctness of all extracted data items. The reviewer (BBN) will be consulted for arbitration in case of any disagreement.

Data simplification

Studies will be primarily grouped based on the backbone ART (nucleoside reverse transcriptase vs. non-nucleoside reverse transcriptase) and reported duration of treatment. In addition, a subgroup analysis based on the gender ratio, and age of included participants will be performed.

Risk of bias and quality assessment

Two independent reviewers (SAM and ZAM) will assess the quality of the included studies using the Quality in prognostic studies (QUIPS) tool[21]. A third reviewer (BBN) will be consulted in cases of disagreements.

Data synthesis

The potential risk factors will be summarized using effect measures which will include the hazards ratio, odds ratio, and mean ratios. A random-effects model will be used in the meta-analysis if there is substantial statistical heterogeneity between studies. The levels of heterogeneity will be assessed using the I^2 statistic and an I^2 >25% will be considered as substantial[22], and a p-value of <0.05 will be considered as significant. To explore the sources of heterogeneity within the reported prognostic effect estimates a subgroup analysis will be performed based on (I) Reported prognostic factor (II)duration of disease or ART usage.

Confirmation of prognostic factors

Only prognostic factors with effect estimates in the same direction across the included studies will be considered as confirmed. Moreover, prognostic factors that are consistently significant in the majority of studies following univariate and multivariate analysis will be considered as confirmed prognostic factors.

Discussion

The use of predictive models based on a single factor usually performs poorly in heterogeneous conditions such as CVD. Whereby multiple mechanisms are associated with the progression and severity of the disease. In the HIV and obesity syndemic, a complex convergence of numerous signalling pathways results in the aberrant expression of several inflammatory and metabolic proteins. Although several biomarkers strongly associated with obesity and CVD, such as the monocyte

chemoattractant protein 1 (MCP-1) and F_2 isoprostanes been reported. Although the c reactive protein (CRP) has been extensively explored in obesity and CVD, the reactive protein is not specific for HIV infection or obesity. The recently reported leucocyte-based biomarkers are rarely measured in longitudinal studies and usually not incorporated in CVD-risk prediction models. Hence, the evaluation of biomarkers that are specific and applicable to PLWH and obesity are needed in the clinical setting. Such biomarkers may be helpful in the prognostication of PLWH, who may be misclassified using the current traditional risk factors.

Authors contribution

SAM and BBN conceptualized, designed the study, and drafted the protocol. ZAM helped draft the protocol. All authors wrote and approved the final manuscript. BBN is the guarantor of the review

Patients and public involvement

There was no contact with patients.

Conflict of interest

The authors declare no conflict of interest

References

1. UNAIDS epidemiological estimates. (2020) doi:10.2307/j.ctt7svxh.14.
2. Forsythe, S. S. *et al.* Twenty years of antiretroviral therapy for people living with hiv: Global costs, health achievements, economic benefits. *Health Aff.* **38**, 1163–1172 (2019).
3. Collaboration, A. T. C. & others. Life expectancy of individuals on combination antiretroviral therapy in high-income countries: a collaborative analysis of 14 cohort studies. *Lancet* **372**, 293–299 (2008).
4. Patel, P., Rose, C., Collins, P., Nuche-Berenguer, B. & Al, E. HHS Public Access Author manuscript AIDS. Author manuscript; available in PMC 2019 February 19. Noncommunicable diseases among HIV-infected persons in low- income and middle-income countries: a systematic review and meta-analysis. *Aids* **32**, S5–S20 (2018).
5. Jespersen, N. A., Axelsen, F., Dollerup, J., Nørgaard, M. & Larsen, C. S. The burden of noncommunicable diseases and mortality in people living with HIV (PLHIV) in the pre-, early- and late-HAART era. *HIV Med.* 1–13 (2021) doi:10.1111/hiv.13077.
6. Negin, J. *et al.* Prevalence of HIV and chronic comorbidities among older adults. *AIDS* **26**, S55 (2012).
7. Bakal, D. R. *et al.* Obesity following ART initiation is common and influenced by both traditional and HIV-/ART-specific risk factors. *J. Antimicrob. Chemother.* **73**, 2177–2185 (2018).
8. Penzak, S. R. & Chuck, S. K. Hyperlipidemia associated with HIV protease inhibitor use: pathophysiology, prevalence, risk factors and treatment. *Scand. J. Infect. Dis.* **32**, 111–123 (2000).
9. Rizzo, M. & Berneis, K. Lipid triad or atherogenic lipoprotein phenotype: a role in cardiovascular prevention? *J. Atheroscler. Thromb.* **12**, 237–239 (2005).
10. Rosenzweig, J. L. *et al.* Primary Prevention of ASCVD and T2DM in Patients at Metabolic Risk: An Endocrine Society* Clinical Practice Guideline. *J. Clin. Endocrinol. Metab.* **104**, 3939–3985 (2019).
11. Dakum, P. *et al.* Prevalence and risk factors for obesity among elderly patients living with HIV/AIDS in a low-resource setting. *Medicine (Baltimore).* **100**, (2021).
12. Han, W. M. *et al.* Association of body mass index with immune recovery, virological failure and cardiovascular disease risk among people living with HIV. *HIV Med.* **22**, 294–306 (2021).
13. Hove-Skovsgaard, M. *et al.* No evidence of a synergistic effect of HIV infection and diabetes mellitus type 2 on fat distribution, plasma adiponectin or inflammatory markers. *BMC Infect. Dis.* **20**, 1–11 (2020).
14. Roozen, G. V. T. *et al.* Cardiovascular disease risk and its determinants in people living with HIV across different settings in South Africa. *HIV Med.* **21**, 386–396 (2020).

15. Mallya, S. D., Sravan Kumar Reddy, T., Kamath, A., Pandey, A. K. & Saravu, K. Determinants of metabolic syndrome and 5-year cardiovascular risk estimates among HIV-positive individuals from an Indian tertiary care hospital. *AIDS Res. Treat.* **2020**, (2020).
16. Dimala, C. A., Blencowe, H. & Choukem, S. P. The association between antiretroviral therapy and selected cardiovascular disease risk factors in sub-Saharan Africa: A systematic review and meta-analysis. *PLoS One* **13**, 1–19 (2018).
17. Behrouz, R. *et al.* Risk of intracerebral hemorrhage in HIV/AIDS: a systematic review and meta-analysis. *J. Neurovirol.* **22**, 634–640 (2016).
18. Moher, D., Liberati, A., Tetzlaff, J., Altman, D. G. & Group, T. P. Preferred Reporting Items for Systematic Reviews and Meta-Analyses : The PRISMA Statement. **6**, (2009).
19. Wannamethee, S. G., Shaper, A. G., Lennon, L. & Morris, R. W. Metabolic syndrome vs Framingham Risk Score for prediction of coronary heart disease, stroke, and type 2 diabetes mellitus. *Arch. Intern. Med.* **165**, 2644–2650 (2005).
20. Kaptoge, S. *et al.* World Health Organization cardiovascular disease risk charts: revised models to estimate risk in 21 global regions. *Lancet Glob. Heal.* **7**, e1332–e1345 (2019).
21. Hayden, J. A. & Co, P. Annals of Internal Medicine Academia and Clinic Evaluation of the Quality of Prognosis Studies in Systematic Reviews. 427–438 (2006).
22. Higgins, J. & Thompson, S. G. Quantifying heterogeneity in a meta-analysis. *Stat. Med.* **21**, 1539–1558 (2002).

APPENDIX B: ETHICS APPROVAL LETTER

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

20 August 2020

Dr S Hanley (993225740)
School of Clinical Medicine
School of Health Sciences
hanley@ukzn.ac.za

Dear Dr Hanley

Title: Integration of Cardiovascular disease screening and prevention in the HIV management plan for women of reproductive age-The ISCHeMia study.
Degree: PhD

We wish to advise you that your correspondence received on 07 July 2020 requesting approval of Amendment for the above study has been **noted and approved** by the Biomedical Research Ethics Committee at a meeting held on 11 August 2020.

The following have been noted and approved:

- Letter of Amendment #2 dated 3 July 2020

This approval is subject to national and UKZN lockdown regulations dated 5th June 2020, see (http://research.ukzn.ac.za/Libraries/BREC/Proposed_UKZN_BREC_revision_to_research_constraints_anticipating_change_to_Level_3_lockdown.sflb.ashx). Based on feedback from some sites, we urge PIs to show sensitivity and exercise appropriate consideration at sites where personnel and service users appear stressed or overloaded.

Yours sincerely

[redacted]

Ms A Marimuthu
(for) Prof D Wassenaar
Chair: Biomedical Research Ethics Committee

UNIVERSITY OF KWAZULU-NATAL

BIOMEDICAL RESEARCH ETHICS COMMITTEE

APPLICATION FOR ETHICS APPROVAL OF AMENDMENTS

NAME OF RESEARCHER: Sherika Hanley

DEPARTMENT: CAPRISA Umlazi Clinical Research Site

TITLE OF STUDY: Integration of Cardiovascular Disease Screening and Prevention in the HIV Management Plan for Women of Reproductive Age-The ISCHeMiA study

ETHICS REFERENCE NO: BFC 220/18

DATE OF ETHICAL APPROVAL OF STUDY: 30 June 2019

DATE OF AMENDMENTS: 3 July 2020

AMENDMENTS REQUESTED:

1. Itemise required amendments in following format:
   (i) original protocol states:
   Secondary objectives: To Compare CVD risk between women receiving Efavirenz versus Lopinavir/r containing ART regimens.
   To assess participant perception of self-body image.
   To evaluate the impact of self-body image perception and other potential barriers to adherence to WHO PEN lifestyle modification interventions

   Amendment requested:
   Secondary objectives: • To measure baseline platelet indices (platelet counts, mean platelet volume, plateletcrit, platelet distribution width in HIV infected obese women on successful antiretroviral therapy
   • To measure baseline levels of platelet activation using flow cytometry in HIV infected virally suppressed obese women

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APPENDIX C: PATIENT'S CONSENTS FORMS

THE ISCHeMiA STUDY INFORMED CONSENT FORM
GROUP 1

Date: _____

Study Title: Integration of Cardiovascular Disease SCreening and Prevention in the HIV MAnagement Plan for Women of Reproductive Age in a Resource-Limited Setting -The ISCHeMiA study

Study Investigator: Dr Sherika Hanley

Dear _____,

You are being invited to consider participating in a sub-study within the PROMOTE study which is currently taking place at Philasande Clinic, Prince Mshiyeni Hospital. The sub-study involves testing the best way to screen and prevent cardiovascular disease. Cardiovascular disease (CVD) is the narrowing of the blood vessels which cause heart disease and stroke, and is becoming very common in persons with HIV infection. The sub-study will look at whether a HIV treatment and care plan that includes steps to identify, prevent and modify risk factors for heart disease is effective in changing the course of CVD in women who are HIV infected and on antiretroviral treatment.

If you choose to enrol in the sub study, your participation will be expected to last 36 months (3 years). The sub-study is funded by the US National Institute of Health.

There will be 2 groups of participants enrolled into the sub study. The 2 groups will have different treatment plans for cardiovascular disease. **You will be in group 1.**

Group 1:
Group 1 is the intervention group which is expected to be made up of 200 women who are currently enrolled in the PROMOTE study. While remaining in the PROMOTE study and having the investigations and physical examinations as planned in the PROMOTE study, the sub-study will involve the following additional procedures.

- Every 6 months, you will be asked a few questions to assess risk of cardiovascular disease, and the physical examination will include an additional measurement of your waist.
- Additional specimens for blood sugar, glucose, cholesterol, and a marker of inflammation called high sensitivity CRP, as well as urine for protein, will be collected once a year when you have your PROMOTE study bloods collected.
- Depending on the examination and results from the additional investigations, you may be commenced on treatment for high blood pressure, high cholesterol, and high blood sugar. You may also receive an exercise and dietary plan and advice on other healthy lifestyle choices.
- **Annually you will also be asked if you are satisfied with your current body image.**
- **If you were unable to follow the exercise and dietary plan, further questions may be asked as to the reasons why you were unable to follow the plan. The research staff will provide guidance and will attempt to assist in accommodating your needs to follow the dietary and exercise plan.**

PEPFAR PROMOTE STUDY INFORMED CONSENT FORM

ENROLLMENT

Study Title: PROMise Ongoing Treatment Evaluation (PROMOTE)
Sponsor: President's Emergency Plan for AIDS Relief (PEPFAR)
Study Investigator: Dr Sherika Hanley

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1. Introduction

- You and your child were enrolled in the PROMISE trial and are now being asked to join the PEPFAR PROMOTE Extended follow up study of PROMISE study participants.
- This consent form explains the PEPFAR PROMOTE extended follow up study – why it is being done, the risks and benefits, and what is expected of you in the study if you decide to join.
- Please read this consent form carefully. You may also have this consent form read to you.
- Please ask questions about things that are not clear to you now or when you think of them later.
- After the study has been fully explained to you and all of your questions have been answered, you can decide freely if you want you and your child to be in the study.
- Your participation in the study is entirely voluntary. You and your child do not have to join the study if you do not want to.
- If your child cannot for any reason participate in the PEPFAR PROMOTE study. You can still participate in the study.
- If you and your child do join the study, and if there were new research or other important information relating to your participation the study staff will let you know of any new information that could affect your choice for you and your child to stay in the study.
- If you decide that you and your child will not join the study or decide later that you and your child will leave the study, you may stop at any time without fear of penalty or loss of benefits of your regular medical care. You will continue to be able to get your anti-HIV medicines and other care from your usual clinic if you decide not to join PROMOTE.
- If you choose not to take part in this study, you can still join another research study later, if there is one and you qualify. You are asked to tell the PROMOTE staff about

PEPFAR PROMOTE STUDY INFORMED CONSENT FORM

SPECIMEN STORAGE AND FUTURE USE

Study Title: PROMIse Ongoing Treatment Evaluation (PROMOTE)

Sponsor: President's Emergency Plan for AIDS Relief (PEPFAR)

Study Investigator: *Dr Shenika Hanley*

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1. Introduction:

You have decided that you and your baby will take part in the PROMOTE study to help us find out more about the effects of anti-HIV medicines on the health of a mother and her baby when used for a long time for preventing mothers passing on HIV to their babies. In addition to the tests that you have as part of the study, we are asking now for your permission to save hair, blood, cells from your blood and any of your baby's blood for testing for HIV related studies looking at immune function, virologic measures including resistance, anti-HIV medication levels, and HIV co-infections. Your child blood may also be saved for later testing to looking at if the anti-HIV drugs might affect your baby's bones, kidneys and liver, as well as lab studies of nutrition and growth. These blood specimens would be saved in a special laboratory with freezers to store the specimens. There are no names on any of the specimens, only a special code. The people who run the storage laboratory and the scientists who later use the specimens will not know your name or your child's name.

2. Why is sample storage for future use being done?

Researchers can learn a lot from a study but as time goes by the tests that they use get better or brand new tests are developed, and more can be learned with these better or new tests by using them on stored specimens. If a researcher wants to do a test on specimens from the storage lab in the future, he or she will write up the idea and it will have to be approved by the study team leaders and other groups to make sure that the research is worthwhile. If the idea is approved, then coded specimens and coded information will be given to the researcher. They would never know your name or your baby's name.

Because of the location of the repositories and/or the place where the tests will be conducted, these stored samples may be shipped to another country for storage and/or future use.

3. How often will these specimens be collected?

At each study visit, some of the hair and blood collected for the study tests that were described to you when you agreed to join the study may be stored for future use. You are not being asked to give additional specimens for long term storage.

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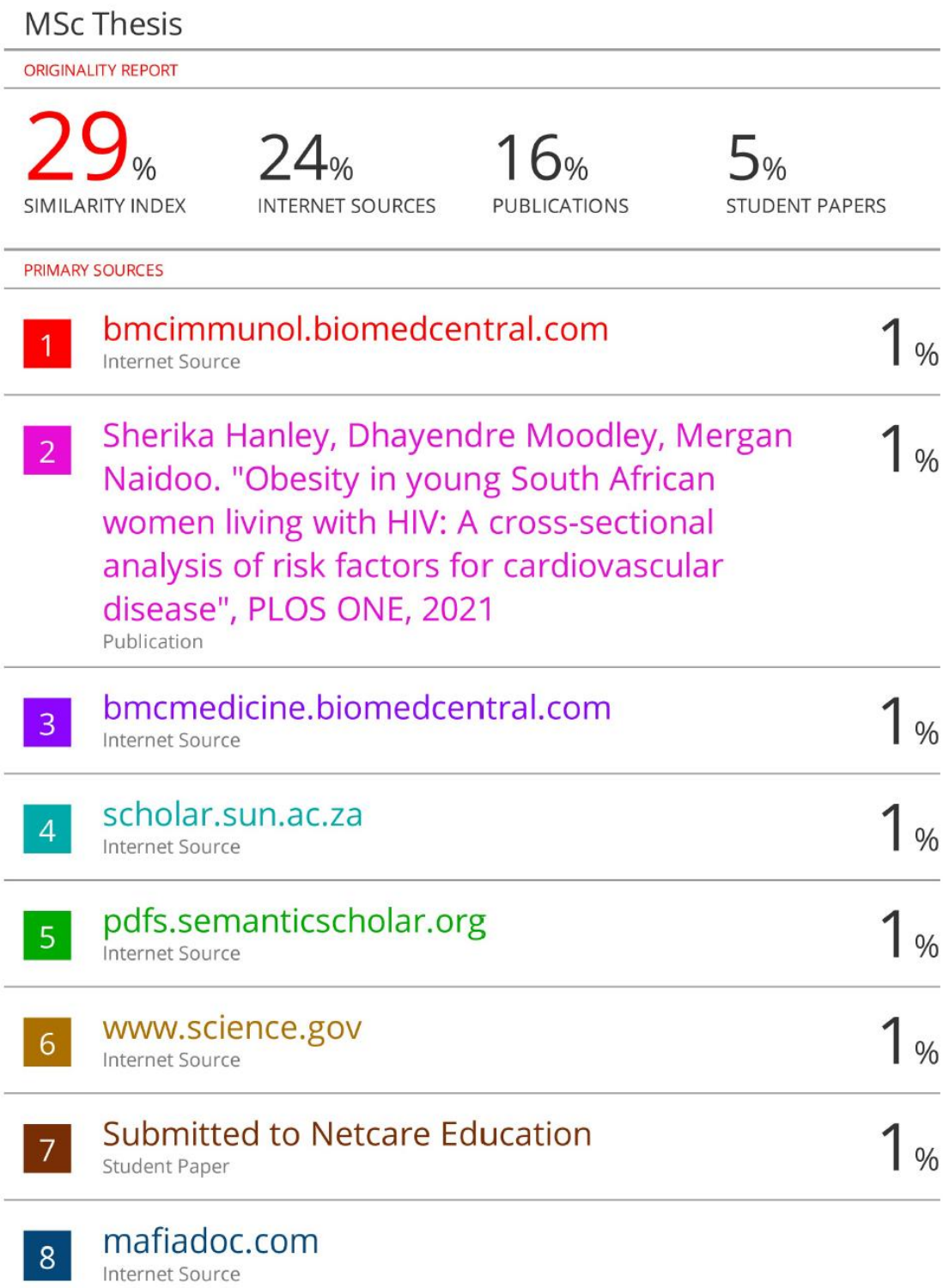
APPENDIX D: TURNITIN REPORT



MSc Thesis

ORIGINALITY REPORT

29%	24%	16%	5%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	bmcimmunol.biomedcentral.com Internet Source	1%
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APPENDIX E: ELISA PROTOCOL

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ELISA methodology

Note: The steps in the ELISA assays performance were somehow similar for CD36, PF-4 and vWF, with a difference in standards and samples dilutions. Also, sP-selectin and ET-1 assay were different.

Reagent's preparations

1X Wash Buffer- Diluted 20 mL of the Wash Buffer Concentrate into 380 mL of deionized or distilled water.

1X Wash Buffer for ET-1- Diluted 15 mL of 20x wash solution concentrate with 285 mL of distilled water.

Diluent B- Diluted 2 mL of 5x diluent B with 10 mL of distilled water (2x to be used for biotin conjugate and streptavidin-HRP preparation)

Diluent D- Diluted 2 mL of 5x diluent B with 10 mL of distilled water (to be used for standards and samples dilution)

Biotin conjugate – Diluted 120 µL of biotin conjugate concentrate and 9570 ul of diluent B to make up for 96 wells

Streptavidin-HRP- Diluted 2000 µL of streptavidin-HRP with 8000 ul of diluent B

1x Assay Buffer for ET-1 – Diluted 14 mL of 5x Assay Buffer with 56 mL distilled water.

Standards dilutions

To make standard dilutions for CD36, 480 µL 1X Assay Diluent D was added into the vial to prepare a 500 ng/mL standard solution. The powder was dissolved thoroughly by a gentle mix. Then we pipetted 300 µL 1X Assay Diluent D into each tube. We thereafter used the 500 ng/mL standard solution to produce a dilution series by mixing each tube thoroughly before the next transfer. 1X Assay Diluent D serves as the zero standard (0 ng/)

To make standard dilutions for sP-selectin, 225 µL of Sample Diluent was added into each tube. A reconstituted standard (80 ng/mL) of 225 µL was pipetted into the first tube and mixed to make a concentration 40 ng/mL. Then, 225 µL of this dilution was transferred into the second tube to produce a dilution series by mixing thoroughly before the next transfer.

To make standard dilutions for PF-4, 400 µL Assay Diluent C was added into the lyophilized standard vial to prepare a 140 ng/mL standard solution. The powder was dissolved thoroughly by gentle mixing. We added 75µL PF-4 standard from the vial of reconstituted standard, into a tube with 625µL Assay Diluent C to prepare a 15,000 pg/mL standard solution. Then we pipetted 400 µL Assay Diluent C into each tube. Assay Diluent C serves as the zero standard (0 pg/mL).

To make standard dilutions for vWF, we added 440 µL Assay Diluent A into the lyophilized standard vial to prepare a 30 ng/mL standard. Standard solution was gently mixed to dissolve the powder thoroughly. We pipetted 300 µL Assay Diluent A into each tube. We used the 30 ng/mL standard solution to produce a dilution series by mixing each tube thoroughly before the next transfer. Assay Diluent A serves as the zero standard (0 ng/mL).

To make standard dilutions for ET-1, we added 10 µL Endothelin-1 Standard to one tube containing 990 µL 1X Assay Buffer and labelled it as 100 pg/mL ET-1. Thereafter, added 150 µL Standard Diluent Buffer to each of 8 tubes labelled as follows: 50, 25, 12.5, 6.25, 3.125, 1.563, 0.781, and 0 pg/mL ET 1.Then we made serial dilutions of the standard by mixing thoroughly between steps to the next transfer.

Samples dilutions

For CD36: 33 µL of sample and 67 µL of Diluent D

For sP-selectin: 10 µL of sample and 90 µL of Sample diluent

For PF-4: 20 µL of sample and 80 µL of Diluent C

For vWF: 2 µL of sample and 398 µL of Diluent A

For ET-1: We were not instructed to dilute our samples

ELISA performance

We made use of the ELISA to detect and quantify the levels of sCD36, PF-4 and vWF. Briefly, 100 µL of standards and 100 µL of diluted samples were added in duplicates to each of the 96-well plate coated with specific human (sCD36 or PF-4 or vWF) antibody (Thermofisher, Scientific, Waltham, MA, USA). The plate was covered and incubated for two and a half hours at 37°C. After incubation, the solution was removed by emptying and adding 300 µL Wash Buffer in each well and blotting the plate against clean towels to remove excess wash buffer. This was repeated four times.

Thereafter, 100 µL of biotin conjugate was added to each well. The plate was incubated for one hour at 37°C with gentle shaking (manually) and was then washed as mentioned before. After incubation and thoroughly washing , 100 µL of streptavidin-HRP solution (prepared by was added to each well. The plate was covered and incubated for 45 minutes at 37°C with gentle shaking and then washed as before. After the incubation and washing of the streptavidin-HRP solution, 100 µL of TMB-substrate was added to each well and incubated for 30 minutes in the dark. A blue colour was developed and was stopped by adding 50 µL of stop solution in each well where colour changed from blue to yellow. The plate was read spectrophotometrically at 450 nm.

To detect and quantify the levels of sP-selectin in the plasma, the 96-well plate was firstly washed two times by adding 400 µL Wash Buffer and discarding the contents. 100 µL of standards and 100 µL of diluted samples were added in duplicates to each of the wells. Thereafter, 50 µL of HRP-conjugate was

added to all wells then covered and incubated for two hours at 37°C. After incubation, the plate was washed two times with 400 µL Wash Buffer. Then immediately added 100 µL TMB substrate solutions to all wells and incubated the plate for 30 minutes in the dark. The enzymatic reaction was stopped by adding 100 µL of Stop Solution in each well. The plate was immediately read on the plate reader (Bio-Rad Laboratories, California, USA) at 450 nm.

To detect the levels of ET-1 in the plasma, 50 µL of diluted standards and 50 µL samples were added in each well. The plate was covered and incubated for 60 minutes at 37°C. The solution was discarded, and the wells were washed by adding 300 µL Wash Buffer and aspirating it and blotting the plate against clean towels four times. Thereafter, 50 µL of ET-1 conjugate was added into each well and incubated for 60 minutes at 37°C. Then washed as mentioned before. After incubation and washing of the conjugate, 100 µL of substrate was added in each well and incubated for 30 minutes. To stop the enzymatic reaction, 50 µL of the stop solution was added into each well. The plate was then read on the microplate reader (Bio-Rad Laboratories, California, USA) at 450 nm.

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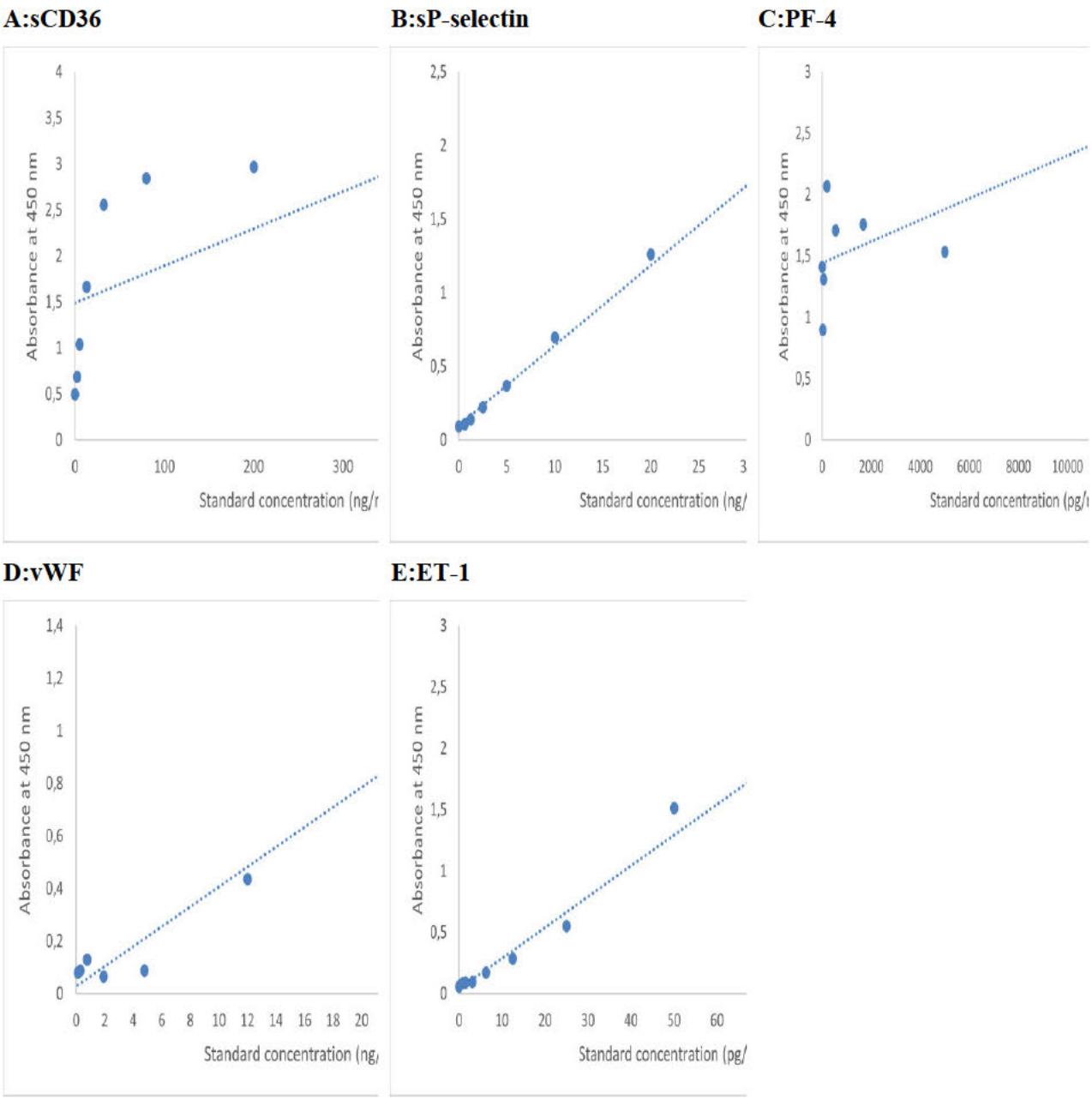


1231 **Figure 1:** Standard curves of platelet and endothelial activation markers produced by the ELISA .
1232 Figure (**A**) demonstrates the standard curve of sCD36 (sGPIV), while figure (**B**) shows the standard
1233 curve of sP-selectin levels and figure (**C**) illustrates the standard curve of platelet factor-4 (PF4). Figure
1234 **(D)** and **(E)** shows the standard curves of von Willebrand Factor (vWF) and Endothelin-1 (ET-1)
1235 respectively.

1236

APPENDIX F: DESCRIPTION OF THE STUDY

`Research design and methods

Overview

1. Study Setting: Umlazi/Philasande Research Clinic and Gateway Umlazi Clinic
2. Study Design: This study is a prospective two-arm, quasi-experimental design comparing a primary health care intervention plan with usual care.
3. Target Population: HIV infected women attending PHC
4. Study population: HIV infected women aged from 18 to 49 years of age and receiving HIV care at PHC clinics.
5. Inclusion criteria
    - HIV infected women
    - On ART for at least 1 year
    - Equal to/older than 18 years of age
    - Equal to/younger than 49 years of age
    - Plans to remain in the study catchment area for at least 3 years
6. Exclusion criteria
    - Participant declines study participation
7. Sampling

• Size of sample

400 HIV-infected women consisting of an intervention arm of 200 women co-enrolled in the PROMOTE study at the Umlazi CRS (see method of sampling for details), and a control arm of 200 women receiving HIV care at the PHC. With a 20-30% estimated prevalence of metabolic syndrome in people living with HIV without any specific intervention in the risk factor modification of cardiovascular disease and a proposed 10-15% estimated incidence of metabolic syndrome in the intervention arm, 132 participants per arm would allow for 80% power. An additional 10% can be added to allow for loss to follow-up. The larger proposed sample size will allow for external validity / generalizability.

- Method of selecting sample

Two HIV-infected female cohorts will be matched for receipt of ART duration >1 year and for age will be compared in the study. Method of selection will be via convenience sampling.

Intervention group: There are 245 HIV infected women between 18 and 49 years of age, who have been on ART for more than 1 year and attending the CAPRISA Research Clinic in Umlazi. These women were enrolled into the PEPFAR PROMise Ongoing Treatment Evaluation (PROMOTE) observational study from May-June 2017. This study has been implemented to provide long-term follow-up data on safety outcomes of widespread use of combination antiretrovirals (cART) among an already well-characterized cohort of HIV infected mothers and their children who previously enrolled in the multi-site PROMISE study. This cohort was selected for the proposed intervention arm because the principal investigator is based at the research clinic. Women will be briefed at their next PROMOTE study visit and the 1st 200 interested candidates meeting all eligibility criteria will be co-enrolled into the Intervention arm of the Sub-study.

Control group: The Tier data base will be used to select HIV infected women aged between 18-49 years, receiving ART for more than 1 year at the Umlazi Gateway PHC. Scheduled clinic visits

at similar time points to the anticipated clinic visits in the intervention group will be used to establish a list of potentially eligible women. Following a matched pool of data, the first 200 women fulfilling the inclusion criteria, who attend the clinic for their next appointment and who consent to study participation will be enrolled.

8. Data sources

- Intervention group: Each participant in the intervention group will be assigned a new Participant identifier number (PID) in addition to the PROMOTE PID. History taking and physical exam, as well as laboratory results and where applicable ultrasound reports, will be entered directly into a data collection tool on a Microsoft Excel spreadsheet (Appendix I).
- Control group: Participants will be assigned a PID in addition to their clinic chart number. Information from clinic patient medical records will be entered directly into the data collection tool (Appendix II). At the final study visit, history, physical exam, lab report finding and ultrasound findings (where applicable), will be source documented.

9. Measures to ensure validity

- Internal
  - The prospective nature of the study, large sample size, careful matching between cohorts by age category and by duration of ART aims to counteract the potential for selection bias.
  - Information bias in chart review will be controlled by training of staff involved in data collection. Missing information
  - Loss to follow up – a retention plan will be placed to send clinic appointment reminders by sms or whatsapp, telephone calls in the case of missed visits and occasionally home visits when deemed necessary.
  - Laboratory sample measurements will be performed by a certified laboratory.
- External -The larger proposed sample size will allow for external validity / generalizability.

10. List of Variables to be measured and schedule of evaluations

11.

CVD risk factors	Intervention Arm (at main study PROMOTE scheduled visits) Year 1(0+6m), Year 2(12+18m), Year 3(24+30m), Final study Visit (36m)	Control Arm	
		**Year 1, 2, 3 (not including end of study visit)	Year 3 (end of study-36months)
Non-modifiable	Age (Categories in 5 year)	Age (Categories in 5 year)	Age (Categories in 5 year)
	Race (A, I, W, C, O)	Race (A, I, W, C, O)	Race (A, I, W, C, O)
	Family history of CVD in first degree relatives		Family history of CVD in first degree relatives

Modifiable	Smoking (Current, Never, Past)		Smoking (Current, Never, Past)
	Unhealthy Diet		Unhealthy Diet
	Exercise (minutes per week)		Exercise (minutes per week)
	ART duration in years	ART duration in years	ART duration in years
	ARV regimen	ARV regimen	ARV regimen
	Viral load	Viral load	Viral load
	CD4 count	CD4 count	CD4 count
	BMI (6monthly)	BMI (6monthly)	BMI
	Waist Circumference (6 monthly)		Waist Circumference
	Systolic BP (6monthly)	Systolic BP	Systolic BP
	Pulse (6monthly)		Pulse
	Fasting Glucose (annual)		Fasting Glucose
	Fasting Lipogram (annual)	Fasting Lipogram	Fasting Lipogram
	hsCRP (annual)		hsCRP
	Urine Microalbumin (annual)		Urine Microalbumin
	*Carotid intima media thickness		Carotid intima media thickness
	CVD risk % by Framingham		CVD risk % by Framingham
	CVD risk % by D:A:D		CVD risk % by D:A:D
	CVD risk % by WHO/ISH		CVD risk % by WHO/ISH

*Only at year 3 in women aged over 40 years **Data collection through chart review

12. Plan for Data collection

- **Intervention group**: The prevalence of CVD risk factors will be determined by data collection through history taking, physical examination, and laboratory and radiology investigations as per the data collection tool. CVD risk assessment will be performed annually using a combination of the WHO and International Society of Hypertension cardiovascular risk prediction (WHO/ISH) and the D:A:D CHD equation. The intervention proposed is a modified WHO PEN algorithm incorporated into HIV management guidelines at study entry. Trends in all risk factors will be monitored, and new risk factors identified, with 6 monthly intervention during the first year, and annually for three years thereafter.

- **Control group**: Following informed consent, data will be collected from the participants ARV clinic medical chart as per the data collection tool. Standard of care HIV management and primary health care will be provided by public sector clinic staff according to current national guidelines. No study investigations or study questionnaires will be carried out at entry visit until the final study visit. There will be regular telephonic follow up with the participants to maintain study retention. Chart review data collection at 6 monthly intervals. CVD risk factors, as per outcomes described above, will be measured by the study at a single 3 year final study visit.

Information obtained will be conveyed to the clinic staff for further management if required.

13. Plan for Data handling/processing

- Data will be analysed using SAS or SPSS software

14. Statistical methods

- Descriptive statistics-continuous variables will be represented by means, medians, prevalence, standard deviation.

  The categorical variables will be represented by N+%
- Analytic statistics

  The continuous variables will be compared by use of t-tests or Wilcoxon rank sum tests.
  The categorical variables will be compared using Chi sq or Fisher's exact test/
- Logistic regression will be applied to identify predictors of cardiovascular disease between the two arms, and between those participants who exhibit atherosclerosis by carotid intima thickness and those who don't.

15. List of possible confounders

- Women in the intervention group are already enrolled in a study with controlled settings. These women may have commenced ART with a higher baseline CD4 counts.
- Multivariate logression analyses will be utilized in order to control for confounders.

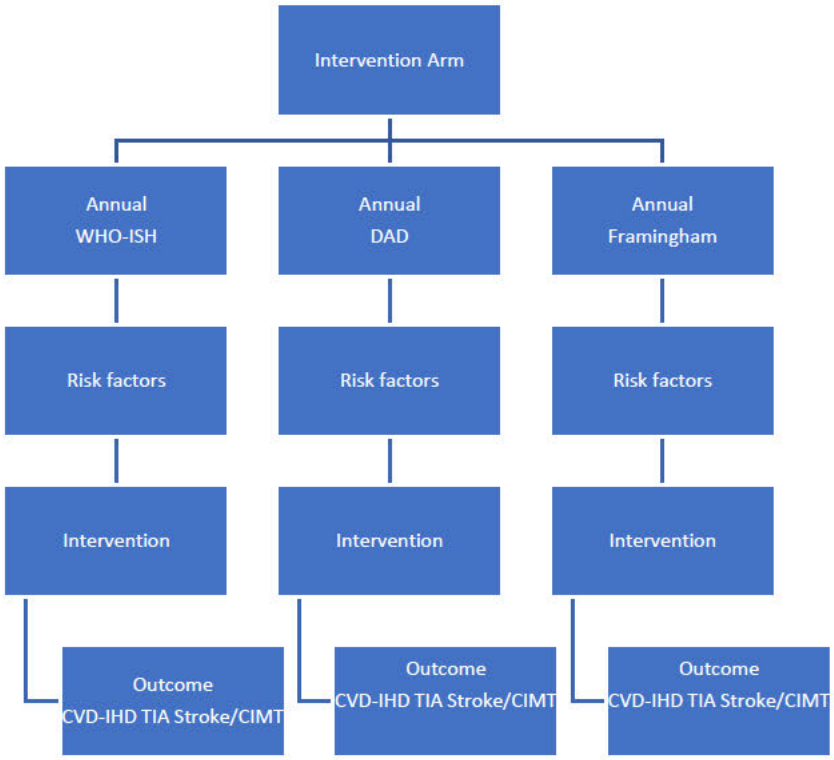
16. List of associations to be measured

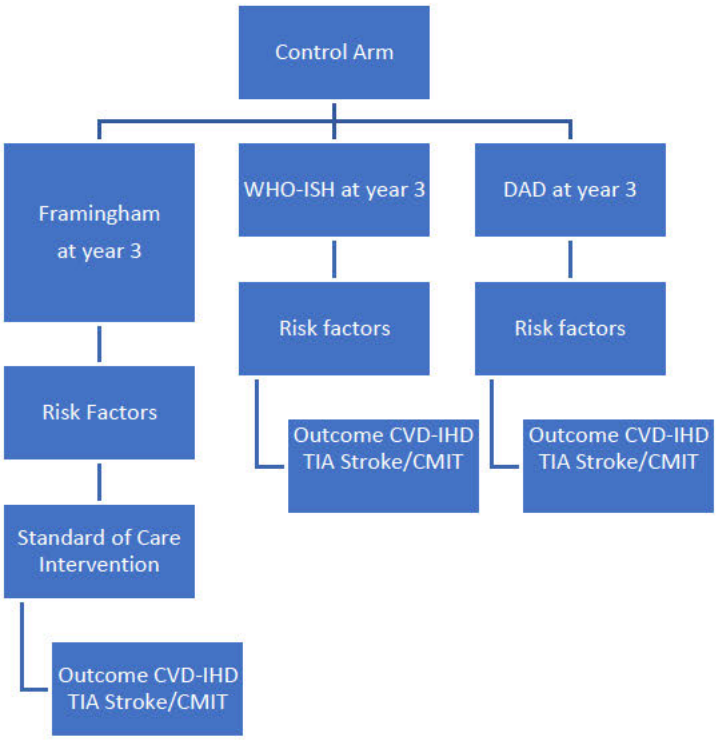
a.

	BMI	WC	Fasting lipogram	Systolic BP	Fasting Glucose	Urine microalbumin	hsCRP	Carotid initima medial thickness
Age	x	x	x	x	x	X	x	X
Family History of CVD	x	x	x	x	x	X	x	X
Tobacco use	x	x	x	x	x	X	x	X
Diet	x	x	x	x	x	X	x	X
Exercise	x	x	x	x	x	X	x	X
ART regimen	x	x	x	x	x	X	x	X
ART duration	x	x	x	x	x	X	x	X
VL	x	x	x	x	x	X	x	X
CD4	x	x	x	x	x	X	x	X
BMI			x	x	X	X	x	X

WC			x	x	X	X	x	X
Fasting Lipogram				x	X	X	x	X
Systolic BP					X	X	x	X
Fasting Glucose						X	x	X
U-microalbumin							x	X
hsCRP								X
CVD risk % by Framingham								X
CVD risk % by D:A:D								X
CVD risk % by WHO/ISH								X

b. Calculate cardiovascular risk as per WHO –ISH, D:A:D and Framingham, annually in intervention group and in the control group at year 3 and compare short term outcomes (presence of subclinical atherosclerosis by means of CMIT, presence of stroke, MI, angina - ischaemic heart disease)




Control Arm
Framingham at year 3
WHO-ISH at year 3
DAD at year 3
Risk Factors
Risk factors
Risk factors
Standard of Care Intervention
Outcome CVD-IHD TIA Stroke/CMIT
Outcome CVD-IHD TIA Stroke/CMIT
Outcome CVD-IHD TIA Stroke/CMIT

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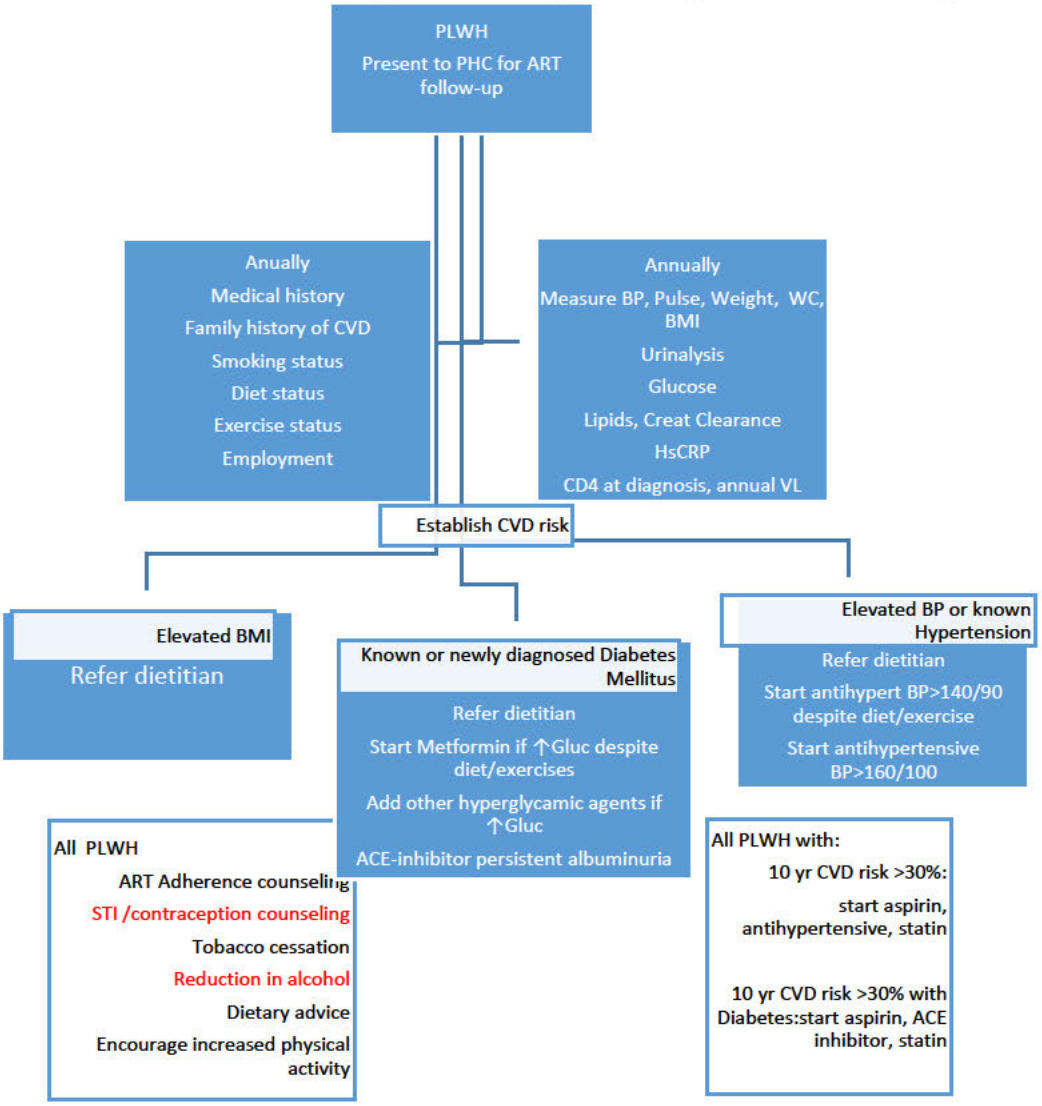
17. Intervention:

DIETARY CHANGES: All individuals encouraged to reduce daily salt intake by at least one third and, if possible, to <5 g



per day, to eat at least 400 g a day of a range of fruits and vegetables as well as whole grains and pulses and to reduce total fat and saturated fat intake

PHYSICAL ACTIVITY: All individuals encourage to do at least 30 minutes of moderate physical activity (e.g. brisk walking) a day, through leisure time, daily tasks and work-related physical activity.
WEIGHT CONTROL: All individuals who are overweight or obese should be encouraged to lose weight through a combination of a reduced-energy diet and increased physical activity

Ethical Considerations

- Permissions needed to conduct the study will be obtained from the University of KwaZulu-Natal Biomedical Research Ethics Committee, Prince Mshiyeni Memorial Hospital, KwaZulu-Natal Department of Health and the PROMOTE protocol team.
- Written informed consent form will be obtained prior to any study procedure.
- All study procedures will be conducted in a manner to protect participant privacy and confidentiality.
- There will be no additional reimbursement in the intervention arm of the proposed sub study. Participants enrolled in the PROMOTE study are reimbursed using PROMOTE funds. There will be no reimbursement in the control arm with the exception of the final study visit, during which additional lab assessments will be carried out during their routine PHC visit. .

APPENDIX G: SUPPLEMENTARY FILE 1.

Supplementary Table 1. Elevated levels of CD36 and vWF in ART-treated WLHIV and obesity (experimental) compared to lean WLHIV (controls)

	Controls (n=30)	Experimental group (n=27)	p-value
PF-4	20085[19629-20335]	19803[19234-20296]	0.1830
CD36	2.79[2.24-3.55]	4.36[2.71-9.53]	**0.0064**
sP-selectin	5.31±2.74	4.43±1.88	0.2054
ET-1	3.94[3.14-4.96]	4.36[3.58-5.04]	0.1491
vWF	5.34[0.65-7.71]	8.83[1.59-9.78]	**0.0009**

Supplementary Table 2. Sensitivity analysis omitting viraemic participants (RNA viral load>20 cp/mL).

	Controls (n=30)	Experimental group (n=27)	p-value
PF-4	20114[19633-20446]	19803[19283-20227]	0.1494
CD36	2.895[2.264-3.641]	4.554[2.816-9.696]	**0.0073**
sP-selectin	5.435±2.813	4.291±4.291	0.1262
ET-1	4.187[3.139-5.208]	4.397[3.541-6.099]	0.2413
vWF	5.492[0.7024-7.670]	8.499[1.524-9.725]	**0.0128**

Supplementary Table 3. Sensitivity analysis omitting smoking.

	Controls (n=28)	Experimental group (n=30)	p-value
PF-4	20094±642.3	19781±748.6	0.1781
CD36	2.913[2.242-3.983]	4.364[2.710-9.532]	**0.0221**
sP-selectin	5.10±2.65	4.42±1.91	0.3786
ET-1	3.768[3.035-4.958]	4.222[3.558-5.063]	0.1211
vWF	5.130[0.529-7.788]	9.022[1.576-9.802]	**0.0144**

Supplementary Table 4. Linear regression analysis between markers of platelet activation, endothelial activation with Age and CD4 count.

Parameter	Age	P-Value	CD4 Count	P-Value
PF-4	0.013	0.937	0.059	0.726
CD36	0.185	0.184	0.129	0.359
sP-selectin	0.072	0.591	0.033	0.804
ET-1	0.227	0.083	0.147	0.266
vWF	0.138	0.272	0.069	0.587