HIV, immune activation and cardiovascular disease in the sub-Saharan African context

A.G. Vos

531207-L-bw-Vos
Processed on: 20-5-2019

HIV, immune activation and cardiovascular disease in the sub-Saharan African context

ISBN:	978-94-028-1519-1
Author:	A.G. Vos
Cover design by:	Edwin Seda, seda.eddie@gmail.com
Layout and design by:	Eduard Boxem, persoonlijkproefschrift.nl
Printed by:	Ipskamp Printing, proefschriften.net

Financial support by the Dutch Heart Foundation for the publication of this thesis is gratefully acknowledged.

Financial support by Gilead Sciences for the publication of this thesis is gratefully acknowledged.

HIV, immune activation and cardiovascular disease in the sub-Saharan African context

HIV, immuun activatie en cardiovasculaire ziekte in de context van sub-Sahara Afrika

(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de
Universiteit Utrecht
op gezag van de
rector magnificus, prof.dr. H.R.B.M. Kummeling,
ingevolge het besluit van het college voor promoties
in het openbaar te verdedigen op

donderdag 20 juni 2019 des middags te 4.15 uur

door

Arnolda Grietje Vos

geboren op 19 december 1985
te Kerkwijk

Promotor:

Prof. dr. D.E. Grobbee

Copromotoren:

Dr. K. Klipstein-Grobusch
Dr. R.E. Barth

Contents

CHAPTER

1

General introduction and thesis outline

The major cause of death in sub-Saharan Africa (SSA) is shifting. Where communicable diseases like human immunodeficiency virus (HIV) and lower respiratory tract infections used to be the main causes of death, non-communicable diseases (NCDs) like cardiovascular disease (CVD), including ischemic heart disease and stroke, are becoming increasingly important[1]. In South Africa, diabetes mellitus (DM) was the second biggest killer after TB in 2016 and stroke and ischaemic heart disease were ranked fourth and fifth for years of life lost in 2015[2,3]. Most deaths due to CVD occur in low- and middle-income countries[4]. The increasing incidence of CVD is driven by a high and still raising burden of classical risk factors for CVD such as hypertension, obesity, and DM type 2[5-7]. In South Africa, for instance, 40% of the population over the age of 15 years was estimated to have hypertension in 2011[8]. In 2016, almost half of the female, rural Malawian population was reported to be obese 44%[9], and a recent meta-analysis based on African studies estimated that a quarter of the population has dyslipidemia [10]. Moreover, the DM type 2 prevalence was reported to be 3% in Africa in 2017[7]. Smoking is another, well-known risk factor for CVD. According to a recent estimate from Kenya, about one in seven people were using tobacco, of which the majority (>80%) were men[11].

Since the emergence of the human immunodeficiency virus (HIV) in the 1980's, HIV heavily burdens SSA. Currently 70% of the world's HIV-positive population is living in SSA, accounting for about 26 million people [12]. From 2002 onwards governmental funded antiretroviral treatment (ART) programs were implemented in Africa[13,14], turning HIV from a deadly disease into a chronic disease. Following the TEMPRANO[15] and START trial[16] in 2015 the World Health Organization recommended initiation of ART immediately, following a diagnosis of HIV infection in all patients[17]. In South Africa, the test-and-treat policy was implemented on the 1st of September 2016[18]. Currently, about 60% of people living with HIV (PLHIV) in Africa have access to ART[19], and this number continues to increase. The result is that life expectancy for PLHIV increased from a maximum survival of eight to 10 years before the ART era to a near-normal life expectancy nowadays[20,21].

As the HIV-positive population is aging, NCDs will be encountered more frequently. Besides, research in high-income countries, mainly from North America and Europe, showed that the incidence of CVD was higher in the HIV-positive population than in the HIV-negative population and HIV infection itself has been associated with an increased risk of myocardial infarction of up to 50% [22-24].

Demographics of the HIV epidemic and CVD risk

The question now is whether SSA is going to be burdened with a dual epidemic of HIV and CVD. The discussion on the role of HIV as a contributor to the development of CVD is

still mainly speculative as data on clinical CVD amongst HIV-positive African populations is scarce. Data regarding the increased risk of CVD in PLHIV are mainly derived from research conducted in North America and Europe and it is questionable whether results from these studies can directly be translated to the African context. The HIV-positive population in high-income countries mainly consists of white men having sex with men, sex workers and intravenous drug users (Figure 1). They typically show high cardiovascular risk profiles reflected by a higher percentage of smokers, hypertension and DM type 2 in PLHIV as compared to HIV-negative populations[25,26]. The HIV-positive population in SSA, on the contrary, consists mainly of black heterosexual women (Figure 1)[27] and they seem to have fewer traditional CVD risk factors than the general population [8,28].

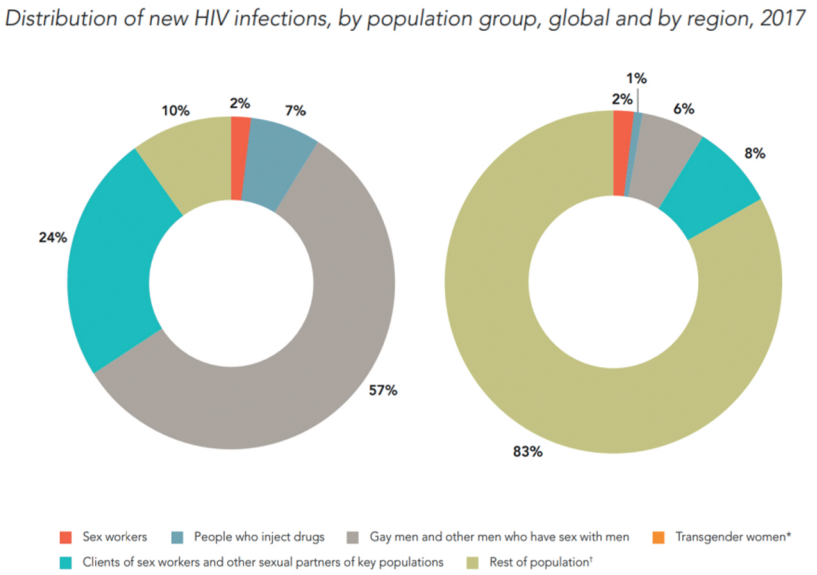


Figure 1. Adapted from: UNAIDS special analysis, 2018. *UNAIDS Data 2018.*

HIV and CVD pathogenesis

CVD is, in the majority of cases, a result of atherosclerosis[29]. The process of atherosclerosis starts with endothelial activation. Following a pro-atherogenic stimuli, the permeability of the arterial wall changes, allowing lipids to enter, and results in the expression of chemokines, like the vascular cell adhesion molecule (VCAM-1). This, in turn, results in migration of leucocytes and macrophages in the intima layer. Once there, the macrophages differentiate into foam cells, which absorb lipids and secrete cytokines. Besides, LDL fragments are presented to naïve T-cells, and an influx

of these cells in the vessel wall leads to changes in macrophages, smooth muscle cells and endothelial cells and, subsequently, into thickening of the intima layer[29,30].

The increased risk of CVD in HIV infection likely relies on the triad of classic CVD risk factors, HIV and ART[31]. First, the HIV-negative population has a substantial burden of classic CVD risk factors, as seen in the general population[25]. Second, HIV infection itself influences the process of atherosclerosis through the inflammatory pathway[32,33]. Upon HIV infection a general, unspecific, T-cell response is seen with aspecific activated CD8+ T-cells and HIV-specific T-cells and both the innate and adaptive immune system are activated[30]. Immune activation is reflected by increased levels of C-reactive protein (CRP), IL-6 and d-dimer compared to the HIV-negative population, and these markers have been associated with CVD and mortality in PLHIV[34]. Besides, HIV infection is accompanied by endothelial activation, as is reflected in increased levels of s-VCAM[35,36]. Macrophages and monocytes likely play a role as well. The subclass of inflammatory monocytes (CD14++ CD16+) is increased in untreated HIV-infected individuals, like is seen in HIV-negative participants with an acute coronary syndrome[37]. The influence of T-cells is less obvious, although nadir CD4-count has been related to CVD risk in a number of studies[24,38]. Third, ART has an effect on CVD risk, which is likely due to metabolic side effects such as lipodystrophy[39], increased insulin resistance[40,41] and dyslipidemia[42]. Initiation of ART tapers inflammation, but immune activation does not normalize to levels observed in the HIV-negative population[33].

The role of immune activation in the relation between HIV and CVD has been addressed in a substantial number of studies. However, there is no consensus yet on how these findings influence the development of CVD as studies reported both positive and negative associations between immune markers and CVD. One of the reasons for this is the paucity of longitudinal studies evaluating the occurrence of clinical CVD. Moreover, little is known about the relation between immune activation and CVD in the SSA HIV-positive population as almost all studies were conducted in high-income countries. Apart from the differences in CVD risk profile, these populations differ with regards to the burden of co-infections like tuberculosis [43]. This underlines the need to investigate the relation between immune activation, HIV and CVD in the SSA HIV-positive population.

Surrogate markers for cardiovascular disease

PLHIV are still relatively young with low CVD event rates. For this reason, surrogate outcomes for CVD have been widely used. These can be divided in structural and functional surrogate CVD outcomes. The most utilized structural surrogate marker is measurement of the carotid intima-media thickness (CIMT). CIMT is an ultrasound-

based measurement of the thickness of the intima-media layer of the carotid artery wall. The thickness of the arterial wall reflects the process of atherosclerosis, as accumulation of lipids and inflammatory cells in the vessel wall increase the thickness. An increase in intima-media thickness is related to an increase in the risk of myocardial infarction and stroke[44]. Examples of functional surrogate markers are pulse wave velocity (PWV) measurement and heart rate variability (HRV). PWV assesses arterial stiffness and HRV is an indicator of the parasympathetic and sympathetic adaptability of the heart[45]. A commonality that all surrogate CVD outcomes share is that abnormal outcomes are closely linked to an increased risk of CVD[46-49].

Thesis objective

The objective of this thesis is to gain insight into the burden of CVD risk factors and CVD risk in a HIV-positive population in sub-Saharan Africa whilst considering the use of antiretroviral therapy, and to investigate to what extent immune activation plays a role in the pathophysiology of CVD in HIV infection.

Setting

The studies described in this thesis were conducted in South Africa. South Africa has the largest HIV epidemic in the world with 7.5 million PLHIV and an estimated HIV prevalence of 19% among adults aged 15 to 49 years[50]. Data were collected at a rural and an urban research site to improve generalizability of our results. CVD risk profile is known to differ between rural and urban populations: urban populations are more likely to have a higher blood pressure, higher body mass index and a higher prevalence of DM type 2 compared to rural populations, though smoking prevalence was reported to be higher in a rural population[9,51].

Research in rural South Africa has been undertaken in Elandsdoorn, Limpopo, and has been embedded in the longitudinal Ndlovu Cohort Study (NCS). The NCS includes both HIV-negative and HIV-positive participants and aims to gain insight into the relation between HIV, ART and CVD whilst considering the role of ongoing immune activation. The NCS was initiated in November 2014 and included 1927 participants until August 2017. The first participants have finished the fourth year of follow-up and the retention rate is currently around 70%. The NCS is an initiative of the Ndlovu Care Group, Elandsdoorn, South Africa, Utrecht University including the University Medical Center Utrecht and the Department of Social Sciences, Utrecht, The Netherlands and the Wits Reproductive Health and HIV Institute (Wits RHI), a research department of the University of the Witwatersrand, Johannesburg, South Africa. The urban research site was initiated by Wits RHI and the Julius Center, UMC Utrecht, and was located at Charlotte Maxeke Johannesburg Academic Hospital in Johannesburg, South Africa. This study aimed to gain insight into the influence of HIV and ART on CVD risk in an urban

population and included PLHIV living in the inner city of Johannesburg and HIV-negative family members or friends of the HIV-positive participants. PLHIV were recruited from finished or ongoing randomized controlled trials investigating different ART regimens. All these trials were supervised by Wits RHI. The urban research site was open between July 2016 and November 2017.

Outline

The ***first part*** of the thesis addresses the relation between markers of immune activation and CVD in PLHIV. The second and third chapters summarize the evidence on the relation between markers of immune activation and surrogate markers of CVD. While the second chapter focuses on the relation between immune markers and CIMT, the third chapter focuses on the relation between immune markers and other CVD surrogate outcomes like PWV, ankle brachial index and flow mediated dilation. The fourth chapter zooms in on one specific marker, Lipoprotein-associated phospholipase A2, and it's utility to detect an increased CVD risk in PLHIV.

The ***second part*** of the thesis focusses on the relation between HIV, ART and CVD risk factors and/or the burden of CVD. It first describes the methods and the rationale of the NCS (Chapter 5). Chapter 6 reports the baseline characteristics of all NCS participants, whilst focusing on the association between HIV, ART and CVD risk including CIMT. Chapter 7 quantifies CVD risk in a sample of the NCS population using heart rate variability.

In chapter 8, the focus is turned towards an urban HIV-positive population, and the results of a study focusing on the role of HIV and ART on CVD risk in this population are presented. Chapter 9 brings chapters 6 and 8 together as it aims to explain the determinants of CVD risk in rural and urban PLHIV. Chapter 10 presents an in-depth analysis of the influence of ART initiation on lipids and glucose metabolism, both risk factors for the development of CVD.

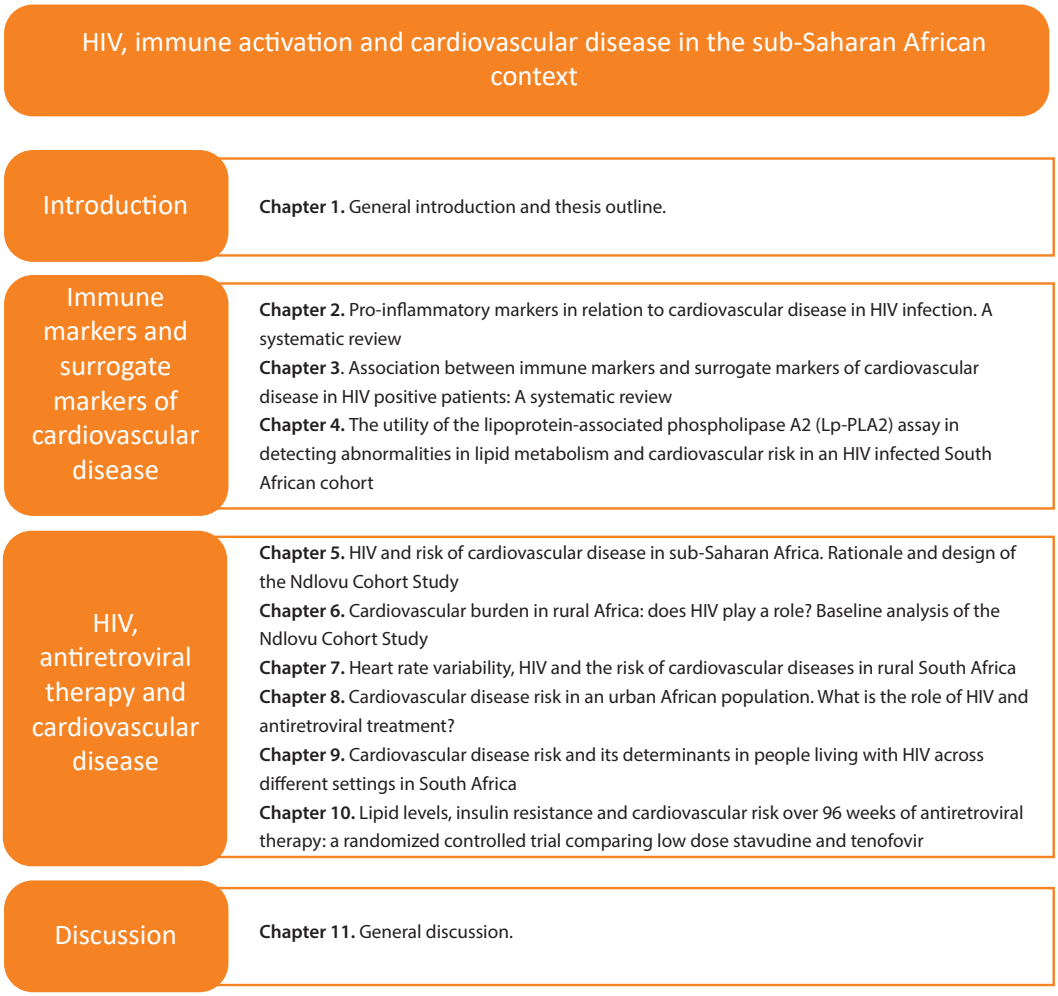


Figure 2. Overview of this thesis

References

1. GBD 2016 Causes of Death Collaborators. Global, regional, and national age-sex specific mortality for 264 causes of death, 1980-2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet. 2017;390: 1151-1210. doi: S0140-6736(17)32152-9.
2. Massyn N, Padarath A, Peer N, Day C. District Health Barometer 2016/17. Health Systems Trust. November 2017.
3. Statistics South Africa. Mortality and causes of death in South Africa, 2016: Findings from death notification. Statistical release P0309.3. 27 March 2018.
4. World Health Organisation. Cardiovascular diseases (CVDs). Key facts. Geneva, 2017, May.
5. NCD Risk Factor Collaboration (NCD-RisC). Worldwide trends in blood pressure from 1975 to 2015: a pooled analysis of 1479 population-based measurement studies with 19.1 million participants. Lancet. 2017;389: 37-55. doi: S0140-6736(16)31919-5.
6. NCD Risk Factor Collaboration (NCD-RisC). Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128.9 million children, adolescents, and adults. Lancet. 2017;390: 2627-2642. doi: S0140-6736(17)32129-3.
7. International Diabetes Federation. IDF DIABETES ATLAS. Brussels, 8th edition 2017.
8. Clark SJ, Gomez-Olive FX, Houle B, Thorogood M, Klipstein-Grobusch K, Angotti N, et al. Cardiometabolic disease risk and HIV status in rural South Africa: establishing a baseline. BMC Public Health. 2015;15: 135-015-1467-1. doi: 10.1186/s12889-015-1467-1.
9. Price AJ, Crampin AC, Amberbir A, Kayuni-Chihana N, Musicha C, Tafatatha T, et al. Prevalence of obesity, hypertension, and diabetes, and cascade of care in sub-Saharan Africa: a cross-sectional, population-based study in rural and urban Malawi. Lancet Diabetes Endocrinol. 2018;6: 208-222. doi: S2213-8587(17)30432-1.
10. Noubiap JJ, Bigna JJ, Nansseu JR, Nyaga UF, Balti EV, Echouffo-Tcheugui JB, et al. Prevalence of dyslipidaemia among adults in Africa: a systematic review and meta-analysis. Lancet Glob Health. 2018;6: e998-e1007. doi: S2214-109X(18)30275-4.
11. Ngaruiya C, Abubakar H, Kiptui D, Kendagor A, Ntakuka MW, Nyakundi P, et al. Tobacco use and its determinants in the 2015 Kenya WHO STEPS survey. BMC Public Health. 2018;18: 1223-018-6058-5. doi: 10.1186/s12889-018-6058-5.
12. Joint United Nations Programme on HIV/AIDS (UNAIDS). UNAIDS DATA 2017. UNAIDS/ JC2910E. 2017.
13. Boulle A, Bock P, Osler M, Cohen K, Channing L, Hilderbrand K, et al. Antiretroviral therapy and early mortality in South Africa. Bull World Health Organ. 2008;86: 678-687. doi: S0042-96862008000900011.

14. Miles K, Clutterbuck DJ, Seitio O, Sebego M, Riley A. Antiretroviral treatment roll-out in a resource-constrained setting: capitalizing on nursing resources in Botswana. Bull World Health Organ. 2007;85: 555-560. doi: S0042-96862007000700014.
15. TEMPRANO ANRS 12136 Study Group, Danel C, Moh R, Gabillard D, Badje A, Le Carrou J, et al. A Trial of Early Antiretrovirals and Isoniazid Preventive Therapy in Africa. N Engl J Med. 2015;373: 808-822. doi: 10.1056/NEJMoa1507198.
16. INSIGHT START Study Group, Lundgren JD, Babiker AG, Gordin F, Emery S, Grund B, et al. Initiation of Antiretroviral Therapy in Early Asymptomatic HIV Infection. N Engl J Med. 2015;373: 795-807. doi: 10.1056/NEJMoa1506816.
17. World Health Organisation. Guideline on when to start antiretroviral therapy and on pre-exposure prophylaxis for HIV. Geneva, 2015, September. ISBN: 9789241509565.
18. Department of Health South Africa. Pillay, Y: Pillay, A. Implementation of the universal test and treat strategy for HIV positive patients and differentiated care for stable patients. DOH letter. 2016.
19. World Health Organisation. Antiretroviral therapy (ART) coverage among all age groups. Global Health Observatory (GHO) data. Geneva, 2017.
20. Legarth RA, Ahlstrom MG, Kronborg G, Larsen CS, Pedersen C, Pedersen G, et al. Long-Term Mortality in HIV-Infected Individuals 50 Years or Older: A Nationwide, Population-Based Cohort Study. J Acquir Immune Defic Syndr. 2016;71: 213-218. doi: 10.1097/QAI.0000000000000825.
21. Pillay-van Wyk V, Msemburi W, Laubscher R, Dorrington RE, Groenewald P, Glass T, et al. Mortality trends and differentials in South Africa from 1997 to 2012: second National Burden of Disease Study. The Lancet Global Health. 2016;4: e642-e653. doi: https://doi.org/10.1016/S2214-109X(16)30113-9.
22. 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;13: 453-468. doi: 10.1111/j.1468-1293.2012.00996.
23. Triant VA, Meigs JB, Grinspoon SK. Association of C-reactive protein and HIV infection with acute myocardial infarction. J Acquir Immune Defic Syndr. 2009;51: 268-273. doi: 10.1097/QAI.0b013e3181a9992c.
24. Freiberg MS, Chang CC, Kuller LH, Skanderson M, Lowy E, Kraemer KL, et al. HIV infection and the risk of acute myocardial infarction. JAMA Intern Med. 2013;173: 614-622. doi: 10.1001/jamainternmed.2013.3728.
25. 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;92: 2506-2512. doi: jc.2006-2190.

26. Mdodo R, Frazier EL, Dube SR, Mattson CL, Sutton MY, Brooks JT, et al. Cigarette smoking prevalence among adults with HIV compared with the general adult population in the United States: cross-sectional surveys. Ann Intern Med. 2015;162: 335-344. doi: 10.7326/M14-0954.
27. UNAIDS. The Gap Report. 2014. Available at: http://www.unaids.org/en/resources/documents/2014/20140716_UNAIDS_gap_report. Date last visited: 28 February 2019.
28. Gaziano TA, Abrahams-Gessel S, Gomez-Olive FX, Wade A, Crowther NJ, Alam S, et al. Cardiometabolic risk in a population of older adults with multiple co-morbidities in rural south africa: the HAALSI (Health and Aging in Africa: longitudinal studies of INDEPTH communities) study. BMC Public Health. 2017;17: 206-017-4117-y. doi: 10.1186/s12889-017-4117-y.
29. Frostegard J. Immunity, atherosclerosis and cardiovascular disease. BMC Med. 2013;11: 117-7015-11-117. doi: 10.1186/1741-7015-11-117.
30. Nou E, Lo J, Grinspoon SK. Inflammation, immune activation, and cardiovascular disease in HIV. AIDS. 2016;30: 1495-1509. doi: 10.1097/QAD.0000000000001109.
31. Zanni MV, Schouten J, Grinspoon SK, Reiss P. Risk of coronary heart disease in patients with HIV infection. Nat Rev Cardiol. 2014;11: 728-741. doi: 10.1038/nrcardio.2014.167.
32. Miedema F, Hazenberg MD, Tesselaar K, van Baarle D, de Boer RJ, Borghans JA. Immune activation and collateral damage in AIDS pathogenesis. Front Immunol. 2013;4: 298. doi: 10.3389/fimmu.2013.00298.
33. Nou E, Lo J, Grinspoon SK. Inflammation, immune activation, and cardiovascular disease in HIV. AIDS. 2016;30: 1495-1509. doi: 10.1097/QAD.0000000000001109.
34. Borges AH, O'Connor JL, Phillips AN, Neaton JD, Grund B, Neuhaus J, et al. Interleukin 6 Is a Stronger Predictor of Clinical Events Than High-Sensitivity C-Reactive Protein or D-Dimer During HIV Infection. J Infect Dis. 2016;214: 408-416. doi: 10.1093/infdis/jiw173.
35. Ross AC, Rizk N, O'Riordan MA, Dogra V, El-Bejjani D, Storer N, et al. Relationship between inflammatory markers, endothelial activation markers, and carotid intima-media thickness in HIV-infected patients receiving antiretroviral therapy. Clin Infect Dis. 2009;49: 1119-1127. doi: 10.1086/605578.
36. Fourie CM, Schutte AE, Smith W, Kruger A, van Rooyen JM. Endothelial activation and cardiometabolic profiles of treated and never-treated HIV infected Africans. Atherosclerosis. 2015;240: 154-160. doi: 10.1016/j.atherosclerosis.2015.03.015.
37. Funderburg NT, Zidar DA, Shive C, Lioi A, Mudd J, Musselwhite LW, et al. Shared monocyte subset phenotypes in HIV-1 infection and in uninfected subjects with acute coronary syndrome. Blood. 2012;120: 4599-4608. doi: 10.1182/blood-2012-05-433946.
38. Post WS, Budoff M, Kingsley L, Palella FJ,Jr, Witt MD, Li X, et al. Associations between HIV infection and subclinical coronary atherosclerosis. Ann Intern Med. 2014;160: 458-467. doi: 10.7326/M13-1754.

39. de Waal R, Cohen K, Maartens G. Systematic review of antiretroviral-associated lipodystrophy: lipoatrophy, but not central fat gain, is an antiretroviral adverse drug reaction. PLoS One. 2013;8: e63623. doi: 10.1371/journal.pone.0063623.

40. Brown TT, Cole SR, Li X, Kingsley LA, Palella FJ, Riddler SA, et al. Antiretroviral therapy and the prevalence and incidence of diabetes mellitus in the multicenter AIDS cohort study. Arch Intern Med. 2005;165: 1179-1184. doi: 165/10/1179.

41. Vos AG, Chersich MF, Klipstein-Grobusch K, Zuithoff P, Moorhouse MA, Lalla-Edward ST, et al. Lipid levels, insulin resistance and cardiovascular risk over 96 weeks of antiretroviral therapy: a randomised controlled trial comparing low-dose stavudine and tenofovir. Retrovirology. 2018;15: 77-018-0460-z. doi: 10.1186/s12977-018-0460-z.

42. Ekoru K, Young EH, Dillon DG, Gurdasani D, Stehouwer N, Faurholt-Jepsen D, et al. HIV treatment is associated with a two-fold higher probability of raised triglycerides: Pooled Analyses in 21 023 individuals in sub-Saharan Africa. Glob Health Epidemiol Genom. 2018;3: 10.1017/gheg.2018.7. Epub 2018 May 8. doi: e7.

43. UNAIDS. UNAIDS 2018 Estimates. 2018. Available at: http://www.unaids.org/sites/default/files/media_asset/unaids-data-2018_en.pdf. Date last visited: 28 February 2019

44. Lorenz MW, Markus HS, Bots ML, Rosvall M, Sitzer M. Prediction of clinical cardiovascular events with carotid intima-media thickness: a systematic review and meta-analysis. Circulation. 2007;115: 459-467. doi: CIRCULATIONAHA.106.628875.

45. Kleiger RE, Miller JP, Bigger JT,Jr, Moss AJ. Decreased heart rate variability and its association with increased mortality after acute myocardial infarction. Am J Cardiol. 1987;59: 256-262. doi: 0002-9149(87)90795-8.

46. Bots ML, Hoes AW, Koudstaal PJ, Hofman A, Grobbee DE. Common carotid intima-media thickness and risk of stroke and myocardial infarction: the Rotterdam Study. Circulation. 1997;96: 1432-1437.

47. Owolabi MO, Agunloye AM, Umeh EO, Akpa OM. Can common carotid intima media thickness serve as an indicator of both cardiovascular phenotype and risk among black Africans? Eur J Prev Cardiol. 2015;22: 1442-1451. doi: 10.1177/2047487314547656.

48. Van Bortel LM, Laurent S, Boutouyrie P, Chowienczyk P, Cruickshank JK, De Backer T, et al. Expert consensus document on the measurement of aortic stiffness in daily practice using carotid-femoral pulse wave velocity. J Hypertens. 2012;30: 445-448. doi: 10.1097/HJH.0b013e32834fa8b0.

49. Sassi R, Cerutti S, Lombardi F, Malik M, Huikuri HV, Peng CK, et al. Advances in heart rate variability signal analysis: joint position statement by the e-Cardiology ESC Working Group and the European Heart Rhythm Association co-endorsed by the Asia Pacific Heart Rhythm Society. Europace. 2015;17: 1341-1353. doi: 10.1093/europace/euv015.

50. Statistics South Africa. Mid-year population estimates 2018. Statistical release P0302. Available at: www statssa gov sa. Date last visited: 28 February 2019.

51. Houehanou YC, Lacroix P, Mizehoun GC, Preux PM, Marin B, Houinato DS. Magnitude of cardiovascular risk factors in rural and urban areas in Benin: findings from a nationwide steps survey. PLoS One. 2015;10: e0126441. doi: 10.1371/journal.pone.0126441.

1

531207-L-bw-Vos
Processed on: 20-5-2019

CHAPTER

2

Pro-inflammatory markers in relation to cardiovascular disease in HIV infection. A systematic review

A.G. Vos, N.S. Idris, R.E. Barth, K.Klipstein-Grobusch,

D.E. Grobbee

PLoS One. 2016 Jan 25;11(1)

Abstract

Background In the past years many inflammatory markers have been studied in association with clinically manifest cardiovascular disease (CVD) and carotid intima-media thickness (CIMT) in HIV-infected patients, to obtain insights in the increased cardiovascular risk observed in HIV infection. This systematic review provides an oversight of the current knowledge.

Methods A search was performed in PubMed, Embase and Cochrane in July 2014, identifying all articles from 1996 onwards addressing the relation between inflammatory markers and CVD or CIMT in HIV-positive adults. Two authors, using predefined criteria, independently conducted the selection of articles, critical appraisal and extraction of the data. Analysis was focused on the immune markers that were most frequently assessed. The review protocol was registered in the PROSPERO database at 11 July 2014 (registration number CRD42014010516). This review was performed according to the PRISMA guideline.

Findings Forty articles were selected; eight addressing cardiovascular disease (CVD) and thirty-two addressing CIMT. C-reactive protein (CRP), interleukin-6 (IL-6) and d-dimer were assessed most frequently in relation to the occurrence of CVD; in four out of eight studies. All three markers were positively related to CVD in three out of four studies. Studies addressing CIMT were too heterogeneous with respect to patient populations, inflammatory markers, CIMT measurement protocols and statistical methods to allow for a formal meta-analysis to obtain summary statistics. CRP, IL-6 and soluble vascular cell adhesion molecule (sVCAM-1) were the most studied markers in relation to CIMT. None of the inflammatory markers showed an association with CIMT.

Discussion This review showed a relation between some inflammatory markers and CVD, however, no consistent relation is observed for CIMT. Statistical approaches that yields effect estimates and standardized CIMT protocols should be chosen. Further research should focus on prospective studies and a selected set of inflammatory markers.

Introduction

When the human immunodeficiency virus (HIV) was discovered in the 1980's, the infection was believed to be immunosuppressive. This view changed in the 1990's, when evidence became available supporting the presence of chronic inflammation rather than primary immunodeficiency.[1]

With the initiation of antiretroviral therapy, mortality patterns in HIV patients changed from AIDS related opportunistic infections and malignancies to cancers not related to AIDS and cardiovascular disease (CVD).[2] Nearly ten years after the introduction of highly active antiretroviral therapy (HAART) non-AIDS defining illnesses were considered to be responsible for almost 50% of deaths in HIV-positive cohorts in North America; seven to 19% of all deaths were attributed to CVD.[3-5]

Chronic immune activation has a pivotal role in the pathogenesis of atherosclerosis in non-HIV infected patients.[6,7] Moreover, a range of studies has reported an association between immune activation and accelerated atherosclerosis in patients who are HIV-infected.[8-10]

The role of immune markers in relation to CVD risk in HIV-positive patients has not been clarified. Evaluating available data concerning the relation between pro-inflammatory parameters and CVD remains difficult if only because of differences in study design and the availability of various immune markers. Moreover, outcome measures vary from clinical relevant outcomes, like the occurrence of myocardial infarction or cardiac death, to surrogate markers of CVD: notably carotid intima-media thickness (CIMT) and markers of arterial stiffness.

The aim of the current review is to summarize the data on the association of pro-inflammatory markers with CVD, including their prognostic value, in HIV-infected patients.

Methods

Search strategy

The review protocol was registered in the PROSPERO database at 11 July 2014 (registration number CRD42014010516). A systematic literature search was conducted in PubMed, EMBASE and Cochrane library (table 1). Words and synonyms related to the domain, determinant and outcome were used. The domain were HIV-infected adults. As determinant, plasma or serum immune markers were included. We excluded cellular blood components (i.e. lymphocyte subsets) and genetic markers. Symptomatical cardiovascular disease or surrogate markers for cardiovascular disease (i.e. CIMT, ankle brachial index) were considered as outcomes (S1 Table). Search terms were limited to title and abstract.

Duplicates were removed by using reference management software, and further checked manually. The review was conducted in accordance to the PRISMA and STROME-ID guidelines.[11,12]

Study selection

Study selection was done in three steps (Fig 1). First, all identified records were screened based on titles and abstracts by one author (AV). Second, full text reports of all abstracts were independently read to assess eligibility by two authors (AV, NI), using preset inclusion criteria. Third, references and citations of the selected articles were screened for additional articles. Discrepancies were discussed in a consensus meeting by two authors (AV, NI).

Agreement could be reached for all but one article as there were different opinions on the question whether there was a relation between the immune marker and outcome, or not. After consulting of a third reviewer (KK), the article was excluded. If the same group of patients was described in more than one article the most detailed report was included. If the reports were complementary both were included and data were combined. For studies describing a group of HIV-positive and HIV-negative individuals, only findings of HIV-positive participants were used.

Table 1. Search strategy.

		Search terms	PubMed (MEDLINE) [title/abstract]	EMBASE [title/abstract]	Cochrane [title/abstract]
#1 Domain	HIV positive patients	HIV human immunodeficiency virus human immuno deficiency virus human immunedeficiency virus human immune deficiency virus aids acquired immunodeficiency syndrome acquired immuno deficiency syndrome acquired immunedeficiency syndrome acquired immune deficiency syndrome			
AND			309067	358649	16040
#2 Determinant	Pro-inflammatory markers	Inflammatory Inflammation Inflamm* Biomarker Biomarkers Immune*			

Table 1. Continued

		Search terms	PubMed (MEDLINE) [title/abstract]	EMBASE [title/ abstract]	Cochrane [title/ abstract]
AND			985859	1262812	34151
#3 Outcome	Cardiovascular disease or surrogate markers of cardiovascular disease.	cardiovascular CVD Myocardial infarction mi Coronary heart disease CHD Stroke Carotid intima media thickness CIMT Arterial stiffness Flow mediated dilation FMD PWV Pulse wave velocity Coronary artery calci* CAC Ankle brachial index ABI			
			570481	476332	57183
Final number of studies by combining #1 AND #2 AND #3			**821**	**246**	**70**
Search date for all databases July 2, 2014				EMBASE (AND [embase]/lim NOT [medline]/lim)	1 cochrane review 68 trials 1 methods study

Validity and data extraction

The following data were extracted: year of publication, study design, follow-up duration, number of patients, country, setting, age, sex, years since HIV diagnosis, CD4 level, viral load, ART use and duration, classic cardiovascular risk factors, inflammatory parameters measured, outcomes and outcome measurement methods. 'In case a database was described in more than one study, baseline characteristics of the most comprehensive article were used.'

Selected studies were critically appraised, particularly for the risk of selection-, detection-, and attrition bias. Bias risk was assigned as likely, unlikely, or unknown. The first author (AV) conducted the data extraction and critical appraisal using a set format. The second author (NI) independently checked all extracted data.

Analysis

As studies were expected to be very heterogeneous, results are descriptive, grouped by outcome and, inflammatory marker. When possible, percentages of common baseline characteristics were calculated. Due to heterogeneity of the data it was impossible to present effect estimates in a clear overview. The only common estimate per study was a p-value; therefore p-values were presented in a figure, stratified by method of analysis and accompanied by the sample size. All p-values of 0.25 or higher were considered to express minimal association. When only 'no significant' was reported, the p-value in the figure was also set at 0.25, when a p-value of <0.05 was reported, a value of 0.03 was displayed in the figure. Outcome data did not allow calculations of summary statistics or prognostic value. A correlation was considered relevant if the Rho value was 0.4 or higher. Relevant correlations were depicted with a circle in the figure. The three most commonly studied inflammatory markers were analyzed separately. Besides the top-three-studied immune markers, findings of the remaining markers assessed at least thrice were summarized in a table. Differences in CIMT measurement protocols were not taken into account. In this review C-reactive protein (CRP) refers to both the regular CRP measurement as to the high-sensitive CRP assays.

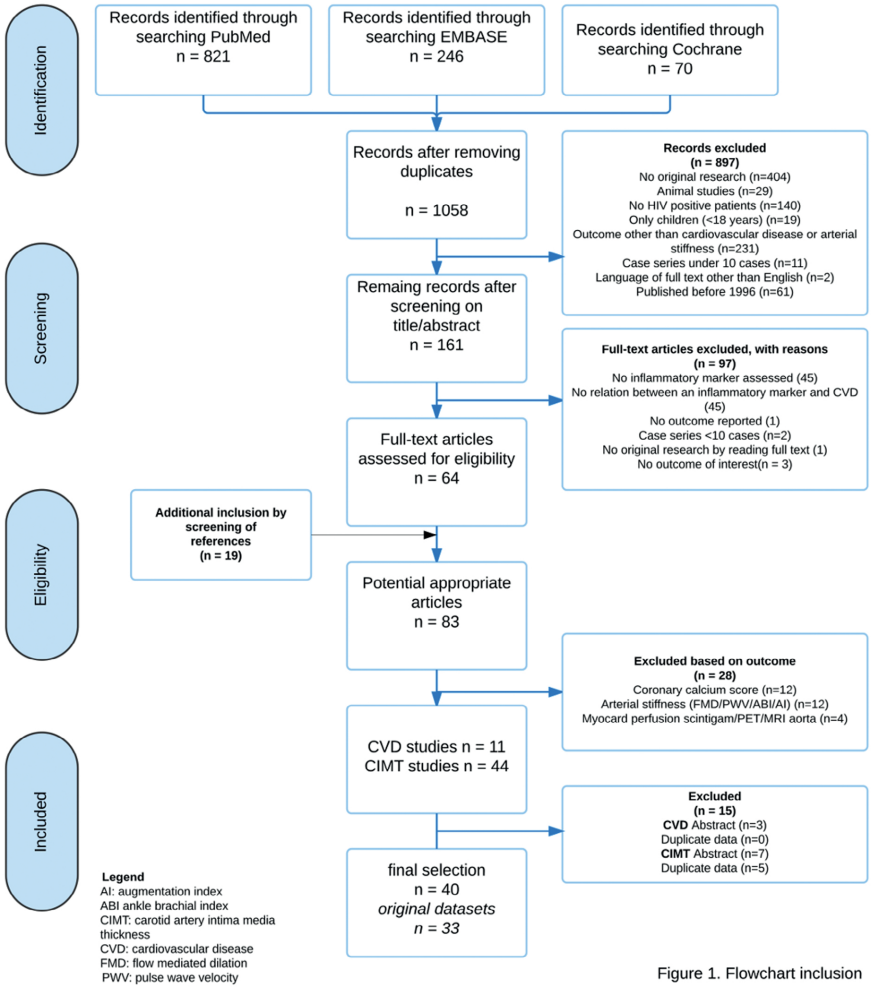


Figure 1. Flowchart inclusion

Figure 1. Flowchart inclusion. AI: augmentation index, ABI: ankle brachial index, CIMT: carotid intima media thickness, CVD: cardiovascular disease, FMD: flow mediated dilation, PWV: pulse wave velocity.

Results

1058 studies were identified, 64 articles remained after screening (Fig 1). Screening of references yielded another 19 articles, which did not mention immune marker measurement (mainly CRP) intitle or abstract. Agreement for inclusion of articles by the two authors (AV, NI) was over 99%.

Due to incomplete information abstracts were excluded (CVD 3 abstracts, CIMT 7 abstracts), in deviation of the initial review protocol. Two studies addressing CVD used the SMART cohort data; both were included in the final analysis since they presented additional information.[9,13] Six populations studied for CIMT were described in more than one article.[14-29] Studies containing additional information remained in the final analysis,[17,19-25,29,30] , studies presenting duplicate data were excluded. [14,16,27,28,31] Finally 40 articles remained (8 assessing CVD, 32 assessing CIMT) [9,10,13,15,17-25,29,30,32-56], including 33 original datasets, describing 48 immune markers.

Baseline characteristics

Almost all studies addressing CVD had a case-control design (appendix 1: Baseline table). The number of cases ranged from 35 to 487 cases [51,56] The majority of patients were men, aged around 47 years. The most frequently assessed markers were CRP, IL-6 and d-dimer; all were assessed in five out of eight studies.

The vast majority of studies addressing CIMT were cross-sectional. Only six out of 32 CIMT studies had a prospective design. The average number of HIV-positive patients per study was 155 (median 129), 80% of which was male. The median age was 46 years and median duration since HIV diagnosis was 9.3 years (interquartile range (IQR) 6·3-13·0). 12 studies had ART coverage of 100%[18,33,37-40,43-45,48-50,57] and three datasets described only ART naïve patients.[19,20,36,47] Average ART coverage in the other studies was 71%, and ART duration was five years (mean and median). Twelve studies were performed in the USA, nine in Europe and one in Africa (Uganda). Nearly 45% of all HIV patients were current smokers and mean body-mass index was 25kg/m^2. About 39% of studies specified that plasma was used, mostly frozen, for immune marker measurement. Protocols for CIMT measurements varied from two-point unilateral measurements to a comprehensive protocol with 12 measurements in each carotid artery.

Critical appraisal

All studies were appraised for eight items (fig 2, S2 Table). The criteria 'homogeneous moment of inclusion' and 'CIMT protocols' are not incorporated in the figure, since they could not be categorized as 'yes' or 'no' due to the different aspects that were covered.

S2 Table shows marked heterogeneity with regard to the patient populations included for CIMT studies. Although all studies had a standardized procedure for measuring immune markers and outcome, these procedures were different between studies.

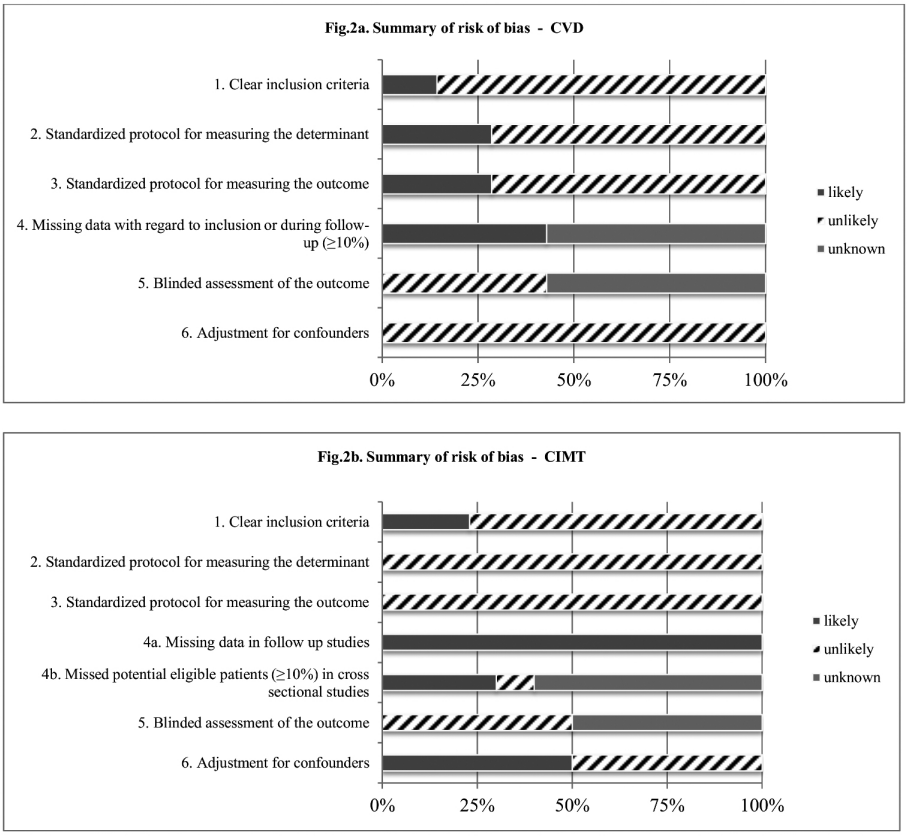


Figure 2. Summary of risk of bias.

Cardiovascular disease

Most frequently assessed markers across eight studies were CRP (five times), IL-6 (five times), d-dimer (five times) and sCD14 (three times). CRP, IL-6 and d-dimer were assessed four times in relation to the occurrence of CVD [9,51, 52,54,56], and one time in relation to fatal versus non-fatal CVD [13] . These markers were found to be significantly associated with the occurrence of CVD in three out of four studies (fig 3). [9,51,52,54,56] One article[52] could not be included in the figure since no odds ratios were presented. The authors did not find a relation between CRP, IL-6 and CVD, but they found an association between d-dimer and CVD; it was increased at both 4 months and 2 years prior to events.

Nordell and colleagues[13] used fatal versus non-fatal CVD as outcome. CRP showed no relation, but an increase in IL-6 or d-dimer increased the risk of a fatal CVD, odds ratio and 95% confidence interval for highest versus lowest tertile at baseline were 2.62 (1.26-6.46) and 2.70 (1.27-5.75) respectively. sCD14 was not associated with CVD in any of the three studies.[52,54,58] All other markers (n=32) were assessed less than three times.

Figure 3a

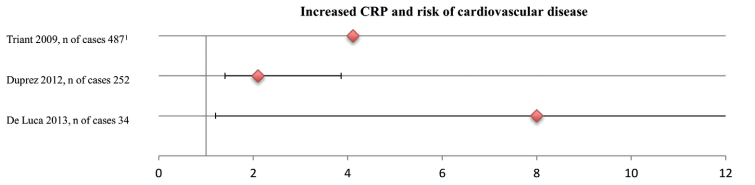


Figure 3b

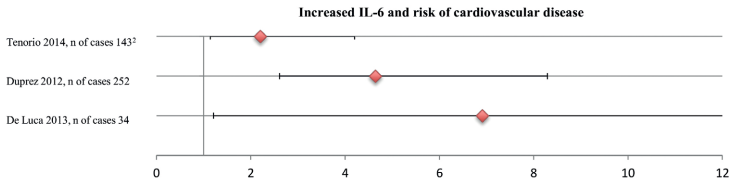
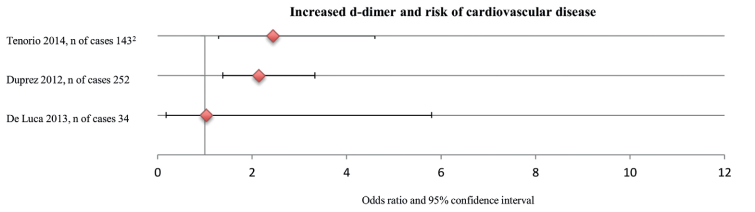


Figure 3c



1. Confidence interval unknown
2. Confidence intervals provided by author on request

Figure 3. Increased CRP, IL-6 and d-dimer and risk of cardiovascular disease. 1. Confidence interval unknown. 2. Confidence interval provided by author on request

Carotid intima-media thickness

In studies using CIMT as the endpoint, the most frequently studied inflammation markers were CRP (23 times), interleukin-6 (IL-6) (13 times) and soluble vascular cell adhesion molecule-1 (sVCAM-1) (10 times).

C-reactive protein

Figure 4a. (Fig 4a) shows the results of all studies addressing the relation between CRP and CIMT. Four out of seven significant results were calculated using correlation coefficients.[37,39,48,50] The correlations, however, were weak; the highest Rho value

was 0.33,[37,48] and were not confirmed in a regression analysis in in two out of four studies.[48,50]

Six studies, describing five patient populations, were prospective with a follow-up duration ranging from 48 to 144 weeks.[19,20,23,30,32,33] The methods that were used to assess the relation between CRP and CIMT differed, varying from a change in CRP versus CIMT progression in a follow-up period of 48 weeks[34], to the association of the baseline level of CRP and CIMT progression in a follow-up period of 48 to 96 weeks[19,20] to the association of the increase of CRP at baseline (in units or doubling of the normal value) versus CIMT increase in millimeters per year[30,59].

The cohort described by Hsue and colleagues[30] showed a significant association between a two-fold increase in CRP at baseline and IMT in univariate analysis, but this association disappeared in multivariable analysis (data not shown).[24,25]

When comparing outcomes from studies including only ART-treated patients (n=10) [18,33,37,40,43-45,48-50] and studies including only ART-naïve patients (n=4) [19,20,36,47], no differences were present.

Only one out of eight studies including patients with a suppressed viral load[33,37,40,42-45,49] found a positive correlation[37].

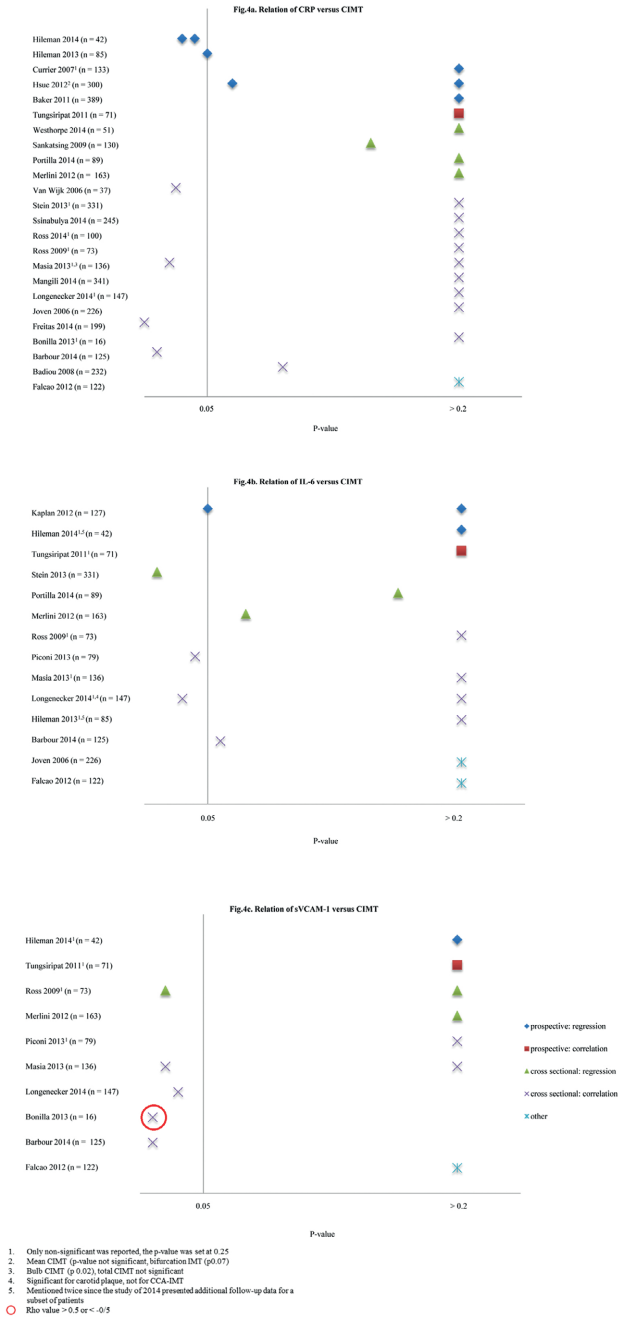


Figure 4. Relation of CRP, IL-6 and sVCAM-1 versus CIMT. 1. Only non-significant was reported, the p-value was set at 0.25, 2. Mean cIMT (p-value not significant, bifurcation IMT p0.07), 3. Bulb CIMT (p0.02), total CIMT not significant, 4. Significant for carotid plaque, not for CCA-IMT, 5. Mentioned twice since the study of 2014 presented additional follow-up data for a subset of patients, Rho value >0.5 or < -0.5.

Interleukin-6

IL-6 was assessed in 13 studies [10,15,18-20,33,34,39-43,47,50] (Fig 4b), six studies only mentioned that the association was non-significant.[18-20,33,39,43]

Of the prospective studies only Kaplan and colleagues[10] reported a positive association of IL-6 with CIMT in a subset of 81 out of 127 patients. However, the association was very modest (3.1 micrometers CIMT difference per 10% increase in biomarker, 95% CI -0.1- 6.3, p 0.05) and only seen following ART initiation.

In a cross-sectional analysis on ART-naïve HIV infected adults, Stein and colleagues[47] found a significant association between IL-6 and carotid lesions (OR 2.1, 95% CI 1.2-3.4), but not for other CIMT segments. Two cross-sectional studies reported a statistically significant but very weak correlation (maximum Rho value 0.22).[18,41]

Soluble Vascular Cellular Adhesion Molecule

Ten studies addressed the relation between sVCAM-1 and CIMT (fig 4c). [18,20,33,34,36,39-41,43,50] In the two prospective studies, no relation was found. [20,33] Although four positive associations were reported in cross-sectional studies [18,36,39,50] only the study of Bonilla and colleagues[36] showed a relevant association for bulb CIMT (Rho-value 0.66). The other correlations were weak, ranging from 0.22 to 0.28 across different CIMT segments.

Other markers

Of the remaining markers, twelve were assessed three times or more and 16 markers were only studied once or twice (table 2a and 2b). As shown in the table, the majority of these markers did not appear to be significantly associated with CIMT.

Discussion

We identified forty articles describing 33 original datasets, that addressed the relation between immune markers and CVD or CIMT in HIV-infected individuals. Increased levels of CRP, IL-6 and d-dimer were associated with an increased risk of CVD. Data did not allow calculation of the average effect size or prognostic value for any of the markers. No clear conclusion can currently be drawn for any of the markers assessed in relation to CIMT. This reflects, among other reasons, the heterogeneity in patient populations, cross-sectional nature of most studies and the variability in methods of data analysis.

The finding that levels of CRP, IL-6 and d-dimer are related to CVD is in line with findings in the general population and in populations with other chronic inflammatory conditions like psoriasis and rheumatoid arthritis.[60-66]

Table 2. Relation between immune markers and CIMT.

	Positive association	Negative association	No association
Inflammation			
TNF- α	2	1	7
sTNFR-1	1	1	5
sTNFR-2	0		6
sCD14	1[1]		8
sCD163	1[2]		3
MCP-1	2		6
MPO	1		3
LPS	1		3
Endothelial activation			
sICAM-1	0		7
Coagulation			
d-dimer	1		6
fibrinogen	1		7
tPAI-1	0		3
Other markers assessed less than 3 times			
CX3CL1	Interleukin-1β	Interleukin-8	Interleukin-10
soluble Interleukin-2 receptor	Mean malonyldialdehyde (MDA)	Matrix metallopeptidase 9 (MMP-9)	Neopterin
Osteoprotegerin (OPG)	Serum amyloid A (SAA)	serum amyloid P component (SAP)	sE-selectin
soluble receptor for advanced glycation end products (sRAGE)	Receptor activator of nuclear factor kappa-B ligand (RANKL)	vascular endothelial growth factor (VEGF)	Von Willebrand Factor (vWF)

1. Positive for yearly rate of change in CIMT versus baseline sCD14, cross-sectionally no association, 2. Positive correlation for total CIMT, not for bulb CIMT. Abbreviations: CIMT: carotid intima media thickness

Given this evidence, one would expect a positive association between inflammatory markers and CIMT as well. In a recent meta-analysis of individual patient data in the general population [67], a significant relation between CRP and fibrinogen and CIMT at baseline was indeed found. However, none of these markers were longitudinally associated with CIMT or CIMT progression after adjustment for classic cardiovascular risk factors, perhaps reflecting the relative healthy population and a short follow-up (mean of 3.9 years).

Baldassarre and colleagues[68] conducted a systematic review on the relation of immune makers to CIMT in the general population. They reported a significant association between CRP and fibrinogen in relation to CIMT based on a Fisher exact test since it was not possible to perform a formal meta-analysis due to the heterogeneity in ultrasound methodologies and statistical approaches. A Fisher exact test can be used to assess whether or not the number of studies reporting a relation between two determinants is larger than expected under the null hypothesis of no association.

When using the Fisher exact test, we similarly found an association between CRP and CIMT (p 0.03), but the use of this test can be questioned. First, as results are simply categorized as 'positive' or 'negative', depending on the p-value, no between-study differences were taken into account. Second, most positive associations were found by correlation analysis. A positive association, however, does not mean that there is indeed a real association given that a very low correlation coefficient can be statistically significant if numbers are large enough.

CRP is lower in individuals with chronic HCV infection.[69] As chronic HCV infection is common among HIV-infected individuals, this might be a confounding variable, explaining why no relation between CRP and CIMT was observed.

For other, less frequently investigated, markers, conclusions on the association with CIMT are even more difficult. We did show a relation between immune activation and CVD, therefore a similar relation for CIMT was expected. The inconclusive results for CIMT are likely due to the already mentioned between-study heterogeneity and the scarcity of prospective data. Besides, only a few markers are analysed in depth as a result of the enormous variety in marker choice, not allowing for firm statements with regard to the majority of markers. From a pragmatic point of view and with an eye on the costs of marker measurement (approximately £5.50/sample), future research should first explore the value of well-established biomarkers, before embarking on a fishing expedition to find any immune-marker 'associated' with CIMT.

Strengths and limitations

To our knowledge this is the first review that provides a full overview of immune-markers in relation to CVD and CIMT in HIV-infected patients. We used a systematic approach covering all available evidence from 1996 onwards, after the initiation of HAART, to July 2014. Since this review directly focuses on the role of immune-markers, it provided a clear, global overview of the current knowledge.

To appreciate the results some limitations need to be mentioned. First, across studies there was a marked heterogeneity in study population, design and methodology of data analysis, limiting the possibilities for a clear summary of outcome data. Second, the vast majority of studies were cross-sectional rather than prospective. Third, the only common measure of association in studies assessing CIMT was a p-value. For reasons of comparability we decided to present the p-value, although we recognize the dependence on the sample size and the lack of parameter estimates. Forth, co-infection with hepatitis C was not taken into account, which may have led to an underestimation of our results. Finally, we did not take into account the differences in protocols for the assessment of CIMT nor the relation of markers for the diverse CIMT segments

(common, bulb, internal). By regarding all segments as being the same we might have overlooked a specific association.

Recommendations

To obtain reliable information on the prognostic value of inflammatory markers in relation to CVD in HIV-infected-patients research addressing hard CVD outcomes in follow-up studies is needed. Currently some large prospective studies are undertaken, like the REPRIEVE trial[70] and the PURE study [71] that will provide data addressing the relation between inflammation, cardiovascular diseases and HIV infection.

As long as these data are not available CIMT could be used as a surrogate, preferably prospectively and with extended follow-up, and choice of immune-markers should focus on a selective set of markers. Furthermore, studies should be optimized with regard to definition of patient population, data-analysis and reporting. Finally, for reasons of comparability, it would be advisable to standardize the CIMT protocols and the definitions of outcomes.

Conclusion

This review gives an overview of available evidence regarding the role of inflammation in relation to CVD and CIMT in HIV-infection. Although an association between three immune-markers (CRP, IL-6 and d-dimer) and CVD was observed, no consistent relation with CIMT could be detected for any of the immune-makers. This might reflect the heterogeneity of the CIMT-studies and the lack of adequate prospective data. In view of the costs and interpretability, the search for immune markers 'associated' with CIMT in cross-sectional studies should be reconsidered. Future research should aim to be of prospective design, utilizing standardized approaches for the selection of participants, immune markers and assessment of the outcome.

References

1. Miedema F, Hazenberg MD, Tesselaar K, van Baarle D, de Boer RJ, Borghans JA. Immune activation and collateral damage in AIDS pathogenesis. Front Immunol 2013 Sep 26;4:298.
2. Lewden C, May T, Rosenthal E, Burty C, Bonnet F, Costagliola D, et al. Changes in causes of death among adults infected by HIV between 2000 and 2005: The "Mortalite 2000 and 2005" surveys [ANRS EN19 and Mortavic]. J Acquir Immune Defic Syndr 2008 Aug 15;48[5]:590-598.
3. Palella FJ,Jr, Baker RK, Moorman AC, Chmiel JS, Wood KC, Brooks JT, et al. Mortality in the highly active antiretroviral therapy era: changing causes of death and disease in the HIV outpatient study. J Acquir Immune Defic Syndr 2006 Sep;43[1]:27-34.
4. Data Collection on Adverse Events of Anti-HIV drugs [D:A:D] Study Group, Smith C, Sabin CA, Lundgren JD, Thiebaut R, Weber R, et al. Factors associated with specific causes of death amongst HIV-positive individuals in the D:A:D Study. AIDS 2010 Jun 19;24[10]:1537-1548.
5. Rodger AJ, Lodwick R, Schechter M, Deeks S, Amin J, Gilson R, et al. Mortality in well controlled HIV in the continuous antiretroviral therapy arms of the SMART and ESPRIT trials compared with the general population. AIDS 2013 Mar 27;27[6]:973-979.
6. Libby P, Ridker PM, Hansson GK, Leducq Transatlantic Network on Atherothrombosis. Inflammation in atherosclerosis: from pathophysiology to practice. J Am Coll Cardiol 2009 Dec 1;54[23]:2129-2138.
7. Weber C, Noels H. Atherosclerosis: current pathogenesis and therapeutic options. Nat Med 2011 Nov 7;17[11]:1410-1422.
8. Triant VA. HIV infection and coronary heart disease: an intersection of epidemics. J Infect Dis 2012 Jun;205 Suppl 3:S355-61.
9. Duprez DA, Neuhaus J, Kuller LH, Tracy R, Belloso W, De Wit S, et al. Inflammation, coagulation and cardiovascular disease in HIV-infected individuals. PLoS One 2012;7[9]:e44454.
10. Kaplan RC, Landay AL, Hodis HN, Gange SJ, Norris PJ, Young M, et al. Pmc3400505; Potential cardiovascular disease risk markers among HIV-infected women initiating antiretroviral treatment. J Acquir Immune Defic Syndr 2012 Aug 1;60:359-368.
11. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gotzsche PC, Ioannidis JP, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. BMJ 2009 Jul 21;339:b2700.
12. Field N, Cohen T, Struelens MJ, Palm D, Cookson B, Glynn JR, et al. Strengthening the Reporting of Molecular Epidemiology for Infectious Diseases [STROME-ID]: an extension of the STROBE statement. Lancet Infect Dis 2014 Apr;14[4]:341-352.
13. Nordell AD, McKenna M, Borges AH, Duprez D, Neuhaus J, Neaton JD. Severity of Cardiovascular Disease Outcomes Among Patients With HIV Is Related to Markers of Inflammation and Coagulation. J Am Heart Assoc 2014;3.

14. Alonso-Villaverde C, Coll B, Parra S, Montero M, Calvo N, Tous M, et al. Atherosclerosis in patients infected with HIV is influenced by a mutant monocyte chemoattractant protein-1 allele. Circulation 2004 Oct 12;110[15]:2204-2209.
15. Joven J, Coll B, Tous M, Ferre N, Alonso-Villaverde C, Parra S, et al. The influence of HIV infection on the correlation between plasma concentrations of monocyte chemoattractant protein-1 and carotid atherosclerosis. Clin Chim Acta 2006 Jun;368:114-119.
16. Coll B, Parra S, Alonso-Villaverde C, de Groot E, Aragones G, Montero M, et al. HIV-infected patients with lipodystrophy have higher rates of carotid atherosclerosis: the role of monocyte chemoattractant protein-1. Cytokine 2006 Apr;34:51-55.
17. Parra S, Coll B, Aragones G, Marsillach J, Beltran R, Rull A, et al. Nonconcordance between subclinical atherosclerosis and the calculated Framingham risk score in HIV-infected patients: relationships with serum markers of oxidation and inflammation. HIV Med 2010 Apr;11:225-231.
18. Longenecker CT, Jiang Y, Orringer CE, Gilkeson RC, Debanne S, Funderburg NT, et al. Soluble CD14 is independently associated with coronary calcification and extent of subclinical vascular disease in treated HIV infection. AIDS 2014 Apr 24;28:969-977.
19. Hileman CO, Carman TL, Longenecker CT, Labbato DE, Storer NJ, White CA, et al. Rate and predictors of carotid artery intima media thickness progression in antiretroviral-naive HIV-infected and uninfected adults: a 48-week matched prospective cohort study. Antivir Ther [Lond] 2013;18:921-929.
20. Hileman CO, Longenecker CT, Carman TL, McComsey GA. C-reactive protein predicts 96-week carotid intima media thickness progression in HIV-infected adults naive to antiretroviral therapy. J Acquir Immune Defic Syndr 2014 Mar 1;65:340-344.
21. Kelesidis T, Kendall MA, Yang OO, Hodis HN, Currier JS. Pmc3475633; Biomarkers of microbial translocation and macrophage activation: association with progression of subclinical atherosclerosis in HIV-1 infection. J Infect Dis 2012 Nov 15;206:1558-1567.
22. Kelesidis T, Kendall MA, Yang OO, Hodis H, Currier JS. Pmc3653400; Perturbations of circulating levels of RANKL-osteoprotegerin axis in relation to lipids and progression of atherosclerosis in HIV-infected and -uninfected adults: ACTG NWCS 332/A5078 Study. AIDS Res Hum Retroviruses 2013 Jun;29:938-948.
23. Currier JS, Kendall MA, Henry WK, Alston-Smith B, Torriani FJ, Tebas P, et al. Progression of carotid artery intima-media thickening in HIV-infected and uninfected adults. AIDS 2007 May 31;21[9]:1137-1145.
24. Hsue PY, Hunt PW, Sinclair E, Bredt B, Franklin A, Killian M, et al. Increased carotid intima-media thickness in HIV patients is associated with increased cytomegalovirus-specific T-cell responses. AIDS 2006 Nov 28;20:2275-2283.

25. Hsue PY, Hunt PW, Schnell A, Kalapus SC, Hoh R, Ganz P, et al. Pmc2691772; Role of viral replication, antiretroviral therapy, and immunodeficiency in HIV-associated atherosclerosis. AIDS 2009 Jun 1;23:1059-1067.
26. Hsue PY, Deeks SG, Hunt PW. Pmc3349295; Immunologic basis of cardiovascular disease in HIV-infected adults. J Infect Dis 2012 Jun;205 Suppl 3:S375-82.
27. Mangili A, Gerrior J, Tang AM, O'Leary DH, Polak JK, Schaefer EJ, et al. Risk of cardiovascular disease in a cohort of HIV-infected adults: a study using carotid intima-media thickness and coronary artery calcium score. Clin Infect Dis 2006 Dec 1;43[11]:1482-1489.
28. Mangili A, Jacobson DL, Gerrior J, Polak JF, Gorbach SL, Wanke CA. Metabolic syndrome and subclinical atherosclerosis in patients infected with HIV. Clin Infect Dis 2007 May 15;44[10]:1368-1374.
29. Mangili A, Ahmad R, Wolfert RL, Kuvin J, Polak JF, Karas RH, et al. Pmc3935500; Lipoprotein-associated phospholipase A2, a novel cardiovascular inflammatory marker, in HIV-infected patients. Clin Infect Dis 2014 Mar;58:893-900.
30. Hsue PY, Scherzer R, Hunt PW, Schnell A, Bolger AF, Kalapus SC, et al. Pmc3487373; Carotid Intima-Media Thickness Progression in HIV-Infected Adults Occurs Preferentially at the Carotid Bifurcation and Is Predicted by Inflammation. J Am Heart Assoc 2012 Apr;1.
31. Longenecker C, Funderburg N, Jiang Y, Debanne S, Storer N, Labbato D, et al. Markers of inflammation and CD8 T-cell activation, but not monocyte activation, are associated with subclinical carotid artery disease in HIV-infected individuals. HIV medicine 2013;14:385-390.
32. Baker JV, Henry WK, Patel P, Bush TJ, Conley LJ, Mack WJ, et al. Progression of carotid intima-media thickness in a contemporary human immunodeficiency virus cohort. Clin Infect Dis 2011 Oct;53[8]:826-835.
33. Tungsiripat M, El-Bejjani D, Rizk N, Dogra V, O'Riordan M, Ross A, et al. Carotid intima media thickness, inflammatory markers, and endothelial activation markers in HIV Patients with lipoatrophy increased at 48 weeks regardless of use of rosiglitazone or placebo. AIDS Res Hum Retroviruses 2011;27:295-302.
34. Falcao Mda C, Zirpoli JC, Albuquerque VM, Markman Filho B, Araujo NA, Falcao CA, et al. Association of biomarkers with atherosclerosis and risk for coronary artery disease in patients with HIV. Arq Bras Cardiol 2012 Nov;99:971-978.
35. Badiou S, Thiebaut R, Aurillac-Lavignolle V, Dabis F, Laporte F, Cristol JP, et al. Association of non-HDL cholesterol with subclinical atherosclerosis in HIV-positive patients. J Infect 2008 Jul;57:47-54.
36. Bonilla H, McShannic J, Goldberg E, Chua D, Conner R, Fiorentino M, et al. Impact of human immunodeficiency virus infection on measures of cardiovascular disease in long-term nonprogressors. Infectious Diseases in Clinical Practice 2013;21:177-180.

37. Freitas P, Carvalho D, Santos AC, Madureira AJ, Martinez E, Pereira J, et al. Carotid intima media thickness is associated with body fat abnormalities in HIV-infected patients. BMC Infect Dis 2014 Jun 23;14:348.

38. Jeong SJ, Kim CO, Song YG, Baek JH, Kim SB, Jin SJ, et al. Low plasma levels of the soluble receptor for advanced glycation end products in HIV-infected patients with subclinical carotid atherosclerosis receiving combined antiretroviral therapy. Atherosclerosis 2011 Dec;219:778-783.

39. Masia M, Robledano C, Ortiz de lT, Antequera P, Lopez N, Gutierrez F. Pmc3662719; Increased carotid intima-media thickness associated with antibody responses to varicella-zoster virus and cytomegalovirus in HIV-infected patients. PLoS One 2013;8:e64327.

40. Merlini E, Luzi K, Suardi E, Barassi A, Cerrone M, Martinez JS, et al. Pmc3459872; T-cell phenotypes, apoptosis and inflammation in HIV+ patients on virologically effective cART with early atherosclerosis. PLoS One 2012;7:e46073.

41. Piconi S, Parisotto S, Rizzardini G, Passerini S, Meraviglia P, Schiavini M, et al. Atherosclerosis is associated with multiple pathogenic mechanisms in HIV-infected antiretroviral-naive or treated individuals. AIDS 2013 Jan 28;27:381-389.

42. Portilla J, Moreno-Perez O, Serna-Candel C, Escoin C, Alfayate R, Reus S, et al. Pmc4021989; Vitamin D insufficiency and subclinical atherosclerosis in non-diabetic males living with HIV. J Int AIDS Soc 2014;17:18945.

43. Ross AC, Rizk N, O'Riordan MA, Dogra V, El-Bejjani D, Storer N, et al. Pmc3895473; Relationship between inflammatory markers, endothelial activation markers, and carotid intima-media thickness in HIV-infected patients receiving antiretroviral therapy. Clin Infect Dis 2009 Oct 1;49:1119-1127.

44. Ross Eckard A, Longenecker C, Jiang Y, Debanne S, Labbato D, Storer N, et al. Lipoprotein-associated phospholipase A and cardiovascular disease risk in HIV infection. HIV Med 2014 Mar 20.

45. Sankatsing RR, Wit FW, Vogel M, de Groot E, Brinkman K, Rockstroh JK, et al. Increased carotid intima-media thickness in HIV patients treated with protease inhibitors as compared to non-nucleoside reverse transcriptase inhibitors. Atherosclerosis 2009 Feb;202[2]:589-595.

46. Ssinabulya I, Kayima J, Longenecker C, Luwedde M, Semitala F, Kambugu A, et al. Pmc3938501; Subclinical atherosclerosis among HIV-infected adults attending HIV/AIDS care at two large ambulatory HIV clinics in Uganda. PLoS One 2014;9:e89537.

47. Stein J, Brown T, Ribaudo H, Chen Y, Yan M, Lauer-Brodell E, et al. Ultrasonographic measures of cardiovascular disease risk in antiretroviral treatment-naive individuals with HIV infection. AIDS 2013;27:929-937.

48. van Wijk JP, de Koning EJ, Cabezas MC, Joven J, op't Roodt J, Rabelink TJ, et al. Functional and structural markers of atherosclerosis in human immunodeficiency virus-infected patients. J Am Coll Cardiol 2006 Mar 21;47:1117-1123.

49. Westhorpe CL, Maisa A, Spelman T, Hoy JF, Dewar EM, Karapanagiotidis S, et al. Associations between surface markers on blood monocytes and carotid atherosclerosis in HIV-positive individuals. Immunol Cell Biol 2014 Feb;92:133-138.

50. Barbour JD, Jalbert EC, Chow DC, Gangcuangco LMA, Norris PJ, Keating SM, et al. Reduced CD14 expression on classical monocytes and vascular endothelial adhesion markers independently associate with carotid artery intima media thickness in chronically HIV-1 infected adults on virologically suppressive anti-retroviral therapy. Atherosclerosis 2014;232:52-58.

51. De Luca A, de GD, Colafigli M, Cozzi-Lepri A, De Curtis A, Gori A, et al. Pmc3846422; The association of high-sensitivity c-reactive protein and other biomarkers with cardiovascular disease in patients treated for HIV: a nested case-control study. BMC Infect Dis 2013;13:414.

52. Ford ES, Greenwald JH, Richterman AG, Rupert A, Dutcher L, Badralmaa Y, et al. Pmc2884071; Traditional risk factors and D-dimer predict incident cardiovascular disease events in chronic HIV infection. AIDS 2010 Jun 19;24:1509-1517.

53. Knudsen A, Moller HJ, Katzenstein TL, Gerstoft J, Obel N, Kronborg G, et al. Pmc3663777; Soluble CD163 does not predict first-time myocardial infarction in patients infected with human immunodeficiency virus: a nested case-control study. BMC Infect Dis 2013;13:230.

54. Tenorio AR, Zheng Y, Bosch RJ, Krishnan S, Rodriguez B, Hunt PW, et al. Soluble Markers of Inflammation and Coagulation but Not T-Cell Activation Predict Non-AIDS-Defining Morbid Events During Suppressive Antiretroviral Treatment. J Infect Dis 2014 May 1.

55. Sandler NG, Wand H, Roque A, Law M, Nason MC, Nixon DE, et al. Pmc3071127; Plasma levels of soluble CD14 independently predict mortality in HIV infection. J Infect Dis 2011 Mar 15;203:780-790.

56. Triant VA, Meigs JB, Grinspoon SK. Association of C-reactive protein and HIV infection with acute myocardial infarction. J Acquir Immune Defic Syndr 2009 Jul 1;51[3]:268-273.

57. Kaplan RC, Landay AL, Hodis HN, Gange SJ, Norris PJ, Young M, et al. Potential cardiovascular disease risk markers among HIV-infected women initiating antiretroviral treatment. J Acquir Immune Defic Syndr 2012 Aug 1;60[4]:359-368.

58. Sandler NG, Wand H, Roque A, Law M, Nason MC, Nixon DE, et al. Plasma levels of soluble CD14 independently predict mortality in HIV infection. J Infect Dis 2011 Mar 15;203[6]:780-790.

59. Baker J, Neuhaus J, Duprez D, Cooper D, Hoy J, Kuller L, et al. Inflammation predicts changes in high-density lipoprotein particles and apolipoprotein A1 following initiation of antiretroviral therapy. AIDS 2011;25:2133-2142.

60. Pirro M, Stingeni L, Vaudo G, Mannarino MR, Ministrini S, Vonella M, et al. Systemic inflammation and imbalance between endothelial injury and repair in patients with psoriasis are associated with preclinical atherosclerosis. Eur J Prev Cardiol 2014 Jun 6.
61. Goodson NJ, Symmons DP, Scott DG, Bunn D, Lunt M, Silman AJ. Baseline levels of C-reactive protein and prediction of death from cardiovascular disease in patients with inflammatory polyarthritis: a ten-year followup study of a primary care-based inception cohort. Arthritis Rheum 2005 Aug;52[8]:2293-2299.
62. Danesh J, Wheeler JG, Hirschfield GM, Eda S, Eiriksdottir G, Rumley A, et al. C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease. N Engl J Med 2004 Apr 1;350[14]:1387-1397.
63. Ridker PM. Clinical application of C-reactive protein for cardiovascular disease detection and prevention. Circulation 2003 Jan 28;107[3]:363-369.
64. Willeit P, Thompson A, Aspelund T, Rumley A, Eiriksdottir G, Lowe G, et al. Hemostatic factors and risk of coronary heart disease in general populations: new prospective study and updated meta-analyses. PLoS One 2013;8[2]:e55175.
65. Lee JK, Bettencourt R, Brenner D, Le TA, Barrett-Connor E, Loomba R. Association between serum interleukin-6 concentrations and mortality in older adults: the Rancho Bernardo study. PLoS One 2012;7[4]:e34218.
66. Su D, Li Z, Li X, Chen Y, Zhang Y, Ding D, et al. Association between serum interleukin-6 concentration and mortality in patients with coronary artery disease. Mediators Inflamm 2013;2013:726178.
67. Willeit P, Thompson SG, Agewall S, Bergstrom G, Bickel H, Catapano AL, et al. Inflammatory markers and extent and progression of early atherosclerosis: Meta-analysis of individual-participant-data from 20 prospective studies of the PROG-IMT collaboration. Eur J Prev Cardiol 2014 Nov 21.
68. Baldassarre D, De Jong A, Amato M, Werba JP, Castelnuovo S, Frigerio B, et al. Carotid intima-media thickness and markers of inflammation, endothelial damage and hemostasis. Ann Med 2008;40[1]:21-44.
69. Shah S, Ma Y, Scherzer R, Huhn G, French AL, Plankey M, et al. Association of HIV, hepatitis C virus and liver fibrosis severity with interleukin-6 and C-reactive protein levels. AIDS. 2015 Jul 7;29[11]:1325-33.
70. Reprieve Trial. Available at: www.reprievetrial.org. Assessed on 10th of November 2015
71. Teo K, Chow CK, Vaz M, Rangarajan S, Yusuf S; PURE Investigators-Writing Group. The Prospective Urban Rural Epidemiology [PURE] study: examining the impact of societal influences on chronic noncommunicable diseases in low-, middle-, and high-income countries. Am Heart J. 2009 Jul;158[1]:1-7.

Supporting File 1. Baseline Table

Author	(Design &) enrollment period	Duration of follow up	Number of HIV-positive patients	Country	Ethnicity	% ♂	Age	Years since HIV diagnosis	CD4 count	Nadir CD4 count	VL	BMI (kg/m2)	Current smokers	ART + (%)	Tx regimen	Duration of ART (months)	Markers assessed	Stored sample used	Time of outcome with regard to biomarker	Methods
Cardiovascular events																				
De Luca 2013	Nested case-control study. Enrollment since 1997	Cases nd Controls ≥5yrs.	35 cases (with CVD event), 74 controls (no CVD event)	Italy	nd	97 / 89	47/45	nd	550/525	nd	2.1/1.7 $\log_{10}$ cp/ml	23.7/23.1	Distribution of matching variable smokers/ diabetics (3;4)	100	nd	NRTI 51/121 NNRTI 3/30 PI 26/52	hscRP, d-dimer, p-selectin, IL-6, t-PA, PAI-1	Yes	Most recent sample:3 months/8 months Older sample: nd	Nephelometry (hsCRP) Immunoturbidimetric assay (d-dimer) ELISA (other) plasma
Ford 2010	Nested case control within NIAID 1995-2009	8.2 / 8.6 years	52 cases 104 controls	USA	African-American 19.2% / 14.4%	98 / 98	50.8 / 50.8	13.4/ 14.0	nd	209 / 229	Peak 4.3/5.4 cp/mL	25.6/ 25.6	49.0 / 25.0	100	nd	107/101 PI exposure 44/48	CRP, IL-6, d-dimer. sCD14, sFT, GM-CSF, INF-Y, IL-1β, IL-10, IL12p70, IL-2, Il-8, TNF-α, eotaxin, eotaxin-3, IP-10, MCP-1, MCP-4, MDC, MIP-1β, TARC, SAA, VCAM-1, ICAM-1, CRP, MPO, TIMP-1, TNRF-II	Yes	4.5 months and 21.6 months prior to event	d-dimer ELISA (Vidas) sCD14, sFT ELISA (R&D) Other ELISA multiplex kits plasma
Knudsen 2013	Nested case control study within Danish HIV Cohort Study 1998-2008	1.3 yr (medium, between first plasma sampel and event)	55 cases 182 controls	Denmark	White 93 / 94%	91 / 92%	49/50	10 /10	496 / 547	nd	<400cp/mL 80/89%	nd	96/97	100	NRTI 100% Abacavir 56/39% NNRTI 73/58% PI 87/84%	72/72	sCD163	Yes	4 samples: 1: before start of ART 2: 3 months after start of ART 3: one year before event 4: sample before event 52 days to event	ELISA plasma

Nordell 2014	Retrospective cohort study of SMART, ESPRIT, SILCAAT	5 yrs (median)	288 cases: 74 fatel, 214 non-fatal 8766 non cases	Resp 33, 25 and 11 countries	Black resp 23/16/19%	86 / 92 / 78	48 / 49/ 42	nd	409/469/487	nd	<500cp/mL 74/77/77	24.0/ 24.3/ 24.3	53/52/41%[3]	100	81/87/84 in SMART, remaining 100%	nd	hsCRP, IL-6, d-dimer	Yes	Baseline samples	hsCRP ELISA (nephelometer, R&D) IL-6 ELISA d-dimer ELISA (Sta-R and VIDAS) plasma
Sandler 2010	Nested case control within SMART	Median of 16 months	120 cases, 238 controls	Enrollment in 33 countries	White 58.3/61.8 Black 42 / 38%	81 / 81	49 / 48	nd	607/638	209/241	≤400 cp/mL (%) 66/59	25.2/25.6	55.0 / 37.4	100	nd	48 months (median among all subjects)	I-FABP, LPS, sCD14, EndoCAb	Yes	Plasma samples taken at baseline	Elisa sCD14 (R&S systems) EndoCAb, I-FABP (Cell sciences) LPS (Limulus assay) Plasma
Tenorio 2014	Case control study ACTG ALLRT participants	2.9 yrs (median, after ART initiation)	143 cases (all causes), 315 controls	USA	White 52/48%W Black 36/28% Hispanic 11/20%	85 / 85	46/44	nd	208/221	nd	4.8 / 4.8 Log_{10} cp/mL	0.92 / 0.92 *(waist to hip ratio, median)*	75 / 55*	100 /100	nd	2.9 yrs *(median, same as follow up duration)*	IL-6, sCD14, IFN-γ, IP-10, sTNFR-1 and II, d-dimer	yes	Samples at baseline (before start of ART), 1 year after start of ART, and pre-event	R&D systems: all, except d-dimer. D-dimer: Diagnostica Stago Plasma
Triant 2009	Retrospective cohort study within Partners HealthCare System 1997-2006	6.0 yrs (mean)	487 HIV+ cases 69870 HIV- cases	USA	White 58% Nonwhite 38%	62.8%	≥55 yrs 24.7%	nd	> CRP: 43% <200 CRP = 28% <200	nd	> CRP: undetectable in 34% CRP =: undectable in 38%	nd	57%*	nd	NNRTI 43% PI: > CRP 67% CRP= 39%	nd	(hs)CRP	no	199 days (median)	Both standard and high sensitivity assays Plasma/serum: nd
Studie describing the same databases as one of the above mentioned studies, but with additional information																				
Duprez 2012 (partly double with Nordell 2014)	Retrospective cohort study of SMART database. Inclusion between 2002-2006	Min. 18 median 29 months	252 cases 4846 non-cases	Enrollment in 33 countries	Black 40 / 29%	81 / 74%	49/43	nd	579/600	236/252	≤400cp/mL 68 / 71%	25.7/25.0	52/41%	100	nd	nd	hsCRP, IL-6, d-dimer	Yes	Median 29 months (same as follow up time)	hsCRP nephelometer IL-6 ELISA (R&D) d-dimer immunoturbidometer plasma
Carotid intima-media thickness; longitudinal																				
Baker 2011	Prospective cohort study March 2004 – June 2006 Measurement at T0 and T1	2 yrs	389	USA	White, non-Hispanic 26% Black, non hispanic 12%	77	42	4.7	485	215	VL <400cp/ml 72%	26	38%	78	NNRTI 39% PI 35% Abacavir 23%	32	hsCRP	yes	=	Immunoturbidimetry Plasma
Currier 2007	Prospective matched cohort Triads of 1)HIV and PI>2yrs 2) HIV without PI use 3) HIV uninfected February 2001 – May 2002	144 weeks	133	USA	White 76% Black 4% Hispanic 16%	90	42	nd	530/481 (PI vs non PI group)	nd	<400cp/ml 75/69% (PI vs non PI group)	nd	45%[2]	97%	PI ±33%	nd	hsCRP	nd	=	nd

Hileman 2013	Matched prospective cohort study July 2008-April 2010	48 wks	85 HIV+ (45 HIV-)	USA	Caucasian 39% African American 58% Latino 2%	78	40 (32-47)	3.3	535	nd	6916 cp/ml *RNA*	27	52%	0	na	na	hsCRP, IL-6, TNFR-1,2, sVCAM-1, sICAM-1, d-dimer, fibrinogen	yes	=	Nephelometry (hsCRP, fibrinogen) Turbidometry (d-dimer); other markers with ELISA Plasma
Hsue 2012	Prospective cohort study (within the SCOPE cohort)nd	2.4 yrs	300 HIV + (47 HIV-controls)	USA	Caucasian 60%, African American 25% Latino 10%	89	47 (41-53)	13	434	172	<75 cp/ml 53%	25	69%[2]	76 (ever)	NRTI use (ever) 76% NNRTI use (ever) 48% PI use (ever) 65%	60	hsCRP	nd		Dade Behring Plasma/serum: nd
Kaplan 2012	Matched prospective cohort (within the WIHS cohort) 1994-1995 (pre-HAART) 2001-2002	2,5yrs	127 HIV+ (127 HIV-controls)	USA	African-American 59% Latina 24% White/ Caucasian 17%	0	37 (33-42)	nd	332 (before start of ART)	nd	4.4 (log VL) (before start of ART)	>25 61%	53%	100	PI 53% NNRTI 35% NRTI 93%	Started during the study period	sCD14, TNF-a, sIL-2rec, IL-6, IL-10, MCP-1, d-dimer, fibrinogen	yes	2004-2005 (≠ timing measurement biomarker)	ELISA (sIL-2R, IL-6, sCD14) Clot-based assay (fibrinogen) Immunoturbidemetric (d-dimer) Bead-based immunoassay (MCP-1, TNF-a, IL-10)
Tungsiripat 2011	RCT (rosiglitazone or placebo) July 2006-December 2007	48 wks	71 *with lipoatrophy*	USA	White 51%	83	47/52	12.2/14.2	595/690	205/123	<50cp/ml 80%	25.3/25.8	nd	100	NNRTI 41% PI 59%	114/105	hsCRP, IL-6, TNF-a, sTNFRI,II, vWF, sVCAM-1, sICAM-1, MPO	no	=	ELISA Plasma/serum: nd
Carotid intima-media thickness; cross-sectional																				
Badiou 2008	Sept-dec 2009 c.s.	na	232	France	nd	75	41 ±9	nd	465	nd	2.9±1.1 (log)	22	70%	80	46% PI containing 31%NNRTI	37±26	hsCRP	yes	=	Immunoturbidimetric Plasma/serum: nd
Barbour 2014	Baseline analysis of a cohort study with a follow up of 5 years. Enrollment period: nd	na	125	Hawaii	White 55%	87	49.5	nd	479	nd	69% undetectable	nd	22%	100	nd	nd	CRP, IL-6, IL-8, IL-10, IL-1B, TNF, MPO, MMP-9, tPAI-1, sVCAM-1, sICAM-1, sE-selectin, MCP-1,VEGF, sCD14, SAA, SAP	nd	nd	High sensitivity Milliplex assay – Luminex Plasma
Bonilla[1] 2013	nd c.s.	na	16 LTNP (16 HIV- controls)	USA	White 62.5%	75	42.8	13.9	586	512	nd	26.4	nd	0	Na	Na	hsCRP, sVCAM-1, sTNFR-II	yes	nd	ELISA Plasma/serum: nd
Falcão 2012	2008-2010 c.s.	na	122	Brazil	nd	61	57.4% >40yrs	nd	≥200 86%	<200 63%	undetectable 41%	Overweight/ obesity 40%	28%	81	PI 35%	<5 yrs 57% >5 yrs 25%	hsCRP, IL-6, TNF-a, IL1B, sVCAM-1, sICAM-1	yes	nd	nephelometry Multiplex bead-array assay Plasma
Freitas 2014	nd c.s.	na	199 117 without -, 82 with LD	Portugal	Caucasian	61/74	45/49	7.2/8.5	503/632	nd	<50 87/90%	25/25	47/33%	100	PI 51/59 NNRTI 50/46 NRTI 97/96	68/95	hsCRP	no	=	'commercial kit', not further specified 'venous blood'
Jeong 2011	nd c.s.	na	76	Korea	'Korean patients'	100	40.5	3.9	324	nd	1,7 (log VL)	23.4	nd	100	On PI 57% On NNRTI 43%	32	sRAGE	yes	=	ELISA Plasma/serum nd

Joven 2006(data per quartile MCP-1)	nd c.s.	na	226 (384 HIV-individuals)	Spain	Caucasian 100%	70	40	nd	369-533	nd	2.5-3.1 (log VL)	18.9-19.7	81-88%	57-75%	PI 27-47% NNRTI 25-48%	nd	hsCRP, MCP-1	no	=	hsCRP 'highly sensity method' ELISA Plasma/serum: nd
Longenecker 2014	From march 2011 c.s.	na	147 HIV RNA <1000 copies/ml	USA	African-American 69%	78	46	12	613	179	<48 cp/ml 70%	27	63%	100	PI use 49%	64	hsCRP, IL-6, sTNFR-1, sCD14, sCD163, sVCAM-1, fibrinogen, d-dimer, OPG, RANKL	no	=	Immunonephelometry (CRP, fibrinogen) Immunoturbidometry (d-dimer) ELISA (other) Plasma/serum: nd
Mangili 2014 (results stratified by LpPLA2 mass)	January 2002-March 2004 c.s.	na	341	USA	White 52% Black 34%	75	44	9.6-10.2	385-470	nd	2.9-3.6 (log VL)	26-28	49%	74 (on HAART)	On PI 44%	25-33	CRP	yes	Within a 3 month time period	Immunoturbidimetry Plasma
Masia 2013	nd c.s.	na	136	Spain	Caucasian 97.1%	99	49	nd	650	nd	All: <200cp/ml	nd	49%	100	PI use 43% NNRTI use 39%	nd	hsCRP, IL-6, TNF-a, sVCAM, sICAM, MCP-1, PAI-1, sCD163, sCD14, d-dimer,MDA	yes	=	Immunometry (CRP) HPLC analysis (MDA) ELISA (other) Plasma
Merlini 2012	nd c.s.	na	163	Italy	Caucasian 94%	82	48	12	496	210	Undetectable 100%	25	48%	100	PI 56% NNRTI 34%	60	hsCRP, IL-6, TNF-a, sCD14, sVCAM-1,LPS	nd	Not clearly defined	ELISA LAL kit (LPS) Plasma
Piconi 2013	nd c.s.	na	76 55 ART+ 21 ART-	Italy	nd	100	46/43	10.3/7.8	546/500	374/169-	<37cp/ml: all ART treated patients	24/20	64% (>10 sig/day)	57	PI use (months) 50 NNRTI use (months) 33 %: nd	98	TNF-a, IL-6, MCP-1, sVCAM-1, sICAM-1, fibrinogen, d-dimer	yes	Not clearly defined	ELISA Plasma
Portilla 2014	March 2009-Oct 2010 c.s.	na	89	Spain	Caucasian 100%	100	42	7.8	467	204	Undetectable in all ART treated patients	24.8	61%	84	PI 44% NNRTI 40%	67	hsCRP, IL-6, TNF-a, sTNFR-I,II, PAI-1	yes	=	Turbidimetry (hsCRP) ELISA Plasma/serum: nd
Ross 2009	nd c.s.	na	73, on ART ≥24 weeks (21 HIV-)	USA	African-American 38% White 45% Hispanic 10%	81	48	13.5	624	162	<50 cp/ml 81%	26	38%	100	nd	Cumulative duration of (months): PI 53 NRTI 96 NNRTI 17	TNF-a, STNFR-I, II, IL-6, hsCRP, MPO,vWF, sICAM-1, sVCAM-1	no	=	ELISA Plasma
Ross 2014	March 2011-August 2012 c.s.	na	100	USA	Black 70% Caucasians 29%	77	47	13	633	199	Undetectable in 80%	27	62%	100	PI 74% NNRTI 52%	75	hsCRP	yes	=	Nephelometry Plasma/serum: nd
Sankatsing 2009	June 2003 – February 2006 c.s.	na	130 (same ART regimen for ≥ 2 yr)	The Netherlands	nd	90	46	nd	nd	nd	Undetectable 100%	23-24 (PI vs NNRTI use)	44%	100	PI based 48% NNRTI based 52%	61/60 (PI vs NNRTI use)	hsCRP	no	Not clearly defined	Immunoturbidimetry Plasma/serum: nd
Ssinabulya 2014	Febr-Oct 2012 c.s.	na	245 (ART naïve or treated)	Uganda	nd	31	37	nd	nd	124	nd	21.5	5%	41	NNRTI 86% 2nd line ART (PI) 14%	ART al least 7 yrs: 41% (median)	hsCRP	yes	Not clearly defined	ELISA Plasma (EDTA)

Stein 2013	nd c.s.	na	331	USA	White 44% Black 32% Hispanic 20%	89	36	0.5	349	nd	4.5 (log VL)	25	38%	0	na	na	hsCRP, IL-6	yes	=	Nephelometry (hsCRP) ELISA Plasma
Van Wijk 2006	nd c.s.	na	37 (15 MS+, 22 MS-)	The Nether lands	nd	100	50/47	8.5/7.5	604/719	nd	undetectable 80/77%	24.4/23.6	13/14%	100	PI 67/68% NNRTI 33/32% NRTI 100/100%	58/50	hsCRP	no	Not clearly defined	Quantes hs-CRP kit Plasma
Westhorpe 2014	nd c.s.	na	51 (+49 HIV-individuals)	Australia	nd	98	49	9.3	705	232	<50cp/ml 100%	nd	39%	100	NNRTI 85% NRTI 100%	nd	hs-CRP, sCD163, sCD14, CX3CL1, MCP-1/CCL2, LPS, neopterin, fibrinogen, d-dimer	yes	Not clearly defined	ELISA (sCD163, neopterin, sCD14, SC3CL1, CCL2) LAL kit (LPS) Plasma
Studies describing the same databases as one of the above mentioned studies, but with additional information																				
Hileman 2014 (additional to Hileman 2013)	July 2008-April 2010 Matched prospective cohort study	96 wks	42 HIV+ (41 HIV- -)	USA	African American 69%	69	40	4.8	630	nd	4900 cp/ml	27.3	67%	0	na	na	hsCRP, IL-6, TNFR-1,2, sVCAM-1, sICAM-1, d-dimer, fibrinogen	yes	=	Nephelometry (hsCRP, fibrinogen) Turbidometry (d-dimer) ELISA Plasma
Hsue 2006 (additional to Hsue 2012)	Cross-sectional (within the SCOPE cohort) ≥1 year off ART or on stable ART	na	93 (37 HIV-)	USA	Caucasian 62%, African American 25% Hispanic 8%	91	48	13	354	nd	Undetectable 57%	25	42%	93.5	PI 88%	For PI users 48	hsCRP	no	CIMT within 4 months after immune marker analysis	CardioPhase assay Plasma/serum: nd
Hsue 2009 (additional to Hsue 2012)	Cross-sectional (within the SCOPE cohort)	na	401 (93 HIV-)	USA	Caucasian ca. 50%	87	48	11-15	452	70-491	<75 cp/ml in 53.1%	nd	66%[2]	92	nd	70 (ART responders) vs 59 (non-responders)	hsCRP	no	nd	Dade Behring Plasma/serum: nd
Kelesidis 2012 2013 (additional to Currier 2007)	nd Historical analysis of a prospective matched cohort study	3 yrs	55 (36 HIV negative -individuals)	USA	White non Hispanic 76% Hispanic 19%	92	41	nd	488	20	<50cp/ml 84%	24.7	25.5/24.2 (HIV/PI vs HIV/non PI)	≥94.5	Ritonavir use (any) 16% NRTI use (any) 57% NNRTI use (any) 31%	39	RANKL, OPG, RANKL/OPG axis sCD14, LPS	yes	=	ELISA Pyro Gene rFactor C assay (LPS) Plasma/serum: nd
Parra 2010 (additional to Joven)	Nd c.s.	na	155 (results grouped on base of atherosclerosis and FRS)	Spain	nd	57	36-47	6.8-7.9	286-364	nd	<40cp/ml 68%	18.8-19.8	78%	nd	nd	On NRTI's 58-65 On PIs 27-30 On NNRTIs 9-11	CRP, IL-6, MCP-1	no	Not clearly defined	High sensitivity method (CRP) Immunoturbidimetry (IL-6) ELISA

1. Case control, but regarded as c.s. since alone the HIV+ group was used, 2. Ever smokers, 3. Data only from SMART database

* Current or past / ever

ACTG: AIDS clinical Trails Group, ART: antiviral therapy, cART: combination ART, c.s.: cross-sectional, EndoCAb: endotoxin core igM antibody, FRS: Framinham Risk Score, I-FABP: Intestinal fatty acid binding protein

LD: lipodystrophy, LTNP: long term non-progressors, na: not applicable, nd: no data, MS: metabolic syndrome, NNRTI: non-nucleoside reverse transcriptase inhibitor, NRTI: nucleoside reverse transcriptase inhibitor,

PI: protease inhibitor

Supporting file 2. Critical appraisal table.

Study	Clear inclusion criteria	Homogenous moment of inclusion/ how were patients included	Standardized protocol for measuring the determinant	Standardized protocol for measuring the outcome	Missing data with regard to inclusion or follow-up	Blinded measurement of determinant / outcome	Outcome	Adjustment for confounders
Cardiovascular events								
De Luca 2013	Yes	Patients of I.Co.N.A and CUSH cohort. Incl: age 35-69yrs, no hx of CVD, no inflammarory disorder. Cases: CVD event, on ART, ≥1sample available before CVD event. Matched based on history of diabetes and smoking status.	No (some samples were initially frozen by -20 degrees, and later on -80 degrees)	No. Definition not clearly specified	Number of potential eligible cases not addressed	Yes	major CVD (acute myocardial infarction; stable or unstable angina or were undergoing myocardial revascularization procedures)	Adjusted for sampling time and –year, HIV related factors, type of ARVs, cholesterol, hepatitis B or C
Ford 2010	No	Participants from the National Institute of Allergy and Infectious Diseases (NIAID). HIV+ matched 2:1 based on HIV+, gender, age, enrollment date	Yes	Yes	Number of potentially eligible patients not addressed	nd	CVD: acute MI, silent MI, coronary revascularization, ACS, CVA, PAD, CV death	Multiple analysis including all significant variables in univariate analysis (p <0.05)
Knudsen 2013	Yes	Patients from the Danish HIV Cohort Study with an ICD-10 diagnosis of IHD. Matched 1:4 to controls without CVD, based on age, duration of ART, gender, smoking	Yes	Yes	Number of potentially eligible patients not addressed	nd	Ischemic Heart disease, based on ICD-10 codes	Adjusted for HIV RNA, abacavir, NNRTIs, PIs.

Nordell 2014	Yes	Patients from the SMART trial, ESPRIT, SILCAAT trial. HIV+, CD4 resp ≥350cells/mm^3, >300cells/mm3, 50-299cells/mm3	Yes	Yes	7.3% loss to FU Potential eligible pts: 13.4% not included; no blood available (11%), history of CVD at entry (2.4%)	Yes	CVD events: deaths attributed to CVD, unwitnessed deaths not otherwise explained, nonfatal MI or stroke, CAD requiring surgery	Adjusted for age, gender, study, time between biomarker and event, race, BMI, HIV RNA, CD4, earlier AIDS event
Sandler 2010	Yes	Patients with a CVD event from the SMART cohort. Age ≥13 yrs, CD4 count >350cells/ mm^3. Matched 1:2 to controls based on age, sex, country, date of enrollment (≤3 months)	Yes	No (Not clearly stated. Definition of diseases not given)	potential eligible participants: 120/142 cases analyzed, based on available blood samples	Yes	CVD event: myocardial infarction, stroke, coronary artery disease, congestive heart failure, peripheral vascular disease, deaths attributed to CVD, unwithnessed deaths	Adjusted for matching factors, HIV-related factors, CV risk factors, Hep B and C, Tx arm. 2nd model: corrected for IL-6, SAA, CRP, d-dimer
Tenorio 2014	Yes	At enrollment in ACTG: Age ≥13 yrs, HIV+, HIV-1 RNA load <400 copies/mL. Cases: death from a non-AIDS related event, MI, stroke, non-AIDS defining malignancy, serious bacterial infection. Controls matched on age, sex, pre-ART CD4 count, ART regimen	Yes	Yes	Number of potentially eligible patients not addressed	nd	Myocardial infarction according to ACTG definitions Stroke	Adjusted for HIV RNA load, CD4 count, Hep B or C, CVD risk factors, injection drug use

Triant 2009	Yes	Between 18 and 84 yrs with ≥1vist at Brigham and Women's Hospital or Massachusetts General Hospital between 1997-2006, and CRP test ≤3 yrs, ≥1week before event	No	Yes	70357 / 1648687 Inclusion based on available CRP test	nd	Acute myocardial infarction	Adjusted for demographic factors and CVD riks factors
Study describing the same databases as one of the above mentioned studies, but with additional information								
Duprez 2012 (*additional to Nordell 2014)*	Yes	Patients with a CVD event from the SMART cohort. Age ≥13 yrs, CD4 count >350cells/ m3. Matched 1:2 to controls based on age, sex, country, date of enrollment (≤3 months)	Yes	Yes	nd	Yes	CVD death, non-fatal MI (clinical and silent), non-fatal stroke, CHF, coronary revascularization, CAD requiring drug treatment, PAD	Corrected for age, gender, race, HIV-related factors, CV risk factors, ECG changes, hep B or C, Tx group.
Carotid intima-media thickness								
Badiou 2008	Yes	Consecutively included HIV+ patients out-/in patients clinic	Yes	Yes	232/423 patients included. Based on available aliquots	nd	IMT of left FW CCA	Age, gender, tobacco consumption
Baker 2011	Yes	HIV, naive to cART or solely cART, expected to survive >2yrs (SUN study). Inclusion if there was a baseline and 2-year CIMT measure	Yes	Yes	270/659 participants at baseline had no 2 yr CIMT data available.	Yes	Difference between baseline and 2 yrs CIMT measurements Far wall of the right distal common carotid artery	No multivariable analysis for hsCRP in relation to CIMT

Barbour 2013	Yes	HIV infected, ≥40yrs, on stable ART ≥6monts	Yes	Yes	125 of potential 158 patients analyzed (other no complete data)	nd	Right CCA IMT	Components of FRS, all potential markers (p <0,15 univariable)
Bonilla 2013	Yes	LTNP: HIV≥5yrs, CD4>300cells/ml, no AIDS defining illness, never on ART	Yes	Yes	13/16 patients had blood samples available. Other 3 not analyzed	Yes	Left and right CCA NW and FW, bulb, ICA. Mean values per segment: CCA/ bulb/ICA	Pearsons correlation
Currier 2007	Yes	From the A5078 prospective cohort: HIV, VL<10.000, all on ART, yes or no PI. Matched extensively, affected generalizability	Yes	Yes	104/134 entered the extension at week 96 till week 144. 103/104 completed the final assessment at week 144	nd	FW right CCA. Progression of CIMT: yearly rate of change of at least 1 SD (≥0.0122mm/ year)	Univariable logistic regression. Only univariable associations with a p-value ≤0.1 were included in multivariable analyis
Falcão 2012	No	Consecutively HIV infected patients coming to an outpatient clinic	Yes	Yes	na nd concerning potential eligible patients	nd	FW of left and right CCA. Atherosclerosis if IMT>0,8mm	Univariable analysis. Only variables with a p-value <0.25 were included in multivariable analysis
Freitas 2014	Yes	HIV+ on stable ART visiting an outpatient clinic	Yes	Yes	na nd concerning potential eligible patients	nd	Mean IMT of left and right CCA Atherosclerosis if IMT>0.8mm	Unadjust analysis for CRP in relation to CIMT (correlation)

Hileman 2013	Yes	HIV, >18yrs, not on ART, not expected to start ART during FU of 48wks (CD4 >400cells/mm3) Excl: CVD, diabetes, active infection, pregnancy, breastfeeding	Yes	Yes	15/85 HIV+ lost to FU (17.6%)	Yes	Mean-mean of left and right FW CCA at three angles and FW of bulb.	Multivariable analysis with all baseline factors with a p-value <0.15
Hsue 2012	No	Consecutive volunteers of the SCOPE cohort, enriched with 'elite' controllers	Yes	Yes	Yes 0-15%: ICA13%, CCA 0%, bulb 4%, mean 15%	Yes	Average of NW and FW of left and right CCA, ICA and bulb Plaque if IMT >1.5mm	Adjusted for demographics, CV risk factors and HIV-characteristics
Jeong 2011	Yes	HIV, cART (>3 drugs) ≥6 mnts. Excl: obesity medication, CVD, malignancy, hypertension, infection, chronic liver or renal disease	Yes	Yes	na nd concerning potential eligible patients	nd	Right and left CCA, bulb and ICA at 3 different points. Mean-IMT: average CCA left and right, max-IMT: greatest value of IMT. Carotid plaque: focal wall thickening ≥50% of the surrounding vessel wall or ≥1.5mm.	Only univariable results
Joven 2006	no	HIV+ from an outpatient clinic	Yes	Yes	na. nd concerning potential eligible patients	Yes	Right and left CCA, bulb, ICA Average CIMT value	Correlation

Kaplan 2012	Yes	WIHS, and first report of HAART and available blood samples including 3 before and 3 after the use of HAART	Yes	Yes	36% (46/127 had no CIMT available)	Yes	IMT of the FW of the right CCA	Adjusted for age, race, smoking, BMI, CD4 count, HIV RNA, class of ART
Longenecker 2014	Yes	HIV infected, ≥18year, without DM or known CVD, on stable ART with VL <1000 copies/ml, increased T-cell activation or CRP >2mg/l, LDL ≤130mg/dl. First 60 subjects of the SATURN trial	Yes	Yes	na nd concerning potential eligible patients	nd	Mean-mean and mean-max CCA-IMT. Plaque: IMT>1.5mm or >50% thicker than the adjacent vessel CCA-IMT >1.0 mm was defined as atherosclerosis	Correlation
Mangili 2014	no	Patients from the CARE cohort. HIV infected patients without baseline diabetes, uncontrolled hypertension, myocardial infarction or stroke within the past 6 months	Yes	Yes	na nd concerning potential eligible patients	nd	Mean of the maximum of near- and far wall CIMT were used for analysis: one for common carotid, one for internal carotid	Adjusted for age, race, FRS, cholesterol spectrum, BMI, homocysteine, Lp-PLA_2, ApoE, NNRTI's, waist circumference and blood pressure
Masia 2013	Yes	Consecutive HIV infected patients, outpatient clinic, VL<200cp/ml, sexual transmitted	Yes	Yes	136/157 patients. Based on availability of serological results for herpesviridae	Yes	Total CIMT: mean of CCA and bulb	Spearman correlation

Merlini 2012	Yes	Consecutively enrolled HIV infected patients, on HAART (≥3 ARVs), >6mnths, VL<40 copies/ml at 2 consecutive assessments	Yes	Yes	12/163 (7%) missing in multivariable analysis	nd	Mean value of the bifurcation, bulb, common carotid artery (left and right) Normal IMT: ≤1mm, pathologic IMT >1mm. Divided in increased IMT and plaque	No confounder adjustment for the biomarkers in relation to CIMT
Piconi 2013	no	Longitudinally enrolled, HIV infected man. Selected on FRS. Excl: use of statins	Yes	Yes	76/79 cases analyzed. 4% 'lost'	Yes	Mean value of the distal CCA left and right.	Correlation
Portilla 2014	Yes	HIV+ men, ≥18yrs, ART naive or ART and VL<50 copies in previous 6mnths, 2NRTI's+PI or NNRTI(-PI). Excl: diabetes, chronic hepatitis C, active AIDS, drug use, psychiatric disorders	Yes	Yes	89/109 potential eligible patients included. No loss to follow up	Yes (CIMT measurements were done automatically)	Mean and max value of left and right CCA	Stepwise regression. All variable's with p<0.05 in univariable analysis were included
Ross 2009	Yes	HIV+, ≥21yrs, on stable ART≥24wks Excl: known CVD, DM, opportunistic infection, cute or a chronic inflammatory condition	Yes	Yes	No nd concerning potential eligible patients	Yes	Mean CCA and ICA left and right.	All variables if P <0.1 or if clinical significant were included in multivariable analysis

Ross 2014	Yes	First 100 subjects who fulfilled the following criteria: HIV+, ≥18yrs, HIV<1000cp/ml, fasting LDL<130mg/dl, cumulative ART duration ≥6mnths,stable ART≥3mnths. Excl: known CVD, statin use	Yes	Yes	No nd concerning potential eligible patients	nd	Mean-mean CCA IMT Plaque IMT >1.5mm	Linear regression: LpPLA1, age, male sex, current smoking, SBP, hsCRP
Sankatsing 2009	Yes	Consecutive HIV patients visiting two outpatients clinics, on PI or NNRTI, stable >2yrs. VL<50cp/ml	Yes	Yes	nd concerning potential eligible patients	Yes	Right and left CCA, bulb, ICA CIMT: average of the sum of the 3 right and left CCA FW	Covariates significantly associated with CIMT in univariable analysis were included in the multivariable analysis
Ssinabulya 2014	Yes	HIV infected from 2 HIV clinics, ≥18years, CD4 >350 or on ART >7 years (41%). Exclusion: malignancy, infection, some drugs. No homogeneous	Yes	Yes	Yes: only 186/245 (76%) underwent CIMT measuring	nd	Overall CIMT for the CCA: mean values of 3 images at 3 different angles Subclinical atherosclerosis: CIMT ≥0.78mm.	No. Bivariate analysis and correlation

Stein 2013	Yes	Baseline evaluation of the AIDS Clinical Trials Group Study A5257. HIV infected, ≥18year, HIV RNA >1000, ART naive. Excl: known CVD, diabetes, uncontrolled hypertension, lipid lowering medication	Yes	Yes	No missing data Number of potential eligible patients not mentioned.	nd	CCA CIMT and bifurcation CIMT at the right sight. Carotid artery lesion: >1.5mm wall thickness	Adjusted: candidate variable selection on the basis of Akaiki Information Criterion, clinical input and effect in univariable analysis
Tungsiripat 2011	Yes	HIV+ with clinical lipoatrophy, stable ART>24months, HIV-Rna<5000. Excl: pregnancy, diabetes, heart failure, cirrhosis, liver and kidney enzyme abnormalities	Yes	Yes	9/71 (12.6%) lost to FU. Excluded from final analysis	Yes	Mean value of CCA and ICA. Plaque defined according to the prococol from the Cardiovascular Health Study	Correlation
Van Wijk 2006	Yes	Men, 18-70yrs, HAART>12months, HIV RNA<10.000. Exclusion: opportunistic infection, malignancy, renal or liver disease, diabetes, lipid-lowering and antihypertensive agents no homogeneous moment	Yes	Yes	na nd concerning potential eligible patients	nd	Average of the left and right CCA	Spearman correlation

Westhorpe 2014	Yes	HIV+, all on ART, VL <50cp/ml. Excl: PI <6mnths, use of statins, HIV RNA>50 <6mnths No homogeneous moment	Yes	Yes	na nd concerning potential eligible patients	Yes	Right and left CCA. The median value was used. Subclinical atherosclerosis: median CIMT >0.7mm	Univariable. None of the markers significant, therefore not included in multivariable analysis.
Studies describing the same databases as one of the above mentioned studies, but with additional information								
Hileman 2014 (additional to Hileman 2013)	Yes	HIV, >18yrs, not on ART, not expected to start ART during FU of 48wks (CD4 >400cells/mm3) Excl: CVD, diabetes, active infection, pregnancy, breastfeeding	Yes	Yes	42/85 analyzed. Other (50.6%) lost to follow up	Yes	Mean-mean of left and right FW CCA at three angles and FW of bulb.	Multivariable analysis with all baseline factors with a p-value <0.15
Hsue 2006 (additional to Hsue 2012)	No	Patients from the SCOPE cohort, HIV+, on ART ≥1 yr or off ART ≥1 yr. Exclusion: acute infection, immune based therapy. 26.8% of HIV patients was involved in a previous study	Yes	Yes	No missing data Potential eligible part not addressed.	nd	Right and left both 6 predefined measurements. Plaque: IMT >1.5mm	Adjusted for traditional CV risk factors
Hsue 2009 (additional to Hsue 2012)	No	Patients from the SCOPE cohort, HIV+, no selection based on CVD risk.	Yes	Yes	No missing data Potential eligible part not addressed.	Yes	Average of NW and FW of left and right CCA, ICA and bulb	Traditional CV risk factors + additional factors associated with IMT in unadjusted analysis

Kelesidis 2012, 2013	Yes	From the A5078 prospective cohort: HIV, VL<10.000, all on ART, whether or not on PI	Yes	Yes	91/133 patients analyzed. FU till 96 weeks for 26% and FU of 144 wks for 74%	nd	Intima media thickness of the far wall of the distal right common carotid artery	Baseline covariates. Included in multivariable analysis if p<.05
Kelesidis 2012, 2013 (additional to Currier 2007)	Yes	From the A5078 prospective cohort: HIV, VL<10.000, all on ART, Yes or no PI	Yes	Yes	91/133 patients analyzed. FU till 96 weeks for 26% and FU of 144 wks for 74%	nd	IMT of FW of distal right CCA	Univariate analysis for markers in relation to CIMT
Parra 2010 (additional to Joven 2006)	Yes	Consecutive HIV-infected patients who came to the clinic, >18yrs. Excl: aids related infection, history of CVD	Yes	Yes	152 of 187 patients analyzed. Exclusion not addressed. 19% 'missing cases'	nd	Median value of CCA, bulb, ICA. Subclinical atherosclerosis: IMT>0.8, plaque: IMT >1.5mm or protruding in the lumen >50% of surrounding CIMT value	Binary analysis. In multivariable analysis cardiovascular risk factors as well as HIV related factors were taken into account

AIDS: acquired immunodeficiency syndrome, ART: antiretroviral therapy, BMI: body mass index, CAD: coronary artery disease cART: combination antiretroviral treatment, CCA: common carotid artery, CHF: Congestive Heart Failure, CIMT: carotid intima-media thickness, CRP: C-reactive protein, CV: cardiovascular, CVD: cardiovascular disease, DM: diabetes mellitus, FRS: Framingham risk score, FU: follow up, FW: far wall, HAART: highly active retroviral therapy, HIV: humane immunodeficiency virus, ICA: internal carotid artery, IHD: Ischemic Heart Disease, IMT: intima-media thickness, LDL: low density lipoprotein, LTNP: Long term non-progressors, MI: myocardial infarction, na: not applicable, nd: no data, NRTI: nucleoside reverse transcriptase inhibitor, NNRTI: non-nucleoside reverse transcriptase inhibitor, NW: near wall, PAD: peripheral arterial disease, PI: protease inhibitor, SBP: systolic blood pressure, SD: standard deviation, Tx: treatment, VL: viral load

Supporting file 3. Prisma checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	2
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	3
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	3
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	3
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	3
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	4
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	4
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	5
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	5

Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	5
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	5
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	5

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	5
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	5
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	5
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	6, S1
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	6, S2
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Figure 1
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	7
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	6, Figure 2
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	7,8 Figure 3,4
DISCUSSION			

Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	9
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	10
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	11
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	12

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

Supporting file 4. Prospero upload version. Review protocol

UNIVERSITY of York
Centre for Reviews and Dissemination

NHS
National Institute for Health Research

PROSPERO International prospective register of systematic reviews

Review title and timescale

1 Review title
Give the working title of the review. This must be in English. Ideally it should state succinctly the interventions or exposures being reviewed and the associated health or social problem being addressed in the review.
Pro-inflammatory markers in relation to cardiovascular disease in HIV infection

2 Original language title
For reviews in languages other than English, this field should be used to enter the title in the language of the review. This will be displayed together with the English language title.
not applicable

3 Anticipated or actual start date
Give the date when the systematic review commenced, or is expected to commence.
02/07/2014

4 Anticipated completion date
Give the date by which the review is expected to be completed.
31/10/2014

5 Stage of review at time of this submission
Indicate the stage of progress of the review by ticking the relevant boxes. Reviews that have progressed beyond the point of completing data extraction at the time of initial registration are not eligible for inclusion in PROSPERO. This field should be updated when any amendments are made to a published record.

The review has not yet started ×

Review stage	Started	Completed
Preliminary searches	Yes	No
Piloting of the study selection process	No	No
Formal screening of search results against eligibility criteria	No	No
Data extraction	No	No
Risk of bias (quality) assessment	No	No
Data analysis	No	No

Provide any other relevant information about the stage of the review here.

Review team details

6 Named contact
The named contact acts as the guarantor for the accuracy of the information presented in the register record.
Alinda Vos

7 Named contact email
Enter the electronic mail address of the named contact.
a.g.vos-8@umcutrecht.nl

8 Named contact address
Enter the full postal address for the named contact.
Julius Center UMC Utrecht Heidelberglaan 100 PO Box 85500 3508 GA Utrecht

9 Named contact phone number
Enter the telephone number for the named contact, including international dialing code.
0887568011

10 Organisational affiliation of the review
Full title of the organisational affiliations for this review, and website address if available. This field may be completed as 'None' if the review is not affiliated to any organisation.

UNIVERSITY of York
Centre for Reviews and Dissemination

NHS
National Institute for Health Research

Julius center for Health Sciences and Primary Care, University Medical Center Utrecht

Website address:
www.juliuscentrum.nl

11 Review team members and their organisational affiliations
Give the title, first name and last name of all members of the team working directly on the review. Give the organisational affiliations of each member of the review team.

Title	First name	Last name	Affiliation
Miss	A.G.	Vos	Julius Center UMC Utrecht
Miss	N.	Idris	Julius Center UMC Utrecht
Dr	R.E.	Barth	UMC Utrecht, department of infectiology
Dr	K.	Klipstein-Grobusch	Julius Center UMC Utrecht
Professor	D.E.	Grobbee	Julius Center UMC Utrecht

12 Funding sources/sponsors
Give details of the individuals, organizations, groups or other legal entities who take responsibility for initiating, managing, sponsoring and/or financing the review. Any unique identification numbers assigned to the review by the individuals or bodies listed should be included.
No funding

13 Conflicts of interest
List any conditions that could lead to actual or perceived undue influence on judgements concerning the main topic investigated in the review.
Are there any actual or potential conflicts of interest?
None known

14 Collaborators
Give the name, affiliation and role of any individuals or organisations who are working on the review but who are not listed as review team members.

Title	First name	Last name	Organisation details

Review methods

15 Review question(s)
State the question(s) to be addressed / review objectives. Please complete a separate box for each question.
To assess whether and which pro-inflammatory markers are associated with cardiovascular disease in HIV infection This relation will be further specified by looking at subgroups of patients - Patients on antiretroviral therapy (ART) versus patients not on ART - Patients with the metabolic syndrome versus patients without the metabolic syndrome (defined by using the criteria of NCEP ATP3 2005)12 - Patients with an early stage of HIV infection versus an advanced stage of HIV infection (measured by CD4 level or viral load) (stage 1 versus stage 2-4)

16 Searches
Give details of the sources to be searched, and any restrictions (e.g. language or publication period). The full search strategy is not required, but may be supplied as a link or attachment.
MEDLINE Restriction: search in title/abstract. Language other than English and studies before 1996 will be excluded. EMBASE Restriction: search in title/abstract, no MEDLINE. Language other than English and studies before 1996 will be excluded. Cochrane Restriction: search in title/abstract. Language other than English and studies before 1996 will be excluded.

17 URL to search strategy
If you have one, give the link to your search strategy here. Alternatively you can e-mail this to PROSPERO and we will store and link to it.
http://www.crd.york.ac.uk/PROSPEROFILES/10516_STRATEGY_20140603.pdf

I give permission for this file to be made publicly available
No

18 Condition or domain being studied
Give a short description of the disease, condition or healthcare domain being studied. This could include health and wellbeing outcomes.
HIV infected patients

19 Participants/population
Give summary criteria for the participants or populations being studied by the review. The preferred format includes details of both inclusion and exclusion criteria.
Inclusion: HIV infected human beings, aged 18 years or older

20 Intervention(s), exposure(s)
Give full and clear descriptions of the nature of the interventions or the exposures to be reviewed
Determinant: pro-inflammatory markers. Markers of inflammation are defined as markers that could be measured in plasma or serum. Exclusion: cellular blood components (i.e. lymphocyte subsets) and genetic markers

21 Comparator(s)/control
Where relevant, give details of the alternatives against which the main subject/topic of the review will be compared (e.g. another intervention or a non-exposed control group).
n.a.

22 Types of study to be included initially
Give details of the study designs to be included in the review. If there are no restrictions on the types of study design eligible for inclusion, this should be stated.
Inclusion: Only original research: Observational cohort studies Cross-sectional studies Case-control studies Intervention studies including the domain: human beings aged 18 years or older including one ot the outcomes: cardiovascular disease or a surrogate marker of cardiovascular disease. Exclusion: animal studies Case series under 10 cases Studies published before 1996

23 Context
Give summary details of the setting and other relevant characteristics which help define the inclusion or exclusion criteria.
n.a.

24 Primary outcome(s)
Give the most important outcomes.
cardiovascular disease: myocardial infarction, cardiac death, stroke

Give information on timing and effect measures, as appropriate.

25 Secondary outcomes
List any additional outcomes that will be addressed. If there are no secondary outcomes enter None.
surrogate markers of cardiovascular disease 1) intima media thickness (carotid or femoral) 2) pulse wave velocity 3) ankel brachial index 4) coronary calcium score 5) flow mediated dilation

Give information on timing and effect measures, as appropriate.

26 Data extraction, (selection and coding)
Give the procedure for selecting studies for the review and extracting data, including the number of researchers involved and how discrepancies will be resolved. List the data to be extracted.
Selection of studies will be done by a three-step wise model. Firstly, all identified records will be screened on title and abstract using above mentioned inclusion criteria. Secondly, of the remaining abstracts full text reports will be read to determine eligibility. This will be done by 2 authors independently (AV and NI) using the following inclusion criteria: 1. assessment of at least one pro-inflammatory marker 2. relation between inflammatory marker and CVD, expressed in a numeric value (e.g. risk, probability data, mean, difference) In case only an abstract is available or outcome data are insufficient or unclear authors will be contacted for additional information. Thirdly, references and citations of the selected articles will be checked for additional eligible articles. Differences in inclusion will be discussed in a consensus meeting. In case no consensus can be reached, a third reviewer (RB) will be consulted. In case of persistent differences a consensus meeting with al authors will be organized where a final decision will be taken. Data extraction will be done independently by 2 authors (AV, NI) using a predefined data extractionform, including the

following items: Year of publication, study design, duration of follow up, number of patients, country and setting will be recorded. For the different subgroups the following data will be extracted: age, sex, HIV status, ART use, duration of HIV infection, duration of ART use, cardiovascular risk factors, inflammatory parameters measured, outcome and outcome measures.

27 Risk of bias (quality) assessment
State whether and how risk of bias will be assessed, how the quality of individual studies will be assessed, and whether and how this will influence the planned synthesis.
Selected studies will be critically appraised to assess the risk of bias. The Cochrane Collaboration's tool for assessing risk of bias will be used, adjusted for descriptive research by using the STROME-ID guideline. The main focus will be on the risk of selection bias, detection bias and attrition bias. A predefined critical appraisal form will be used. Critical appraisal will be done by 2 authors independently (AV, NI).

28 Strategy for data synthesis
Give the planned general approach to be used, for example whether the data to be used will be aggregate or at the level of individual participants, and whether a quantitative or narrative (descriptive) synthesis is planned. Where appropriate a brief outline of analytic approach should be given.
Study designs and pro-inflammatory parameters included will be very heterogeneous. If data allow a meta-analysis will be performed and results will be presented in a forest plot. If this is not the case a quantitative synthesis will be performed. Results will be grouped by outcome and type of inflammatory marker. Besides this, there will be stratification by study design: cross-sectional versus longitudinal.

29 Analysis of subgroups or subsets
Give any planned exploration of subgroups or subsets within the review. 'None planned' is a valid response if no subgroup analyses are planned.
In case of sufficient data, outcomes will be reported per pre-defined subgroup of patients (antiretroviral therapy yes or no, metabolic syndrome yes or no, early stage or advanced stage of HIV)

Review general information

30 Type of review
Select the type of review from the drop down list.
Prognostic

31 Language
Select the language(s) in which the review is being written and will be made available, from the drop down list. Use the control key to select more than one language.
English

Will a summary/abstract be made available in English?
Yes

32 Country
Select the country in which the review is being carried out from the drop down list. For multi-national collaborations select all the countries involved. Use the control key to select more than one country.
Netherlands

33 Other registration details
List places where the systematic review title or protocol is registered (such as with he Campbell Collaboration, or The Joanna Briggs Institute). The name of the organisation and any unique identification number assigned to the review by that organization should be included.
none

34 Reference and/or URL for published protocol
Give the citation for the published protocol, if there is one.
n.a.

Give the link to the published protocol, if there is one. This may be to an external site or to a protocol deposited with CRD in pdf format.

UNIVERSITY of York
Centre for Reviews and Dissemination

NHS
National Institute for Health Research

I give permission for this file to be made publicly available
Yes

35 Dissemination plans
Give brief details of plans for communicating essential messages from the review to the appropriate audiences.
The results will be presented in a paper, with the intent to publish the review in an international journal.

Do you intend to publish the review on completion?
Yes

36 Keywords
Give words or phrases that best describe the review. (One word per box, create a new box for each term)
HIV

inflammation

markers of inflammation

cardiovascular disease

surrogate markers of cardiovascular disease

37 Details of any existing review of the same topic by the same authors
Give details of earlier versions of the systematic review if an update of an existing review is being registered, including full bibliographic reference if possible.
n.a.

38 Current review status
Review status should be updated when the review is completed and when it is published.
Ongoing

39 Any additional information
Provide any further information the review team consider relevant to the registration of the review.

40 Details of final report/publication(s)
This field should be left empty until details of the completed review are available.
Give the full citation for the final report or publication of the systematic review.
Give the URL where available.

CHAPTER

3

Association between immune markers and surrogate markers of cardiovascular disease in HIV positive patients: A systematic review

A.G. Vos, A. Hulzebosch, D. Grobbee, R. Barth, K. Klipstein-Grobusch

PLoS One. 2017 Jan 13;12(1)

Abstract

Background HIV infection is associated with an increased risk of cardiovascular disease (CVD). Chronic low-grade immune activation is likely one of the driving mechanisms. This systematic review provides an overview of the evidence addressing the relation between immune markers and surrogate markers of CVD (except CIMT) in HIV infection.

Methods A systematic search was performed in PubMed, Embase and Cochrane Library identifying all articles from 1996 to April 2015. It addressed the relation between immune markers and surrogate markers of CVD (except Carotid Intima-media Thickness) in HIV-positive adults. Two authors, using predefined criteria, independently conducted the selection of articles, critical appraisal and extraction of the data. Analysis focused on immune markers that were assessed most frequently. The review was conducted according to the PRISMA guideline and performed as part of an overarching review registered with PROSPERO (CRD42014010516).

Findings Twenty-nine articles were selected, describing 34 immune markers and nine different CVD surrogate outcomes: coronary calcium score (13 times) and flow-mediated dilation (10 times) were used most frequently. Twenty-seven studies had a cross-sectional design. CRP, IL-6 and sVCAM-1 were assessed most frequently. None of the immune markers were clearly associated with any of the surrogate CVD outcomes. No effect estimate could be calculated due to marked heterogeneity in study populations, immune markers, outcomes and statistical approaches.

Interpretation This review could not identify a clear association between any of the immune markers and surrogate CVD outcomes. This may reflect a true lack of association, or may be explained by heterogeneity across studies and lack of follow-up data. Future research should focus on longitudinal studies measuring a select set of immune markers and surrogate CVD outcomes awaiting the primary outcome of clinical cardiovascular events.

Background

Highly active antiretroviral therapy (HAART) has markedly increased life expectancy among persons infected with human immunodeficiency virus (HIV). However, it has become clear that patients infected with HIV have an increased risk for developing cardiovascular disease (CVD)[1]. Multiple factors contribute to the increased risk for non-AIDS morbidity. An excess burden of traditional risk factors and direct toxic effects of antiretroviral therapy (ART) are the most likely drivers of the pathogenesis and HIV replication has been shown to contribute to the process of atherosclerosis through chronic inflammation and endothelial dysfunction[2,3]. Increased concentrations of immune biomarkers, like C-reactive protein (CRP), IL-6 and D-dimer, indicative of inflammatory processes, have been associated with increased risk of atherosclerosis and mortality in the general population[4-6]. Research in this area has intensified and several studies have addressed the role of immune activation in the occurrence of CVD in HIV infected individuals, identifying a likely association between CRP, IL-6, d-dimer and clinical CVD[7].

To provide insight in the burden of cardiovascular disease in HIV infected individuals while awaiting results from longitudinal studies, a multitude of surrogate markers for CVD have been used. A recent summary of the current body of evidence regarding potential associations between markers of immune activation and carotid intima media thickness (CIMT)could not draw a clear conclusion due to the heterogeneity in data.[7] This systematic review therefore investigates the relation between markers of immune activation and further surrogate markers of CVD like coronary artery calcium score, flow mediated dilation and pulse wave velocity in HIV infected individuals.

Methods

This systematic review was conducted according to the guidelines provided by PRISMA(8) and it is part of a larger review registered in the PROSPERO registry for systematic reviews (Registration number CRD42014010516). Results addressing the relation of markers of immune activation, CVD and CIMT were published in January 2016.[7] http://www.crd.york.ac.uk/PROSPERO/display_record.asp?ID=CRD42014010516)

Search strategy

A systematic search was performed in PubMed, EMBASE and Cochrane Library on April 29th 2015 using terms and synonyms for HIV, immune markers and surrogate markers for CVD other than CIMT, covering all evidence from 1996 onward. Terms were limited to title and abstract (Table 1). The search strategy was an update of the search described in the PROSPERO protocol and published previously[7].

Table 1. Search strategy.

		Search terms
#1 Domain	**HIV-positive patients**	**HIV**
		human immunodeficiency virus
		human immuno deficiency virus
		human immunedeficiency virus
		human immune deficiency virus
		aids
		acquired immunodeficiency syndrome
		acquired immuno deficiency syndrome
		acquired immunedeficiency syndrome
		acquired immune deficiency syndrome
AND		
#2 Determinant		**Inflammatory**
		Inflammation
		Inflamm*
		Biomarker
		Biomarkers
		Immune*
AND		
#3 Outcome	**Cardiovascular disease or surrogate markers of cardiovascular disease.**	**cardiovascular**
		CVD
		Myocardial infarction
		mi
		Coronary heart disease
		CHD
		Stroke
		Carotid intima media thickness
		CIMT
		Arterial stiffness
		Flow mediated dilation
		FMD
		PWV
		Pulse wave velocity
		Coronary artery calci*
		CAC
		Ankle brachial index
		ABI

Selection

Selection was done in a stepwise process (Fig 1). Firstly, studies were screened on the basis of title and abstract by one author (AH). Studies describing original research including HIV positive patients aged 18 years or above and one of the following surrogate CVD outcomes, were included; CT coronary angiography, coronary artery calcium score (CAC), MRI of blood vessels, arterial inflammation measured by 18FDG PET scan, myocardial perfusion scintigraphy (SPECT), flow mediated dilation (FMD), pulse wave velocity (PWV), pulse wave analysis (PWA), ankle brachial index (ABI) and carotid artery stiffness. Animal studies, articles in a language other than English and case-series describing less than 10 cases were excluded. Secondly, two authors (AH, AV) independently evaluated full-text articles using the following exclusion criteria: only poster abstracts, no relation described between immune marker and no description of an outcome of interest. In case of uncertainty regarding the relation between immune marker and outcome, the corresponding author of the study was contacted once for additional data. Discrepancies in inclusion were discussed in a consensus meeting between two reviewers (AV, AH). A third reviewer (KK) would have been available in case of discrepancies, but agreement could be reached for all inclusions.

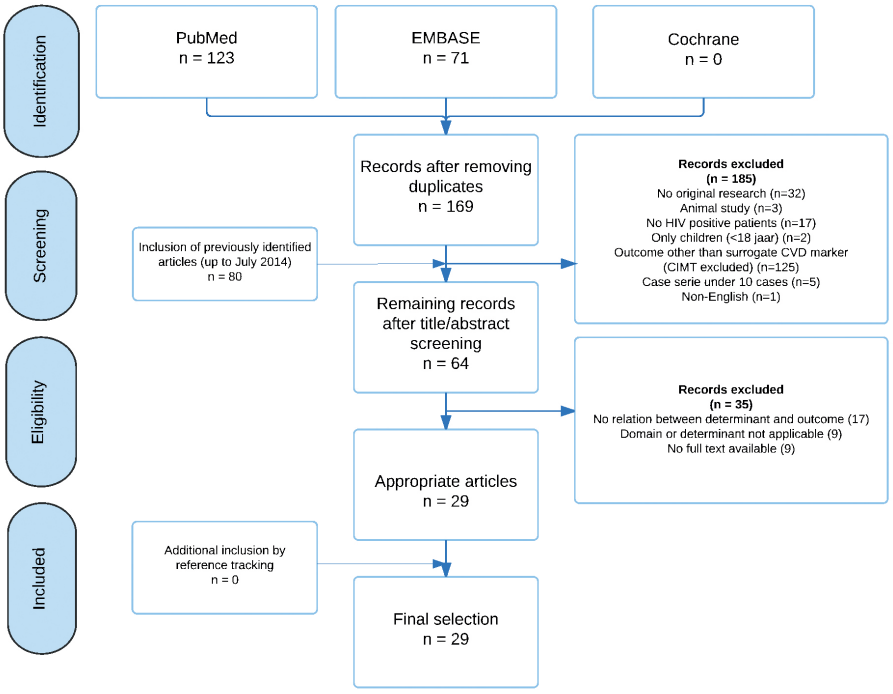


Figure 1. Flowchart inclusion. CIMT: carotid intima media thickness, CVD: cardiovascular disease.

Data extraction and critical appraisal

The following data were extracted: study design and enrollment period, duration of follow up, sample size, country, ethnicity, gender, mean age, duration since HIV diagnosis in years, CD4 count, nadir CD4 count, viral load, BMI, percentage of current smokers, percentage of participants on ART, ART regimen, duration of ART in years, immune markers assessed, use of a stored sample, time of measuring outcome versus immune markers and laboratory methods for immune markers. In case a study used more than one surrogate outcome, only the outcome that was related to immune markers was included in the review.

Selected studies were critically appraised, particularly for the risk of selection-, detection-, and attrition bias. Bias risk was assigned as likely, unlikely, or unclear using an adapted Cochrane Collaboration tool. Data extraction and critical appraisal was performed independently by two authors (AH, AV) using a set format.

Analysis

Given heterogeneity of studies, a descriptive analysis, grouped by outcome and most frequently assessed immune markers, was conducted. When possible, percentages of common baseline characteristics were calculated.

Surrogate outcomes of CVD were divided in two groups: outcomes using imaging techniques and outcomes assessing arterial stiffness. Imaging techniques were subdivided in two main categories: 1) coronary angiography and coronary artery calcium score, and 2) all other imaging techniques, namely 18FDG PET scan, SPECT and MRI scan of aortic and carotid arteries. Arterial stiffness outcomes were FMD, PWV, PWA, ABI and carotid artery stiffness. Differences in outcome protocols were not taken into account in this review.

Outcome was reported as a positive association, an inverse association, no association or no data. To combine information from various types of studies reporting often more than one risk estimate per outcome, data were reduced by choosing only one effect estimate per immune marker per surrogate CVD outcome per article. To select the effect estimate, the following hierarchical order was used: 1) outcome of multivariable analysis. If not reported, 2) outcome of univariable analysis. If there was no significant outcome in multi-or univariable analysis, the outcome was scored as 3) 'No association'. If no data were reported, although the methods section specified that the relation between immune marker and outcome has been studied, the outcome was reported as 4) 'no data'. In this review C-reactive protein (CRP) refers to both the regular CRP measurement and to the high-sensitive CRP assays.

Results

The updated search identified 169 articles, 64 articles were screened using full text, and 29 articles were finally selected (Fig 1). Articles were excluded if either no relation was described between the immune marker and the surrogate CVD outcome (n=17), the domain or determinant turned out to be not applicable during full text screening (n=9) or there was no full text available (n=9).

Baseline characteristics

A total of 3,559 HIV positive patients were included, whereof the majority were male (median 80%, interquartile range 59.5-92.0%) (S1 Table); mean age across studies was 45.3 ± 5.4 years, and on average there were almost as many black as white people included in the studies. All except two studies were conducted in the United States of America or in Europe (one study in Australia[9] and in Ethiopia[10]). Two studies had a longitudinal design with a maximum follow-up duration of 24 weeks[11,12]. Average BMI was 25.1kg/m^2, (standard deviation (SD) 1.9kg/m^2) and nearly 40% of all participants were current smokers. Duration of HIV infection ranged between 24 weeks and 16 years. Ten studies[9,13-20] included only participants on ART and three studies included only ART naïve participants[11,12,21].

CAC score was used as endpoint in 13 studies[6,13,14,17,22-30], in seven of which a coronary angiography was performed as well[6,22-27]. Five studies used other imaging techniques to assess cardiovascular burden; two studies used 18FDG PET scan[30,31], two used a myocardial perfusion scintigraphy[19,32] and one used an MRI scan of aortic and carotid arteries[33]. Arterial stiffness was assessed in 13 studies[9-12,15-18,20,21,34-36]; 10 studies used FMD[9-12,15-17,20,21,34], two PWV[10,20], one PWA[18], one ABI[35] and one used carotid artery stiffness[36].

Critical appraisal

All studies were critically appraised on eight items; clarity of inclusion criteria; moment of inclusion; standardization of measurement of determinant and outcome; missing data at baseline or follow-up; missing data on potential eligible participants; and blinding and adjustment for confounders (S2 Table). A risk of bias summary is presented in Fig 2. The criterion 'homogeneous moment of inclusion' is not incorporated in the figure, since it could not be categorized as 'likely' or 'unlikely' due to the different aspects that were covered. Risk of bias was low in general, except for the missed potential eligible participants. Although most studies had a standardized procedure for measuring immune markers and outcomes, these procedures were different between studies.

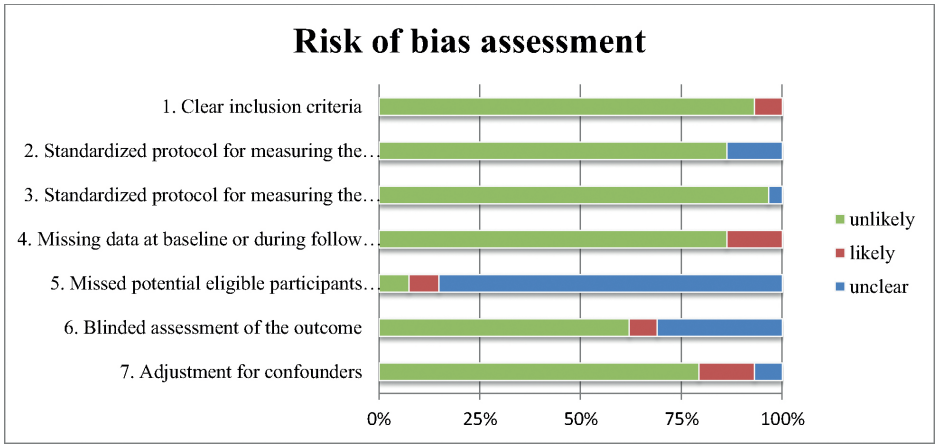


Figure 2. Risk of bias assessment.

Overall outcome

Figure three provides an overview of the number of studies that assessed any immune marker in relation to the surrogate CVD outcomes. CRP, IL-6 and sVCAM-1 were assessed most frequently. None of the markers were clearly associated with surrogate markers of CVD. Only CRP and sCD163 were at least three times positively associated: CRP 5 times (out of 26 studies)[10,13,18,22,29], of which three times in univariable analysis[13,22,29], sCD163 three times (out of 7 studies)[6,27,30], of which two times in univariable analysis[6,30].

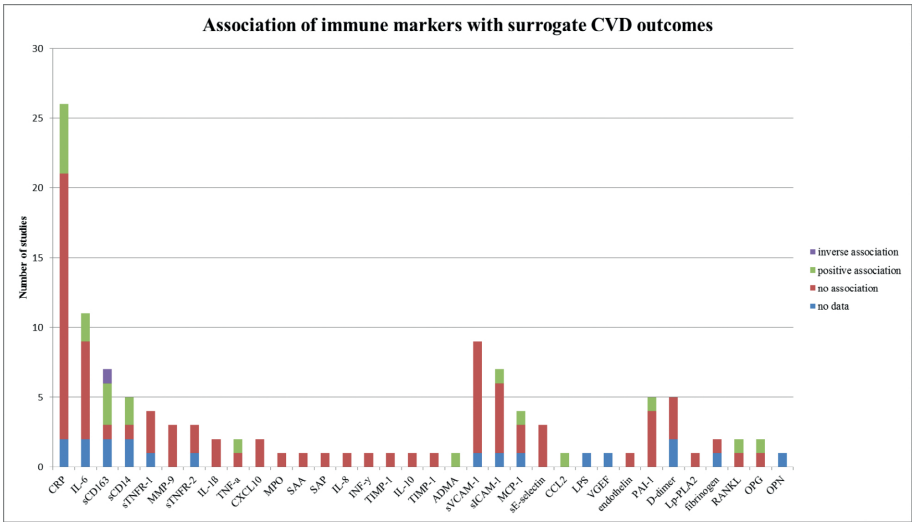


Figure 3. Association of immune markers with surrogate CVD outcomes. CVD: cardiovascular disease.

Imaging

CT coronary angiography and coronary calcium score

Six markers were evaluated three or more times in relation to coronary calcium score or coronary angiography (fig 4a). CRP was assessed 10 times[6,13,14,17,22-26,29], of which two studies showed a significant, but weak correlation with severity of obstruction on angiography (Rho 0.29, p 0.038)[22] and with CAC (Rho value 0.16, p 0.003)[29]. sCD163 was positively related in three out of five studies; two times to non-calcified plaque[6,23] and one time to coronary calcium score and coronary artery stenosis of more than 50%[27]. However, the association with non-calcified plaque disappeared in multivariable analysis in one out of two studies[6]. sCD14 showed a significant association in two out of five studies. A high level of sCD14 compared to a low level of sCD14 was associated with an odds ratio of 3.3 (95% CI 1.1 - 9.7) for coronary artery stenosis[27], and levels of sCD14 were related to the presence of coronary artery calcium in multivariable analysis[13]. MCP-1 was borderline significantly related to severity of plaque (Rho value 0.23, p 0.047) and weakly related to the Agatston Calcium score (Rho value 0.27, p 0.02) in one study[26].

Other imaging techniques

CRP was assessed in four studies using 18FDG PET scan[30,31] or SPECT scan as outcome[19,32]; no study showed a significant association. None of the other markers were assessed more than three times. sCD163 was assessed twice in relation to PET scan outcomes, both studies showed a significant association (association with FDG uptake in the descending aorta Rho value -0.517, p 0.007[31] and with Target Background Ration, Rho value 0.31, p 0.04)[30].

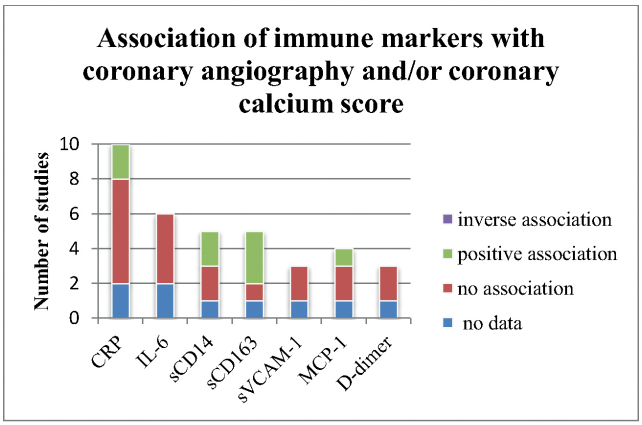
Arterial stiffness measurement

Six markers were evaluated three times or more in relation to arterial stiffness (Fig 4b). CRP was positively associated with PWV in multivariate analysis (parameter estimate 0.0209, p 0.01)[10], and mean levels of CRP were significantly higher in individuals with definite peripheral artery disease compared to participants with normal ABI (8.5 versus 7.2 mg/L)[11,12,35]. Levels of IL-6 corresponded with the lowest quartile of FMD (OR 1.17, 95% CI 1.01-1.35, p 0.04) and IL-6 was the best predictor of FMD in a multivariable linear model[16]. On the other hand, Stein et al.,[21] found an inverse relation in univariate analysis; higher IL-6 levels were associated with higher FMD (indicative of a lower CVD risk) and lower levels of IL-6 were associated with increase in brachial diameter (indicative of a higher CVD risk). None of the other markers were significantly associated with arterial stiffness. Three studies using FMD as outcome included only ART naïve individuals[11,12,21]. No association with CRP was found (assessed in all

three studies), and associations with IL-6 (assessed in two studies) showed contradictory results.

Composite endpoint for vascular disease

Longenecker et al.,[13] used a composite endpoint for vascular disease consisting of CAC score >0, endothelial dysfunction according to FMD and carotid disease. They found that CRP, sCD14 and fibrinogen were all significantly associated with vascular disease[13].



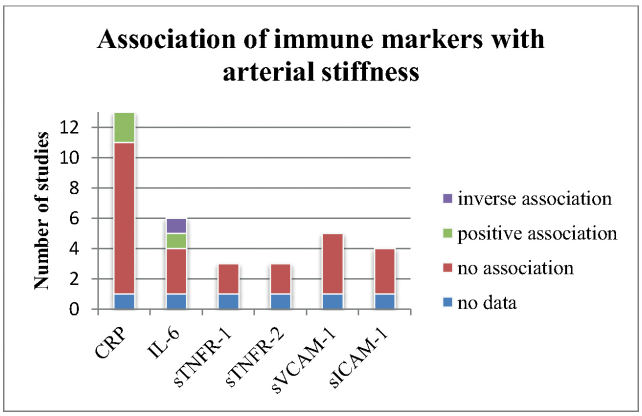


Figure 4. Association of immune markers with coronary angiography, coronary calcium score and arterial stiffness.

Discussion

A large number of immune markers have been investigated for predictive value in relation to surrogate markers of cardiovascular disease in HIV positive patients. CRP, IL-6 and sVCAM-1 were addressed most frequently; neither CRP, IL-6, sVCAM-1 nor any of the other markers showed a clear relation with any of the surrogate CVD markers.

These results complement the findings of a recent review assessing immune marker choice in relation to CVD or CIMT. CRP, IL-6 and s-VCAM-1 were also the most frequently assessed markers in relation to CIMT, and the lack of a clear relation between any of these immune markers and surrogate CVD outcomes that is currently found is in line with the findings in relation to CIMT[7].

Although there is ample evidence that levels of CRP[37,38] and IL-6[39,40] are related to CVD both in the general population as well as in the HIV-infected population[5,41-43], the current review does not indicate any consistent relation of CRP and IL-6 to coronary calcium score, coronary stenosis, SPECT, 18FDG PET scan or measurements of arterial stiffness.

Although the prognostic role of s-VCAM-1 levels in the prediction of CVD in not as clear, an association with signs of endothelial damage (FMD, PWV) or inflammation (MRI, PET) was to be expected as s-VCAM-1 is expressed on the endothelial surface in case of endothelial inflammation[44]. However, no single positive association with any of the surrogate CVD outcomes was detected in this review.

The two possible explanations why this review did not show an association between any of the markers and surrogate CVD outcomes could be that either there is no association, or associations are not yet clear due to methodological and qualitative constraints in the available evidence.

The first explanation, no association, could be due to the fact that, despite surrogate outcomes and overt CVD being clearly related[45-53] and CRP, IL-6 and overt CVD having a clear relation, associations between markers of immune activation and surrogate markers of CVD may be weak.

The second explanation, lack of an association due to methodological and qualitative constraints, could be due to a marked heterogeneity in choice of both immune markers, surrogate outcomes and ways of analysis (effect estimates vary from correlations, mean markers levels, odds ratio's and multivariable analysis). In addition, studies were not primarily designed or powered to detect an association between immune markers and outcome and study designs were mainly cross-sectional (27 out of 29 studies). Follow-up time of the two prospective studies was too short to detect significant vascular

alterations and all studies included a relatively young population (average age 45 years), in which atherosclerotic vascular changes and – burden are expected to be low.

Finally, possible associations might be masked by heterogeneity in patient populations, duration of HIV diagnosis, use and duration of antiretroviral therapy (ART), viral suppression rates and CD4 counts, as they differ substantially in, and between, studies. All these factors have been previously reported to influence both the inflammatory response and the risk of cardiovascular morbidity and mortality[53-55]. Moreover, statin use is not taken into account in most of the included studies, whereas recent evidence suggests that statin use can improve surrogate cardiovascular outcomes[56,57].

Strengths and limitations

This systematic review synthesizes the available information on immune-markers in relation to surrogate markers of CVD (except CIMT) in HIV-infected patients from 1996 up to April 2015. The main focus of this review was to provide an overview of which immune markers were assessed, how frequently these markers were assessed and which surrogate CVD outcomes were chosen, as well as to summarize the current evidence on the relation between immune markers and outcomes.

The most frequently assessed immune markers (CRP and IL-6) have been shown to be related to CVD morbidity and mortality in both the general population and in the HIV-infected population, and surrogate CVD outcomes have been shown to be related to CVD in the general population. This is the first time that the relation between immune markers and surrogate CVD outcomes is addressed in a systematic way in the context of HIV infection. The major strength of this review is that it contributes to a clear, global understanding of existing knowledge regarding immune markers and surrogate CVD outcomes.

Limitations to be considered include that only one outcome per marker per article was considered which might have resulted in an overly optimistic impression of associations as negative outcomes are underreported; that positive outcomes include univariable associations which might in part be confounded; and that outcomes were grouped in categories (CT-angiography/CAC, other imaging techniques and arterial stiffness) which might have resulted in specific associations (to specific parts of an outcome) being overlooked.

Conclusion

This review provides an overview of the current literature regarding the association between immune markers and surrogate markers of cardiovascular disease other than CIMT in HIV-positive individuals. Most frequently assessed immune markers were CRP, IL-6 and s-VCAM-1; most frequently assessed surrogate markers for CVD were CAC and FMD.

No relation between any of the immune markers and any of the surrogate outcomes could be detected. This may be due to the cross-sectional design, heterogeneity in patient populations, the variety in immune marker choice and surrogate CVD outcomes. The search for the association of immune markers in relation to surrogate CVD outcomes in a cross-sectional study design should be reconsidered.

Future research should focus on longitudinal studies measuring immune markers and surrogate CVD outcomes awaiting the primary outcome of clinical cardiovascular events.

Acknowledgements

There are no acknowledgements' to be made.

References

1. Barbaro G. HIV infection, highly active antiretroviral therapy and the cardiovascular system. Cardiovasc Res 2003 Oct 15;60(1):87-95.
2. Armah KA, McGinnis K, Baker J, Gibert C, Butt AA, Bryant KJ, et al. Pmc3493182; HIV status, burden of comorbid disease, and biomarkers of inflammation, altered coagulation, and monocyte activation. Clin Infect Dis 2012 Jul;55:126-136.
3. Baker JV, Lundgren JD. Pmc3076665; Cardiovascular implications from untreated human immunodeficiency virus infection. Eur Heart J 2011 Apr;32:945-951.
4. 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 21;5(10):e203.
5. Triant VA, Meigs JB, Grinspoon SK. Association of C-reactive protein and HIV infection with acute myocardial infarction. J Acquir Immune Defic Syndr 2009 Jul 1;51(3):268-273.
6. Burdo TH, Lo J, Abbara S, Wei J, DeLelys ME, Preffer F, et al. Pmc3203384; Soluble CD163, a novel marker of activated macrophages, is elevated and associated with noncalcified coronary plaque in HIV-infected patients. J Infect Dis 2011 Oct 15;204:1227-1236.
7. Vos AG, Idris NS, Barth RE, Klipstein-Grobusch K, Grobbee DE. Pro-Inflammatory Markers in Relation to Cardiovascular Disease in HIV Infection. A Systematic Review. PLoS One 2016 Jan 25;11(1):e0147484.
8. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gotzsche PC, Ioannidis JP, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. BMJ 2009 Jul 21;339:b2700.
9. Nolan D, Watts GF, Herrmann SE, French MA, John M, Mallal S. Endothelial function in HIV-infected patients receiving protease inhibitor therapy: does immune competence affect cardiovascular risk? QJM 2003 Nov;96:825-832.
10. Gleason RL,Jr, Caulk AW, Seifu D, Parker I, Vidakovic B, Getenet H, et al. Current Efavirenz (EFV) or ritonavir-boosted lopinavir (LPV/r) use correlates with elevate markers of atherosclerosis in HIV-infected subjects in Addis Ababa, Ethiopia. PLoS One 2015 Apr 27;10(4):e0117125.
11. Gupta S, Mi D, Dubé M, Saha C, Johnson R, Stein J, et al. Pentoxifylline, inflammation, and endothelial function in HIV-infected persons: a randomized, placebo-controlled trial. PloS one 2013;8:e60852.
12. Torriani FJ, Komarow L, Parker RA, Cotter BR, Currier JS, Dube MP, et al. Endothelial function in human immunodeficiency virus-infected antiretroviral-naive subjects before and after starting potent antiretroviral therapy: The ACTG (AIDS Clinical Trials Group) Study 5152s. J Am Coll Cardiol 2008 Aug 12;52(7):569-576.

13. Longenecker CT, Jiang Y, Orringer CE, Gilkeson RC, Debanne S, Funderburg NT, et al. Soluble CD14 is independently associated with coronary calcification and extent of subclinical vascular disease in treated HIV infection. AIDS 2014 Apr 24;28:969-977.
14. Shikuma CM, Barbour JD, Ndhlovu LC, Keating SM, Norris PJ, Budoff M, et al. Plasma monocyte chemoattractant protein-1 and tumor necrosis factor-(alpha) levels predict the presence of coronary artery calcium in HIV-infected individuals independent of traditional cardiovascular risk factors. AIDS Res Hum Retroviruses 2014;30:142-146.
15. Hileman CO, Longenecker CT, Carman TL, Milne GL, Labbato DE, Storer NJ, et al. Elevated D-dimer is independently associated with endothelial dysfunction: a cross-sectional study in HIV-infected adults on antiretroviral therapy. Antivir Ther (Lond) 2012;17:1345-1349.
16. Masia M, Padilla S, Garcia N, Jarrin I, Bernal E, Lopez N, et al. Endothelial function is impaired in HIV-infected patients with lipodystrophy. Antivir Ther (Lond) 2010;15:101-110.
17. Ross Eckard A, Longenecker C, Jiang Y, Debanne S, Labbato D, Storer N, et al. Lipoprotein-associated phospholipase A and cardiovascular disease risk in HIV infection. HIV Med 2014 Mar 20.
18. Sevastianova K, Sutinen J, Westerbacka J, Ristola M, Yki-Jarvinen H. Arterial stiffness in HIV-infected patients receiving highly active antiretroviral therapy. Antivir Ther 2005;10(8):925-935.
19. Kristoffersen US, Lebech AM, Wiinberg N, Petersen CL, Hasbak P, Gutte H, et al. Pmc3743817; Silent ischemic heart disease and pericardial fat volume in HIV-infected patients: a case-control myocardial perfusion scintigraphy study. PLoS One 2013;8:e72066.
20. van Wijk JP, de Koning EJ, Cabezas MC, Joven J, op't Roodt J, Rabelink TJ, et al. Functional and structural markers of atherosclerosis in human immunodeficiency virus-infected patients. J Am Coll Cardiol 2006 Mar 21;47:1117-1123.
21. Stein JH, Brown TT, Ribaudo HJ, Chen Y, Yan M, Lauer-Brodell E, et al. Pmc3664137; Ultrasonographic measures of cardiovascular disease risk in antiretroviral treatment-naive individuals with HIV infection. AIDS 2013 Mar 27;27:929-937.
22. D'Ettorre G, Ceccarelli G, Francone M, Vullo F, Mewamba S, Mancone M, et al. High prevalence of coronary stenosis detected by Coronary CT angiography in asymptomatic HIV-infected subjects with low cardiovascular risk. Infection 2010;38:48.
23. Fitch K, Srinivasa S, Abbara S, Burdo T, Williams K, Eneh P, et al. Noncalcified coronary atherosclerotic plaque and immune activation in HIV-infected women. J Infect Dis 2013;208:1737-1746.
24. Hwang JJ, Wei J, Abbara S, Grinspoon SK, Lo J. Receptor activator of nuclear factor-kappaB ligand (RANKL) and its relationship to coronary atherosclerosis in HIV patients. J Acquir Immune Defic Syndr 2012 Nov 1;61(3):359-363.

25. Lai S, Bartlett J, Lai H, Moore R, Cofrancesco J,Jr, Pannu H, et al. Long-term combination antiretroviral therapy is associated with the risk of coronary plaques in African Americans with HIV infection. AIDS Patient Care STDS 2009 Oct;23(10):815-824.
26. Lo J, Abbara S, Shturman L, Soni A, Wei J, Rocha-Filho JA, et al. Increased prevalence of subclinical coronary atherosclerosis detected by coronary computed tomography angiography in HIV-infected men. AIDS 2010 Jan 16;24(2):243-253.
27. McKibben RA, Grinspoon S, Li X, Palella F, Kingsley LA, Witt MD, et al. Elevated levels of monocyte activation markers, soluble CD14 and CD163, are associated with subclinical atherosclerosis in the multicenter aids cohort study (MACS). Circulation 2013;128.
28. Jang JJ, Berkheimer SB, Merchant M, Krishnaswami A. Asymmetric dimethylarginine and coronary artery calcium scores are increased in patients infected with human immunodeficiency virus. Atherosclerosis 2011 Aug;217:514-517.
29. Mangili A, Ahmad R, Wolfert RL, Kuvin J, Polak JF, Karas RH, et al. Pmc3935500; Lipoprotein-associated phospholipase A2, a novel cardiovascular inflammatory marker, in HIV-infected patients. Clin Infect Dis 2014 Mar;58:893-900.
30. Subramanian S, Tawakol A, Burdo TH, Abbara S, Wei J, Vijayakumar J, et al. Arterial inflammation in patients with HIV. JAMA 2012 Jul 25;308(4):379-386.
31. Knudsen A, Hag AM, Loft A, von Benzon E, Keller SH, Moller HJ, et al. HIV infection and arterial inflammation assessed by (18)F-fluorodeoxyglucose (FDG) positron emission tomography (PET): a prospective cross-sectional study. J Nucl Cardiol 2015 Apr;22(2):372-380.
32. Mariano-Goulart D, Jacquet JM, Molinari N, Bourdon A, Benkiran M, Sainmont M, et al. Should HIV-infected patients be screened for silent myocardial ischaemia using gated myocardial perfusion SPECT? Eur J Nucl Med Mol Imaging 2013 Jan;40:271-279.
33. Floris-Moore M, Fayad ZA, Berman JW, Mani V, Schoenbaum EE, Klein RS, et al. Association of HIV viral load with monocyte chemoattractant protein-1 and atherosclerosis burden measured by magnetic resonance imaging. AIDS 2009 May 15;23(8):941-949.
34. Solages A, Vita JA, Thornton DJ, Murray J, Heeren T, Craven DE, et al. Endothelial function in HIV-infected persons. Clin Infect Dis 2006 May 1;42(9):1325-1332.
35. Jang JJ, Schwarcz AI, Amaez DA, Woodward M, Olin JW, Keller MJ, et al. Pmc2859852; Elevated osteoprotegerin is associated with abnormal ankle brachial indices in patients infected with HIV: a cross-sectional study. J Int AIDS Soc 2010;13:12.
36. Kaplan RC, Sinclair E, Landay AL, Lurain N, Sharrett AR, Gange SJ, et al. Pmc3139014; T cell activation predicts carotid artery stiffness among HIV-infected women. Atherosclerosis 2011 Jul;217:207-213.
37. Ridker PM. Clinical application of C-reactive protein for cardiovascular disease detection and prevention. Circulation 2003 Jan 28;107(3):363-369.

38. Danesh J, Wheeler JG, Hirschfield GM, Eda S, Eiriksdottir G, Rumley A, et al. C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease. N Engl J Med 2004 Apr 1;350(14):1387-1397.

39. IL6R Genetics Consortium Emerging Risk Factors Collaboration, Sarwar N, Butterworth AS, Freitag DF, Gregson J, Willeit P, et al. Interleukin-6 receptor pathways in coronary heart disease: a collaborative meta-analysis of 82 studies. Lancet 2012 Mar 31;379(9822):1205-1213.

40. Interleukin-6 Receptor Mendelian Randomisation Analysis (IL6R MR) Consortium. The interleukin-6 receptor as a target for prevention of coronary heart disease: a mendelian randomisation analysis. Lancet 2012 Mar 31;379(9822):1214-1224.

41. Duprez DA, Neuhaus J, Kuller LH, Tracy R, Belloso W, De Wit S, et al. Inflammation, coagulation and cardiovascular disease in HIV-infected individuals. PLoS One 2012;7(9):e44454.

42. De Luca A, de GD, Colafigli M, Cozzi-Lepri A, De Curtis A, Gori A, et al. Pmc3846422; The association of high-sensitivity c-reactive protein and other biomarkers with cardiovascular disease in patients treated for HIV: a nested case-control study. BMC Infect Dis 2013;13:414.

43. Tenorio AR, Zheng Y, Bosch RJ, Krishnan S, Rodriguez B, Hunt PW, et al. Soluble Markers of Inflammation and Coagulation but Not T-Cell Activation Predict Non-AIDS-Defining Morbid Events During Suppressive Antiretroviral Treatment. J Infect Dis 2014 May 1.

44. Liyama K, Hajra L, Iiyama M, Li H, DiChiara M, Medoff BD, et al. Patterns of vascular cell adhesion molecule-1 and intercellular adhesion molecule-1 expression in rabbit and mouse atherosclerotic lesions and at sites predisposed to lesion formation. Circ Res 1999 Jul 23;85(2):199-207.

45. Pletcher MJ, Tice JA, Pignone M, Browner WS. Using the coronary artery calcium score to predict coronary heart disease events: a systematic review and meta-analysis. Arch Intern Med 2004 Jun 28;164(12):1285-1292.

46. Budoff MJ, Dowe D, Jollis JG, Gitter M, Sutherland J, Halamert E, et al. Diagnostic performance of 64-multidetector row coronary computed tomographic angiography for evaluation of coronary artery stenosis in individuals without known coronary artery disease: results from the prospective multicenter ACCURACY (Assessment by Coronary Computed Tomographic Angiography of Individuals Undergoing Invasive Coronary Angiography) trial. J Am Coll Cardiol 2008 Nov 18;52(21):1724-1732.

47. Detrano R, Guerci AD, Carr JJ, Bild DE, Burke G, Folsom AR, et al. Coronary calcium as a predictor of coronary events in four racial or ethnic groups. N Engl J Med 2008 Mar 27;358(13):1336-1345.

48. Inaba Y, Chen JA, Bergmann SR. Prediction of future cardiovascular outcomes by flow-mediated vasodilatation of brachial artery: a meta-analysis. Int J Cardiovasc Imaging 2010 Aug;26(6):631-640.

49. Lekakis J, Abraham P, Balbarini A, Blann A, Boulanger CM, Cockcroft J, et al. Methods for evaluating endothelial function: a position statement from the European Society of Cardiology Working Group on Peripheral Circulation. Eur J Cardiovasc Prev Rehabil 2011 Dec;18(6):775-789.

50. Laurent S, Cockcroft J, Van Bortel L, Boutouyrie P, Giannattasio C, Hayoz D, et al. Expert consensus document on arterial stiffness: methodological issues and clinical applications. Eur Heart J 2006 Nov;27(21):2588-2605.

51. Vlachopoulos C, Aznaouridis K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. J Am Coll Cardiol 2010 Mar 30;55(13):1318-1327.

52. Pedersen KK, Pedersen M, Troseid M, Gaardbo JC, Lund TT, Thomsen C, et al. Microbial translocation in HIV infection is associated with dyslipidemia, insulin resistance, and risk of myocardial infarction. J Acquir Immune Defic Syndr 2013 Dec 15;64:425-433.

53. 53 Nou E, Lo J, Grinspoon SK. Inflammation, immune activation, and cardiovascular disease in HIV. AIDS 2016 Jun 19;30(10):1495-1509.

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

55. D'Ascenzo F, Cerrato E, Appleton D, Moretti C, Calcagno A, Abouzaki N, et al. Prognostic indicators for recurrent thrombotic events in HIV-infected patients with acute coronary syndromes: use of registry data from 12 sites in Europe, South Africa and the United States. Thromb Res 2014 Sep 134(3):558-64.

56. Lo J, Lu MT, Ihenachor EJ, Wei J, Looby SE, Fitch KV, et al. Effects of statin therapy on coronary artery plaque volume and high-risk plaque morphology in HIV-infected patients with subclinical atherosclerosis: a randomised, double-blind, placebo-controlled trial. Lancet HIV 2015 Feb;2(2):e52-63.

57. Ballocca F, Gili S, D'Ascenzo F, Marra WG, Cannillo M, Calcagno A, et al. HIV Infection and Primary Prevention of Cardiovascular Disease: Lights and Shadows in the HAART Era. Prog Cardiovasc Dis 2016 Mar-Apr;58(5):565-76.

Supporting information

Supporing file 1. Baseline table

Author	Title	Design, enrollment period and follow-up duration	HIV+ Patients (n)	Country	Ethnicity	% ♂	Age	Years since HIV diagnosis	CD4 count	Nadir CD4 count	Viral load (log copies /mL)	BMI (kg/m2)	Current smokers	ART	Tx regimen	Duration of ART (years)	Markers assessed	Stored sample used	Time of outcome - biomarker	Methods
CT coronary angiography and coronary artery calcium score (CAC)																				
Burdo,T. H.; Lo,J.; Abbara,S.;	Soluble CD163, a novel marker of activated macrophages, is elevated and associated with noncalcified coronary plaque in HIV-infected patients	Cross-sectional	102	USA	white 63%, black 22%, hispanic 12%, Asian 1%, Native American 3%	100	46.6 ± 6.4	13.8 ± 6.4	530 ± 287	202 ± 173	<50 (<50 to <50) 81% <50	26.3 ± 4.7	41%	95%	52% PI, 92% NRTI, 47% NNRTI	7.2 ± 5.0	sCD163, LPS, sCD14, Osteopontin, MCP-1, hsIL-6, CRP, d-dimer	no	=	ELISA, LAL assay (LPS), immunoturbidometry (d-dimer)
D'Ettorre,G.; Ceccarelli,G.; Francone,M.;	High prevalence of coronary stenosis detected by Coronary CT angiography in asymptomatic HIV-infected subjects with low cardiovascular risk	Cross-sectional	55	Italy	nd	86	47.6 ± 8.7	9.5 ± 5.5	493 ± 223	193 ± 126	2.3 ± 1.1, 74.5% <50	22.2 ± 2.5	nd	89.1	nd	9.4±5.0	hsCRP	nd	?	ELISA
Fitch,Kv; Srinivasa,S.; Abbara,S.;	Noncalcified coronary atherosclerotic plaque and immune activation in HIV-infected women	Cross-sectional	60	USA	white 25%, nonwhite 75%	-	47 ± 7	15 ± 6	597 ± 297	191 ± 160	4.1 ± 0.9. 84% undetectable viral load	28 ± 6	50%	98	58% PI, 92% NRTI, 17% NNRTI	8 ± 5	hsCRP, hsIL-6, sC163, sCD14, CXCL10, MCP-1	no	=	ELISA
Hwang,J. J.; Wei,J.; Abbara,S.;	Receptor activator of nuclear factor-kappaB ligand (RANKL) and its relationship to coronary atherosclerosis in HIV patients	Cross-sectional	78	USA	white 68%, black 18%, asian 1%, hispanic 9%, native American 4%	100	46.5 ± 6.5	13.5± 6.1	523 ± 282	169 (54 - 263)	<50 (<50 to <50) 81% <50	26.1 ± 4.3	35%	95	53% PI	7.1 ± 4.6	RANKL, osteoprotegerin, CRP	nd	?	ELISA
Lai,S.; Bartlett,J.; Lai,H.;	Long-term combination antiretroviral therapy is associated with the risk of coronary plaques in African Americans with HIV infection	Cross-sectional, Aug 2003 to Dec 2007	176	USA	African American 100%	63	44.2 (39.9-47.3)	nd	344 (177-498)	nd	581 (30-14789) cp/mL	23.8 (21.5-27.3)	86.4%	nd	nd	In months: NRTI's 0 (0-12), NNRTIs 0 (0-0), PIs 0 (0-12), All 3 (0-24)	hsCRP	nd	=	nd

Lo,J.; Abbara,S.; Shturman,L.	Increased prevalence of subclinical coronary atherosclerosis detected by coronary computed tomography angiography in HIV-infected men	Cross-sectional	78	USA	white 68%, black 18%, asian 1%, hispanic 9%, native American 4%	100	46.5 ± 6.5	13.5 ± 6.1	523 ± 282	169 (54 - 263)	<50 (<50 to <50) 81% <50	26.1 ± 4.3	35%	95	53% PI, 91% NRTI, 49% NNRTI	7.1 ± 4.6	MCP-1, CRP, IL-6	nd	?	ELISA. No data for IL-6
McKibben,R A.; Margolick,J B.; Grinspoon,S;	Elevated levels of monocyte activation markers are associated with subclinical atherosclerosis in men with and those without HIV infection	Cross sectional. Jan 2010-Aug 2013	566	USA	white 51.9%, African American 34.3%, Hispanic/other 13.8%	100	53.0 ± 6.5	nd	599 (426-751)	251 (144-335)	81% <50 copies/mL, rest: 537 (131-12200) copies/mL	26.2 ± 4.5	31.3%	95.9	nd	12.3 (8.8-14.1)	sCD163, sCD14, CCL2	yes	=	ELISA for sCD163 and sCD14, Luminex-based singleplex cytokine panel for CCL2
Coronary calcium score (CAC)																				
Jang,J. J.; Berkheimer,S. B.; Merchant,M.;	Asymmetric dimethylarginine and coronary artery calcium scores are increased in patients infected with human immunodeficiency virus	Case control, from Jan 2007 to Dec 2008	37	USA	nd	73	45.0 ± 8.0	nd	nd	nd	nd	27.5 ± 7.5	past or current: 54.0%	nd	nd	nd	ADMA	nd	?	ADMA by chromotography
Longenecker,C. T.; Jiang,Y.; Orringer,C. E.;	Soluble CD14 is independently associated with coronary calcification and extent of subclinical vascular disease in treated HIV infection	Cross-sectional, substudy of SATURN-HIV trial	147	USA	African american 69%	78	46 (40-53)	12 (6.2-18)	613 (425-853)	179 (86-298)	70% <48 copies/mL	27 (23-30)	63%	100%	49% PI, 5% ZDV or D4T, 5% abacavir	5.3 (3.2-9.8)	sCD14, sCD163, hsCRP, IL-6, sTNFR-I, sVCAM-1, D-dimer, fibrinogen, OPG, RANKL.	nd	=	ELISA, nephelometer (hsCRP and fibrinogen), immunoturbidometric assay (d-dimer), singleplex immunoassay (RANKL).
Mangili,A.; Ahmad,R.; Wolfert,R. L.	Lipoprotein-associated phospholipase A2, a novel cardiovascular inflammatory marker, in HIV-infected patients	Cross-sectional, CAC computed from Jan 2002 to Dec 2003	LpPLA2 mass <235: 84	USA	76% non white	69	44 ± 7	10.2 ± 4.9	385 ± 256	nd	3.6 ± 1.2 (36% undetectable)	28 ± 6	54%	64%	36% on PI	25 ± 25 months	Lp-PLA2 mass, CRP	yes	unclear	ELISA, immunoturbidometry (CRP)
			LpPLA2 mass >235: 257		39% non white	76	44 ± 7	9.6 ± 4.8	470 ± 308		2.9 ± 1.0 (62% undetectable)	26 ± 5	47%	77%	47% on PI	33 ± 26 months				

Shikuma,C. M.; Barbour,J. D.; Ndhlovu,L. C.;	Plasma monocyte chemoattractant protein-1 and tumor necrosis factor-(alpha) levels predict the presence of coronary artery calcium in HIV-infected individuals independent of traditional cardiovascular risk factors	Cross sectional. Substudy of Hawaii Aging with HIV Cardiovascular (HAHC-CVD) study	130	USA	60% white, 3.1% African American, 2.3% Native american/ alaskan, 12.3% Native hawaiian/ pacific islanders, 7.7% asian, 14.6% mixed	88	51 (46-57)	nd	492 (341-635)	154 (30-250)	86.9%<50 copies/ml	25.8 (23.9-28.3)	22.3% (of patients with clinical CVD)	100%	nd	nd	sE-selectin, sVCAM-1, sICAM-1, MMP-9, MPO, tPAI-1, CRP, SAA, SAP, IL-1B, IL-6, IL-8, IL-10, TNF-a, MCP-1, VEGF, IFN-γ	yes	unclear	Multiplex (Milliplex Human Cardiovascular disease panels)
Flow mediated dilation (FMD)																			
Gupta,S. K.; Mi,D.; Dube,M. P.;	Pentoxifylline, inflammation, and endothelial function in HIV-infected persons: a randomized, placebo-controlled trial	Randomized double blinded placebo controlled trial FU 8 weeks	Placebo: 13	USA	62% black race	85	34 ± 10.9	nd	583 ± 175	nd	4.0 ± 1.2	27.7 ± 5.9	38%	0	na	na	hsCRP, IL-6, sTNFRI, sTNFRII, MCP-1, TIMP-1, IP-10, PAI-1 Ag, sVCAM-1	yes	=	hsCRP, IL-6, IP-10, lipids and insulin: serum. MCP-1, sTNFRI and II, sVCAM-1 and TIMP-1: EDTA plasma and PAI-1 Ag : citrated plasma. No information on technique.
			Pentoxifylline: 13		62% black race	62	40 ± 11.6		524 + 165		4.0 ± 0.7	26.1 ± 4.6	46%	0						
Hileman,C. O.; Longenecker,C. T.; Carman,T. L	Elevated D-dimer is independently associated with endothelial dysfunction: a cross-sectional study in HIV-infected adults on antiretroviral therapy	Cross-sectional	98	USA	African american 52%, Caucasian 42%	88	47.5 (43-52)	11.3 (7.2-16.2)	579 (431-789)	130 (32-238)	all <400 copies/ml	26 (23.3-30)	47%	100	PI 52%, NNRTI 48%	nd	IL-6, sTNFR-I, sTNFR-II, hsCRP, sICAM-1, sVCAM-1, fibrinogen, d-dimer	yes	=	ELISA, immunonephelometry (hsCRP, fibrinogen), immunoturbidometry (d-dimer)

3

Masia,M.; Padilla,S.; Garcia,N.;	Endothelial function is impaired in HIV-infected patients with lipodystrophy	Cross-sectional, Jan - June 2008	No lipodystrophy: 55	Spain	nd	80	46.0 (36.3-50.3)	nd	445 (337.5-645.0)	nd	75% <50 copies/ml	23.7 (21.8-27.3)	61.8%	100%	25.5% NN analogue based regimen, 69.1% PI based regimen, 32.7% abacavir containing regimen, 16.4% thymidine analogue containing regimen	7 (4-9.25)	sICAM-1, sVCAM-1, sE-selectin, hsCRP, IL-6, TNF-a, PAI-1	yes	=	ELISA, IMMULITE 2000 analyzer (CRP)
			Lipodystrophy: 55			82	45.5 (41.9-52.2)		550 (347.5-805.0)		76.7% <50 copies/ml	24.6 (21.7-27.2)	52.7%		51.9% NN analogue based regimen, 44.4% PI based regimen, 36.4% abacavir containing regimen, 5.5% thymidine analogue containing regimen	11 (9-13)				
Nolan,D.; Watts,G. F.; Herrmann,S. E.;	Endothelial function in HIV-infected patients receiving protease inhibitor therapy: does immune competence affect cardiovascular risk?	Cross-sectional	24	Australia	nd	100	42.6 ± 9.5	nd	469 ± 240	134 ± 157	75% <50 copies/ml (mean: log 3.97 ± 1.1 copies/mL)	24.1 ± 2.7	41.6%	100%	100% PI	30.5 ± 4.9 months (PI)	CRP	no	=	Immunonephelometry
Solages,A.; Vita,J. A.; Thornton,D. J.;	Endothelial function in HIV-infected persons	Cross-sectional	75	USA	Black 56%, white 21%, hispanic 20%	56	44.2 ± 8.4	nd	nd	270 ± 250	90	26.6 ± 4.5	63%	84%	43% PI, 57% non PI regimen	nd	hsCRP	nd	=	nd
Stein,Jh; Brown,Tt; Ribaudo,Hj;	Ultrasonographic measures of cardiovascular disease risk in antiretroviral treatment-naive individuals with HIV infection	Cross-sectional	331	USA	44% white, 32% black, 20% hispanic	89	36 (28-45)	0.5 (0.2-2.6)	349 (207-455)	nd	4.5 (4.0-5.1)	25 (22-28)	38%	0%	na	na	hsCRP, IL-6	yes	unclear	Nephelometry (hsCR)), ELISA (IL-6)

Torriani,F. J.; Komarow,L.; Parker,R. A.;	Endothelial function in human immunodeficiency virus-infected antiretroviral-naive subjects before and after starting potent antiretroviral therapy: The ACTG (AIDS Clinical Trials Group) Study 5152s	Prospective, multicenter study, Oct 2002 to Dec 2004 FU 24 weeks	82	USA	54% white, 32% black or asian, 15% hispanic	91	35 (30-40)	nd	245 (119-356)	nd	4.8(4.49-5.32)	25.1 (22.8-27.7)	44%	0%	33% PI sparing regimen, 33% NNRTI sparing regimen, 33% NRTI sparing regimen	24 weeks	hsCRP	no	=	nd
Gleason,R L.,Jr; Caulk,AW.; Seifu,D;	Current Efavirenz (EFV) or Ritonavir-Boosted Lopinavir (LPV/r) Use Correlates with Elevate Markers of Atherosclerosis in HIV-Infected Subjects in Addis Ababa, Ethiopia	Cross-sectional	HAART naive: 51	Ethiopia	nd	27	38 (32-45)	1.6 (0.3-3.7)	395 (182-546)	nd	3.6 (2.2-4.7)	22 (20-26)	2%	81.9%	100% NRTI backbone, 66.2% NNRTI, 15.7% PI		hsCRP, sVCAM-1, sICAM-1	nd	=	ELISA
			Efavirenz: 91			25	38 (34-45)	5.7 (3.4-7.0)	349 (232-481)		<1.6(<1.6-<1.6)	21 (19-24)	0%			5.0 (3.2-6.2)				
			Nevarapine: 95			22	37 (32-42)	6.0 (5.6-7.4)	390 (271-534)		<1.6(<1.6-<1.6)	23 (20-25)	2%			5.7 (3.9-6.2)				
			Lopinavir: 44			32	39 (35-44)	6.5 (5.3-8.3)	285 (147-453)		<1.6(<1.6-<1.6)	22 (18-25)	0%			5.9 (4.2-6.8)				
van Wijk,J. P.; de Koning,E. J.; Cabezas,M. C.;	Functional and structural markers of atherosclerosis in human immunodeficiency virus-infected patients	Cross-sectional	Metabolic syndrome: 15	Netherlands	nd	100	50 ± 3	8.5 ± 0.8	604 ± 105	nd	1114 ± 824 copies/ml, 80% <50cp/mL	24.4 ± 0.5	13%	100%	67% PI, 33% NNRTI, 100% NRTI	4.8 ± 0.5	hsCRP	nd	unclear	high sensitivity kit: Quantex hs-CRP kit
			No metabolic syndrome: 22				47 ± 2	7.5 ± 5.2	719 ± 58		813 ± 201 copies/ml, 77% <50cp/mL	23.6 ± 0.4	14%		68% PI, 32% NNRTI, 100% NRTI	4.2 ± 0.6				
Ross Eckard,A.; Longenecker,C.; Jiang,Y	Lipoprotein-associated phospholipase A2 and cardiovascular disease risk in HIV infection	Cross-sectional, March 2011 to Aug 2012	100	USA	Caucasian 29%, Black 70%, other 1%	77	47 (25-68)	13 (1.5-26.8)	633 (142-1683)	199 (0-614)	100% <1000 copies/ mL. 80% undetectable	27 (7-17)	62%	100%	47 PI%, 52% NNRTI, 6% NRTI	6.3 (0.8-21.7)	Lp-PLA2, Il-6, sTNRF-1, sTNFR-II, sVCAM-1, sICAM-1, d-dimer, fibrinogen, hsCRP, sCD14, sCD163.	yes	=	ELISA, nephelometer (hsCRP, fibriongen), STA-R Coagualtion Analyzer (d-dimer)
Sevastianova,K.; Sutinen,J.; Westerbacka,J.;	Arterial stiffness in HIV-infected patients receiving highly active antiretroviral therapy	Cross-sectional	Lipodystrophy: 42	Finland	100% caucasian	93	44 ± 1	8.6 ± 0.6	567 ± 40	160 ± 20	1.8 ± 0.1	23.3 ± 0.4	36	100%	69% stavudine, 78% PI	6.2 ± 0.4	hsCRP	no	=	high sensitivity commercial kit (Ultrasensitive CRP Kit)
			No lipodystrophy: 17			76	41 ± 2	8 ± 1.2	533 ± 60	210 ± 20	1.5 ± 0.1	23.1 ± 0.9	35		18% stavudine, 59% PI	4.8 ± 0.8				

3

Jang,J. J.; Schwarcz,A. I.; Amaez,D. A	Elevated osteoprotegerin is associated with abnormal ankle brachial indices in patients infected with HIV: a cross-sectional study	Cross-sectional, Dec 2005 to May 2006	102	USA	45% Hispanic, 44% African American, <1% Asian	57	48.4 ± 9.1	141.1 ± 63.6 months	565.4 ± 416.3	nd	10,856.5 ± 47,682.8 copies/mL	27.4 ± 6.0	75% ever smokers	nd	nd	52.7 ± 56.6 months	CRP, IL-1B, IL-6, OPG	nd	?	IMMAGE 800 assay for CRP and ELISA for IL-1B, IL-6 and OPG
Kaplan,R. C.; Sinclair,E.; Landay,A. L.;	T cell activation predicts carotid artery stiffness among HIV-infected women	Cross-sectional. Substudy of Women's Interagency HIV study.	114	USA	White/other 9%, hispanic 27%, African American 64%	-	46 (43-50)	nd	384 (227-582)	210 (109-339)	1.3 (0.08-16) (thousands)	32% <25, 39% 25-30, 29% >30	48%	64%	63% NRTI, 24% NNRTI, 27% 1 PI, 18% 2 PI's	nd	CRP	nd	several months	nephelometry
18FDG PET																				
Knudsen,A; Hag,A;Loft,A	HIV infection and arterial inflammation assessed by (18) F-fluorodeoxyglucose (FDG) positron emission tomography (PET): a prospective cross-sectional study	Cross-sectional, March 2011 to June 2013	26	Denmark	nd	100	50.5 ± 2.4	13.9 (10.8-16.8)	636 (549-717)	nd	19 (19-31) copies/mL	24.0 (22.9-25.1)	19%	100%	≥2 NRTIs + 1 NNRTI (81%), ≥2 NRTIs + ≥1 PI (15%), other 4%	9.9 (8-11.7)	hsCRP, sCD163, sE-selectin, sVCAM-1, sICAM-1, MMP-9, PAI-1	nd	=	Siemens STRATUS CS (hsCRP), ELISA (sCD163), multiplex assay (sE-selectin, sVCAM-1, sICAM-1, MMP-9 and PAI-1)
18FDG PET and CAC																				
Subramanian,S.; Tawakol,A.; Burdo,T.	Arterial inflammation in patients with HIV	Cross-sectional, Nov 2009 to July 2011	27	USA	nd	93	51.6 (49.5-53.6)	15.5 ± 5.7	641 ± 288	99 (50, 250)	<48 (<48-<48) 81% <48 copies/mL	nd	22%	100%	41% PI, 96% NRTI, 52% NNRTI	12.3 ± 4.3	hsCRP, D-dimer, sCD163	nd	nd	hsCRP: immunochemiluminometric assay. sCD163: ELISA. D-dimer: immunoturidimetric assay

MRI of thoracic aorta and carotid arteries																				
Floris-Moore,M.; Fayad,Z. A.; Berman,J. W.;	Association of HIV viral load with monocyte chemoattractant protein-1 and atherosclerosis burden measured by magnetic resonance imaging	Cross sectional	viral load <75 copies/mL: 38	USA	black 65.8%, latino 28.9%, white/other 5.3%	55	54.7 ± 1.0	nd	<200: 7.9%, 200-499: 44.7%, >500 47.4%	nd	61% detectable viremia	25.2 ± 0.7	47.4%	84.2%	57.9% PI based, 26.3% non-PI	PI users: median 66 months (42-98)	MCP-1, CCL2	yes	=	ELISA
			viral load ≥75 copies/mL: 60		black 70%, latino 15%, white/other 15%	65	54.9 ± 0.7		<200: 25.0%, 200-499: 46.7%, >500 28.3%	nd		26.9 ± 0.8	68.3%	56.7%	41.7% PI based, 15.0% non-PI					
Mariano-Goulart,D.; Jacquet,J. M.; Molinari,N	Should HIV-infected patients be screened for silent myocardial ischaemia using gated myocardial perfusion SPECT?	Cross-sectional, Nov 2009 to Jan 2012	94	France	nd	87	55 ± 8	16 ± 7	555 ± 261	nd	19% >20cp/mL	24 ± 4	64%	nd	NRTI 89%, NNRTI 33%, Integrase Inhibitor 14%, PI 67%	12 ± 6	CRP	nd	nd	nd
Kristoffersen,U. S.; Lebech,A. M.; Wiinberg,N.;	Silent ischemic heart disease and pericardial fat volume in HIV-infected patients: a case-control myocardial perfusion scintigraphy study	Cross-sectional, Sept 2008 to July 2010	105	Denmark	nd	89	47.4 ± 0.83	12.3 ± 0.64	636 ± 25	171 ± 11	90 % < 40 copies/mL	24.7 ± 0.33	37%	100%	≥2 NRTIs + 1 NNRTI (62%), ≥2 NRTIs + ≥1 PI (24%), NRTI + NNRTI + PI (6%), other (9%)	8.9 ± 0.41	sICAM-1, sVCAM-1, MMP-9, tPAI-1, hsCRP, endothelin.	nd	=	Fluorescent bead-based immunoassay (sICAM-1, sVCAM-1,MMP9, hsCRP, tPAI-1). ELISA (Endothelin)

Supporing file 2. Critical appraisal table.

Author	Title	Clear inclusion criteria	Homogenous moment of inclusion/how were patients included	Standardized: determinant	Standardized: outcome	Missing data with regard to inclusion or follow-up	Blinded measurement of determinant / outcome	Outcome	Adjustment for confounders
CT coronary angiography and coronary artery calcium score (CAC)									
Burdo,T. H.; Lo,J.; Abbara,S.;	Soluble CD163, a novel marker of activated macrophages, is elevated and associated with noncalcified coronary plaque in HIV-infected patients	yes	HIV+ men recruited from HIV clinics, community health care centers and newspaper advertisements. Aged 18-58 years without known cardiac diseaese or symptoms suggestive of cardiac disease. Excl: known renal disease or creat levels >1.5 mg/dL or estimated GFR <70. Patients on ART were required to have been on stable therapy for >3 months.	yes	yes	nd on potential eligible pts	Outcome	Coronary plaque (calcified/ non-calcified) on CT coronary angiography, based on consensusreading by 2 investigators, and CAC (agatson). Severe coronary artery stenosis was defined as luminal obstruction 70% diameter	age, race, lipids, blood pressure, glucose, smoking, HIV infection, lipid lowering therapy, sCD14
D'Ettorre,G.; Ceccarelli,G.; Francone,M.;	High prevalence of coronary stenosis detected by Coronary CT angiography in asymptomatic HIV-infected subjects with low cardiovascular risk	yes	Asymptomatic HIV+ patients referred by physicians with a low cardiovascular risk, no HCV co-infection.	yes	yes	nd on potential eligible pts	No	Coronary plaque on CT coronary angiography. Read independently by two radiologists. Luminal narrowing >50%: clinicallsy significant coronary stenosis, than: coronary angiography. Coronary calcium score (agatson).	not clear if CRP was used in multivariable analysis. Only univariate data
Fitch,Kv; Srinivasa,S.; Abbara,S.;	Noncalcified coronary atherosclerotic plaque and immune activation in HIV-infected women	yes	HIV+ women recruited from HIV clinics, community health centers and newspaper advertisements. Aged 18-60 years, without symptoms or history of CVD, stable ART >3 months.	yes	yes	nd on potential eligible pts	Outcome	Coronary plaque (calcified/ non-calcified) on CT coronary angiography, based on consensusreading by 2 investigators, and CAC (agatson). Severe coronary artery stenosis was defined as luminal obstruction 70% diameter	No

Hwang,J. J.; Wei,J.; Abbara,S.;	Receptor activator of nuclear factor-kappaB ligand (RANKL) and its relationship to coronary atherosclerosis in HIV patients	yes	Substudy of SATURN-HIV trial. 18-55 years, without symptoms or history of cardiac disease. In case of ART use stable >3 months.	yes	yes	nd on potential eligible pts	Outcome	Coronary plaque on CT coronary angiography and CAC (agatson)	Framingham risk score, HIV related factors
Lai,S.; Bartlett,J.; Lai,H.;	Long-term combination antiretroviral therapy is associated with the risk of coronary plaques in African Americans with HIV infection	yes	HIV infected patients, consecutively enrolled in a hospital, 25-54 years, African American race without hypertension or cardiac disease	ND	yes	nd on potential eligible pts	Outcome	MDCT for CAC and CT coronary angiography	Univariable analysis. Factors with p ≤0.10 were included in multivariable analysis
Lo,J.; Abbara,S.; Shturman,L.	Increased prevalence of subclinical coronary atherosclerosis detected by coronary computed tomography angiography in HIV-infected men	yes	HIV infected patients recruited from HIV clinics and ads in newspapers, aged 18-55 years, without (symptoms of) CVD. In case of ART stable >3 months	yes	yes	nd on potential eligible pts	Outcome	Coronary plaque on CT coronary angiography. CAC (agatson)	HIV related parameters and cardiovascular risk factors
McKibben,R A.; Margolick,J B.; Grinspoon,S;	Elevated levels of monocyte activation markers are associated with subclinical atherosclerosis in men with and those without HIV infection	yes	Substudy of MACS. HIV infected homosexual patients, aged 40-70 years, no prior cardial intervention.	yes	yes	yes >10%	Outcome	Coronary plaque on noncontrast cardiac CT, CAC (agatson). Moderate stenosis 50-69%, severe stenosis ≥70%.	CVD risk factors, age, race, HIV-associated factors

Coronary calcium score (CAC)									
Jang,J. J.; Berkheimer,S. B.; Merchant,M.;	Asymmetric dimethylarginine and coronary artery calcium scores are increased in patients infected with human immunodeficiency virus	no	HIV+ patients ≥18 years and controls matched for age and gender.	yes	yes	ND	ND	CAC	HIV status, creatinine and HDL
Longenecker,C. T.; Jiang,Y.; Orringer,C. E.;	Soluble CD14 is independently associated with coronary calcification and extent of subclinical vascular disease in treated HIV infection	yes	Substudy of SATURN-HIV trial, aged ≥18 years, stable ART ≥12 weeks, HIV RNA <1000 cp/mL, hs-CRP≥2mg/l, no coronary disease or diabetes.	yes	yes	nd on potential eligible pts	ND	CAC, FMD (CAC present : >5 pixels >130HU)	age, sex, race, nadir CD4+ cell count, LDL-C, limb fat
Mangili,A.; Ahmad,R.; Wolfert,R. L.	Lipoprotein-associated phospholipase A2, a novel cardiovascular inflammatory marker, in HIV-infected patients	yes	Substudy within the CARE trial. HIV-infected patients without diabetes, uncontrolled hypertension , myocardial infarction or stroke < 6 months.	yes	yes	nd on potential eligible pts	ND	CAC. CAC stratified into 0, 1-100, >100.	standard risk factors
Shikuma,C. M.; Barbour,J. D.; Ndhlovu,L. C.;	Plasma monocyte chemoattractant protein-1 and tumor necrosis factor-(alpha) levels predict the presence of coronary artery calcium in HIV-infected individuals independent of traditional cardiovascular risk factors	yes	Substudy of HAHC-CVD study, patients baseline data used. Inclusion: HIV infected patients on stable ART≥6months, ≥40yrs.	yes	unclear	nd on potential eligible pts. 7/130 patients have partially missing data.	Outcome	CAC. CAC present in case of Agatston score >0.	If univariable significant, multivariable adjusted for age, gender, CD4 percent, hypertension, diabetes, smoking history, total cholesterol/HDL ratio.
Flow Mediated Dilation (FMD)									
Gupta,S. K.; Mi,D.; Dube,M. P.;	Pentoxifylline, inflammation, and endothelial function in HIV-infected persons: a randomized, placebo-controlled trial	yes	Patients with HIV not requiring ART per DHHS Guidelines, aged ≥18yrs, CD4 cell count≥350 uL at screening. Excl: known cardiovascular disease and risk factors of CVD	yes	yes	yes >10%	Outcome	FMD and nitroglycerin-mediated dilation (NTGMD)	correlation
Hileman,C. O.; Longenecker,C. T.; Carman,T. L.;	Elevated D-dimer is independently associated with endothelial dysfunction: a cross-sectional study in HIV-infected adults on antiretroviral therapy	yes	HIV infected, stable ART >3months, HIV-1 RNA <400cp/mL, FMD measurement available. Part of a study at the HIV Metabolic Research Center	yes	yes	nd on potential eligible pts	ND	FMD	age, sex, race, BMI, CD4+ T-cell count, whether on a protease inhibitor and smoking status and all variables with P<0,25 in univariable analysis
Masia,M.; Padilla,S.; Garcia,N.;	Endothelial function is impaired in HIV-infected patients with lipodystrophy	yes	Consecutive healthy HIV adults with lipodystrophy if ART >2 years, stable >6months, random control subject (1:1) without lipodystrophy mached by age (±5 years) and sex.	yes	yes	yes >10% in potential eligible patients	Yes	FMD	traditional CVD risk factors, pro-atherosclerotic biomarkers and factors associated with HIV infection
Nolan,D.; Watts,G. F.; Herrmann,S. E.;	Endothelial function in HIV-infected patients receiving protease inhibitor therapy: does immune competence affect cardiovascular risk?	yes	Subgroup of the Western Australian HIV cohort. Incl: HIV+ men, referred to lipid disorder clinic, PI >9 monhts.	yes	yes	nd on potential eligible pts	Outcome	FMD	smoking status, BMI, lipid and lipoprotein levels, fastin insulin, HOMA-R duration of PI therapy, age, mean arterial pressure, baseline arterial diameter, pulse pressure, %CD4 T cell count.

Solages,A.; Vita,J. A.; Thornton,D. J.	Endothelial function in HIV-infected persons	yes	HIV+ from a cohort of Hep C infected patients. Excl: hemodialysis, uncontrolled hypertension	ND	yes	nd on potential eligible pts. 1/76 patients with missing data.	Outcome	FMD	Age, BMI, smoking, total cholesterol, fasting blood glucose, seks and factors with P≤0,15 in univariate analysis
Stein,Jh; Brown,Tt; Ribaudo,Hj;	Ultrasonographic measures of cardiovascular disease risk in antiretroviral treatment-naive individuals with HIV infection	yes	Baseline evaluation of ART naive HIV infected patients enrolled in a randomized ART treatment trial (AIDS clinical trials group study A5257). Age ≥18 yrs, HIV RNA >1000cp/mL. Excl: known CVD	yes	yes	nd on potential eligible pts	Outcome	FMD	selection of variables based on Akaiki Information Criteria. For the final model selection was done based on clinical input, colllinearity, final model r2 values.
Torriani,F. J.; Komarow,L.; Parker,R. A.	Endothelial function in human immunodeficiency virus-infected antiretroviral-naive subjects before and after starting potent antiretroviral therapy: The ACTG (AIDS Clinical Trials Group) Study 5152s	yes	Substudy of ACTG5142, subjects recruited consecutively from six sites.Incl: HIV+, HIV RNA >2.0 log10 cp/mL. Excl: o.a. CVD	ND	yes	<10% on baseline, >10% at 24 weeks follow up. Nd on potential eligible pts	Outcome	FMD	correlation
Flow mediated dilation (FMD) and pulse wave velocity (PWV)									
Gleason,R L.,Jr; Caulk,AW.; Seifu,D	Current Efavirenz (EFV) or Ritonavir-Boosted Lopinavir (LPV/r) Use Correlates with Elevate Markers of Atherosclerosis in HIV-Infected Subjects in Addis Ababa, Ethiopia	yes	HIV+ and HIV- patients recruited from a referral hospital, 18-65 years, on the same ART regimen >2 months. No AIDS defining illnesses or diabetes mellitus	yes	yes	No data on potential eligible patients. Biomarkers mssing in >10% of subjects	Outcome	PWV and FMD	Relevant study parameters or p <0.05 in correlation analysis
van Wijk,J. P.; de Koning,E. J.; Cabezas,M. C.	Functional and structural markers of atherosclerosis in human immunodeficiency virus-infected patients	yes	HIV infected men, 18-70 years, recruited from the Department of Infectious Diseases. HIV-RNA <10.000cp/mL, HAART ≥12 months.	yes	yes	nd on potential eligible pts	ND	PWV and FMD	Correlation, significant variables were enterd in regression analysis
Flow mediated dilation (FMD) and coronary calcium score (CAC)									
Ross Eckard,A.; Longenecker,C.; Jiang,Y.;	Lipoprotein-associated phospholipase A2 and cardiovascular disease risk in HIV infection	yes	First 100 subjects. HIV+, age≥18yars, LDL≤130mg/dL, ART≥ 6 months, stable ≥ 3 months, HIV-1 RNA <1000cp/mL, no CVD or statin use	yes	yes	no	Outcome	CAC, FMD	only correlation for analysis of interest
Pulse wave analysis (PWA)									
Sevastianova,K.; Sutinen,J.; Westerbacka,J.;	Arterial stiffness in HIV-infected patients receiving highly active antiretroviral therapy	yes	HIV patients recruited from HIV outpatient clinic. HAART ≥18 months, stable ≥3 months	yes	yes	nd on potential eligible pts	ND	PWA	All baseline variables with a p-value ≤0.20 were enterd in regression analysis
Ankle brachial index (ABI)									

Jang,J. J.; Schwarcz,A. I.; Amaez,D. A.;	Elevated osteoprotegerin is associated with abnormal ankle brachial indices in patients infected with HIV: a cross-sectional study	yes	HIV-infected patients ≥18years recruited from primary HIV clinic on consecutive clinic days. No vascular disease of non-atherosclerotic origin.	yes	yes	nd on potential eligible pts	ND	ABI. Definite peripheral arterial disease: ABI ≤0.90	age, sex, BMI, smoking, diabetes mellitus, total cholesterol, HDL, low densitiy lipoprotein, triglycerides, CRP, cardiovascular disease, family cardiac history, duration of HIV and duration of PI use.
Carotid artery stiffness (carotid artery distensibility and Young's elastic modulus)									
Kaplan,R. C.; Sinclair,E.; Landay,A. L.	T cell activation predicts carotid artery stiffness among HIV-infected women	no	Substudy within WIHS (enrollment 1994-1995 and 2001-2002). Random sample of women aged≥40yrs, without CVD, with available carotid ultrasounds within several months of collection of peripheral blood cells.	yes	yes	nd on potential eligible pts	ND	Carotid arterial stiffness (distensibility, Young's elastic modulus)	age, cardiovascular risk factors, HIV RNA, CD4+ T-cell count
18FDG PET									
Knudsen,A; Hag,A; Loft,A	HIV infection and arterial inflammation assessed by (18) F-fluorodeoxyglucose (FDG) positron emission tomography (PET): a prospective cross-sectional study	yes	Recruited from routine visits out-patient clinic department infectious diseases. Incl: HIV+, men, age ≥18 years, ART >12 months, no CVD.	yes	yes	nd on potential eligible pts	No	18FDG PET/CT with concominant measurement of intima-media thickness	Only the marker that was significantly different between HIV+ and HIV- was analyzed in multiple regression.
Subramanian,S.; Tawakol,A.; Burdo,T. H.;	Arterial inflammation in patients with HIV	yes	Prospectively enrolled HIV+ patiens without known CVD, stable ART ≥3 months. Unclear were cases were recruited.	yes	yes	nd on potential eligible pts	Outcome	18FDG PET/CT, CAC	Correlation
MRI of thoracic aorta and carotid arteries									
Floris-Moore,M.; Fayad,Z. A.; Berman,J. W.	Association of HIV viral load with monocyte chemoattractant protein-1 and atherosclerosis burden measured by magnetic resonance imaging	yes	Participants with HIV with CIMT ≥ 0.7mm, ≥45 years, not on lipid lowering drugs.	yes	yes	yes	Outcome	MRI of thoracic aorta and carotid arteries	age, BMI, current cigarette smoking.
Myocardial perfusion scintigraphy SPECT									
Mariano-Goulart,D.; Jacquet,J. M.; Molinari,N.;	Should HIV-infected patients be screened for silent myocardial ischaemia using gated myocardial perfusion SPECT?	yes	HIV+ patients with CV risk factors without signs of CVD after cardiac examination. Prospectively recruited from hospital (outpatient).	ND	yes	yes, 5 of 99 recruited patients not included. ND on potential eligible pts	ND	Myocardial SPECT. Ischemia: reversible perfusion defect and necrosis as a fixed significant defect with abnormal wall thickening	values with p< 0.25 were tested in multivariable analysis
Myocardial perfusion scintigraphy (MPS), CAC, pericardial fat volume measurement (PFVM)									
Kristoffersen,U. S.; Lebech,A. M.; Wiinberg,N.;	Silent ischemic heart disease and pericardial fat volume in HIV-infected patients: a case-control myocardial perfusion scintigraphy study	yes	Consecutive HIV patients, age 18-70 years, receiving cART >12 months, prospectively recruited at routine visits at outpatient clinic.	yes	yes	nd on potential eligible pts	Outcome	Myocardial perfusion scintigraphy, coronary artery calcium score, pericardial fat volume measurement, carotid intima media thickness	metabolic syndrome, smoking status, age, gender, cholesterol, triglycerides, glucose and systolic blood pressure

CHAPTER

4

The utility of the lipoprotein-associated phospholipase A2 (Lp-PLA_2) assay in detecting abnormalities in lipid metabolism and cardiovascular risk in an HIV infected South African cohort.

E.S Mayne, S. Louw, H. Moabi, D.E. Grobbee, R.E. Barth,

K.Klipstein-Grobusch, W.S. Stevens, A.G. Vos

Submitted

Abstract

Background People living with HIV (PLWH) show an increased prevalence of cardiovascular disease (CVD). Traditional CVD risk-assessment tools may have limited utility in PLWH. Lp-PLA2 catalyses the synthesis of metabolically-active lipid mediators which recruit monocytes, key effectors of atherosclerosis. Lp-PLA2 levels correlate with plaque burden and instability.

Methods 98 participants were recruited (49 uninfected, 27 ART-naïve HIV-infected and 22 ART-treated participants) in a cohort study looking at CVD in PLWH in Johannesburg, South Africa. Lp-PLA2, lipogram, glucose, physical measurements, HIV viral load (VL) and CD4+ T-cell count and carotid intimal-medial thickness (CIMT) were measured. Demographic and CVD risk factors were documented and the Framingham risk score (FRS) computed.

Results Lp-PLA2 levels were increased in participants on protease inhibitor(PI)-containing ART regimens (median 50.5 vs 127.0, $p=0.05$) and correlated with age ($r=0.25$, $p<0.001$), BMI ($r=0.73$, $p=0.04$) and LDL-cholesterol ($r=0.40$, $p<0.001$). Significance did not persist with correction for LDL levels. Lp-PLA2 was not related to FRS or CIMT in any group. Lp-PLA2 correlated with VL ($r=0.323$, $p=0.025$) and this strengthened if patients with undetectable VL were excluded ($r=0.653$, $p<0.001$). Lp-PLA2 correlated inversely with CD4+ T cell count in patients with detectable VL ($r=-0.727$, $p<0.001$).

Conclusion Lp-PLA2 was increased in HIV-infected participants on PIs and reflected LDL levels which may indicate the PI-associated dyslipidaemia. Lp-PLA2 did not correlate with CIMT or FRS but correlated strongly with VL and CD4+ cell count. This may suggest that HIV-associated inflammation is linked to increased Lp-PLA2 levels providing a mechanistic link between HIV and CVD.

Introduction

Access to antiretroviral therapy (ART) has increased the life expectancy of people living with HIV (PLWH).(1) Although there has been a decrease in the prevalence of opportunistic infections, PLWH are more likely to develop non-communicable diseases including cardiovascular disease (CVD). CVD was the cause of mortality in patients undergoing planned interruption of ART, focusing research interest on validation of biomarkers to assess CVD risk in these patients.(2) The pathogenesis of CVD in PLWH is multifactorial. Immune activation and chronic inflammation, associated with low-grade viral replication, microbial translocation and opportunistic diseases, directly impacts the endothelial surface and activates leukocytes, specifically monocytes, which are implicated in plaque formation.(3) PLWH may have underlying traditional risk factors and are more likely to use tobacco.(4) Although suppression of viral replication generally protects against CVD, certain ART drugs including the protease inhibitors (PI) cause disorders in lipid metabolism.

CVD risk assessment tools, validated in the general population, such as the Framingham risk score convert clinical risk factors into a summary estimate of the likelihood of a CVD event over a specified period. The scores can underestimate the individual patient risk (5) and may have limited utility in PLWH as HIV-specific risk factors including the use of PI and viral burden, are not always included.

Various biomarkers have been assessed in CVD risk evaluation. In PLWH, inflammatory changes (cytokines and leukocyte activation), changes in coagulation proteins, presence of oxidized lipids and adhesion markers have been measured to identify a robust predictive model.(6) Lipoprotein-associated phospholipase A2 (Lp-PLA_2), an enzyme secreted by leukocytes and liver cells, circulates primarily complexed to low-density lipoprotein (LDL).(7) Lp-PLA_2 hydrolyses phospholipids to produce metabolically active lipid mediators including proinflammatory free fatty acids. These products activate platelets and recruit T-cells and monocytes, key effectors of atherosclerotic plaque formation. Lp-PLA_2 is over-expressed in macrophages in the fibrous cap of unstable atherosclerotic coronary lesions.(8)

Lp-PLA_2 has been extensively evaluated in clinical cohorts, correlates with plaque formation and CVD risk, and identifies patients at risk for recurrent cardiovascular events.(8) Lp-PLA_2 was incorporated into the American Association of Clinical Endocrinologists/American College of Endocrinology Guidelines for management of dyslipidaemia and prevention of CVD as a non-traditional risk factor. CVD risk is elevated in patients with raised Lp-PLA_2 and C-reactive protein levels, even in the presence of a moderately increased LDL.(9) Studies suggest PLWH have higher Lp-PLA_2 levels than uninfected patients, independent of triglyceride and LDL levels. The elevated levels

persist with viral suppression. It may also guide both ART regimen choice (tenofovir may reduce Lp-PLA_2 levels) and use of ancillary risk modifiers like statins. Most studies measured Lp-PLA_2 levels in patients with controlled HIV viraemia. (10)

The aim of this study was to investigate Lp-PLA_2 levels of HIV-infected patients (ART naïve and treated) enrolled in large cross-sectional study in South Africa and to investigate relationships between this marker, other measures of CVD risk and markers of viral control.

Methods

Patient recruitment

The study was approved by the Human Research Ethics Committee (Medical) of the University of the Witwatersrand (Protocol number M160131). 98 patients, enrolled in a larger study to evaluate CVD risk in PLWH in Johannesburg, South Africa from 2016-17, were included. 27 HIV-infected, ART-naïve participants and 22 HIV-infected participants on stable protease inhibitor (PI)-containing ART were compared with 49 age- and sex-matched uninfected controls. Participants were considered ART-naïve if they had received no ART or ART for less than 6 weeks. The ART-treated participants, treated with a PI-containing regimen for at least 48 weeks, showed viral suppression. Demographic and cardiovascular risk factors were collected using a modified version of the WHO STEPS instrument.(11) Blood was collected for measurement of fasting glucose and lipid levels and Lp-PLA_2. Physical examination included measurement of height, weight, waist and hip circumference and blood pressure. The Framingham risk score (FRS) was computed to estimate a cumulative 10-year CVD risk. Left and right carotid intima-medial thickness (CIMT) was measured sonographically at 3 standardized angles using the Meijer's Arc. Measurements were semi-automated but manually corrected if required.

Plasma Lp-PLA_2 levels were measured on the Roche® Cobas analyser using an enzymatic assay (Diazyme Laboratories®, USA).

Statistical analysis

Because of the small number of participants per group continuous variables were expressed using median with minimum and maximum values or count with percentage. Baseline variables across the three groups were compared using a Kruskal Wallis test for continuous outcomes and a chi square test for categorical outcomes. LP-PLA_2 was correlated to age, BMI, waist-to-hip circumference, lipid sub-components and glucose using a Pearson's correlation coefficient.

In a multivariable regression LP-PLA_2 levels in the three groups wasadjusted for age, gender, LDL levels, body mass index (BMI) and smoking. The relationship between Lp-PLA_2 levels and FRS or CIMT was examined in a multivariable regression considering HIV and ART status. Finally, the relationship between LP-PLA_2 levels of all viraemic participants and log-viral load and CD4-cell count were analysed using a Pearsons correlation coefficient. A p-value of 0.05 was considered significant. Statistical analyses were performed using IBM SPSS Statistics Version 25™ (SPSS, Chicago, Illinois, USA).

Results

The baseline characteristics for the three participant groups are included in table 1. As expected, patients on PI treatment had significantly higher LDL, triglycerides and total cholesterol levels. Lp-PLA_2 levels were increased in patients on PI-containing ART regimens (median of 150.5 versus 127.0, p=0.05). Lp-PLA_2 was correlated to age (r=0.25, p <0.001), BMI (r=0.73, p=0.04), total cholesterol (r=0.21, p=0.04) and LDL-cholesterol (r=0.40, p<0.001).

The significant higher levels of Lp-PLA_2 for participants on ART did not persist when LDL levels were considered. Lp-PLA_2 was not related to FRS or CIMT in any of the groups. Lp-PLA_2 did, however, show a strong correlation with viral load (r=0.323, p=0.025). When patients with undetectable viraemia were excluded, the correlation became more pronounced (r=0.653, p<0.001). There was an inverse relationship with CD4+ T cell count, but this reached only significance in the virally unsuppressed participants (r=-0.727, p<0.001).

Table 1. Participant characteristics.

	HIV negative (n=49)	HIV positive ART naïve (n=27)	HIV positive PI ART (n=22)	*p*
Female (n,%)	24 (49.0)	14 (51.9)	12 (54.5)	0.91
Age (years)	38.9 (25.1– 64.3)	38.9 (24.2 – 56.5)	40.5 (26.5 – 57.7)	0.97
University education (n,%)	7 (14.3)	2 (7.9)	2 (9.1)	0.62
Stable relationship (n,%)	16 (33.3)	3 (11.1)	12 (54.4)	0.005
Employed (n,%)	18 (36.7)	15 (55.6)	14 (63.6)	0.07
Current smoking (n,%)	12 (25.0)	8 (29.6)	2 (9.1)	0.20
Systolic BP (mmHg)	123.5 (99.5–190.0)	119.5 (87.5–164)	120.5 (91.5–161.0)	0.35
Diastolic BP (mmHg)	78.5 (81.5)	74.5 (49.5)	83.0 (51.5)	0.26

Table 1. Continued

	HIV negative (n=49)	HIV positive ART naïve (n=27)	HIV positive PI ART (n=22)	*p*
BMI (kg/m^2)	25.7 (17.0 – 47.2)	23.7 (17.5 – 35.0)	25.0 (18.7 – 42.1)	0.49
Waist to hip ratio	0.88 (0.69 – 1.00)	0.88 (0.76 – 0.98)	0.88 (0.62 – 0.97)	0.82
CD4 (cells/mm3)	-	276 (15 - 532)	480 (121 - 997)	<0.01
Log_VL (cp/ml) (median, IQR)	-	9.53 (3.66– 14.15)	3.66 (3.66 – 8.72)	<0.01
Total cholesterol (mmol/L)	4.32 (3.60 – 6.40)	3.66 (2.50 – 7.40)	4.43 (3.60 – 6.40)	0.002
HDL cholesterol (mmol/L)	1.32 (0.90 – 3.20)	1.18 (0.60 – 3.90)	1.29 (0.80 - 1.80)	0.56
LDL cholesterol (mmol/L)	2.23 (0.60 – 4.40)	2.16 (1.10 – 3.40)	2.74 (1.80 – 4.70)	0.02
Triglycerides (mmol/L)	1.04 (0.50 – 3.70)	0.97 (0.60 – 1.90)	1.58 (0.80 – 4.90)	0.006
Random glucose (mmol/l)	4.85 (2.60 – 9.60)	4.40 (3.40 – 7.80)	4.50 (4.30 – 9.4)	0.033
Framingham risk score (%)	3.17 (0.59 – 40.99)	2.50 (0.51 – 13.52)	2.73 (0.51 – 22.37)	0.77
Mean CCA (mm)	0.514 (0.447–1.024)	0.512 (0.446 – 0.758)	0.547 (0.477 – 0.738)	0.58
LpPLA2 (IU/L)	127.0 (52.0– 196.0)	127.0 (62.0– 17.0)	150.5 (47.0 - 219)	0.05

All outcomes are in median with minimum and maximum values, unless otherwise specified. ART, antiretroviral therapy; BP, blood pressure; BMI, body mass index; CCA, common carotid artery; HDL, high density lipoprotein; LDL, low density lipoprotein.

Discussion and conclusion

Lp-PLA$_2$ is a phospholipase which complexes with LDL and catalyses the release of proinflammatory lipid mediators. It has been linked to CVD risk (plaque formation and instability) specifically in patients with underlying inflammatory disease. Studies, generally performed on patients with controlled viraemia have demonstrated elevated levels of Lp-PLA$_2$.

In this study, we assessed Lp-PLA$_2$ utility when compared with traditional CVD risk factors and also in patients both on a PI regimen and ART naïve. Lp-PLA$_2$ were increased in HIV infected participants in this cross-sectional study although only significantly

in patients on a PI. This increase reflected LDL levels and may indicate the generally dyslipidaemia associated with PI -containing drug regimens. Lp-PLA_2 correlated with LDL and total cholesterol which are traditional risk factors for atherosclerosis although there was no convincing correlation with the Framingham risk score of More importantly, Lp-PLA_2 correlated strongly with viral load and CD4+ cell count, specifically in those patients with detectable viraemia. This suggests that inflammation linked to the virus may be driving increased levels of Lp-PLA_2 and provides a link between active viraemia and the mechanistic development of CVD in these patients.

This study has a number of limitations. We did not access patients who were not on a PI-containing regimen which may explain the discrepancies of our findings and other studies looking at Lp-PLA_2 in HIV-infected patients. In addition, this study was cross-sectional and it would be useful to examine the patients with increased Lp-PLA_2 levels longitudinally to assess the contribution to CVD risk. Finally, we did not include measurement of hs-CRP which would have been an important indicator of inflammation. Despite these limitations, we are of the opinion that this enzyme may drive atherosclerosis in HIV-infected patients and its utility in clinical care should be investigated in prospective studies.

References

1. Hyle EP, Mayosi BM, Middelkoop K, Mosepele M, Martey EB, Walensky RP, et al. The association between HIV and atherosclerotic cardiovascular disease in sub-Saharan Africa: a systematic review. BMC Public Health. 2017;17(1):954.
2. Olmo M, Saumoy M, Alonso-Villaverde C, Penaranda M, Gutierrez F, Romeu J, et al. Impact of antiretroviral therapy interruption on plasma biomarkers of cardiovascular risk and lipids: 144-week final data from the STOPAR study. HIV Med. 2012;13(8):488-98.
3. Pinto DSM, da Silva M. Cardiovascular Disease in the Setting of Human Immunodeficiency Virus Infection. Curr Cardiol Rev. 2018;14(1):25-41.
4. Younas M, Psomas C, Reynes J, Corbeau P. Immune activation in the course of HIV-1 infection: Causes, phenotypes and persistence under therapy. HIV Med. 2016;17(2):89-105.
5. Karmali KN, Lloyd-Jones DM. Implementing Cardiovascular Risk Prediction in Clinical Practice: The Future Is Now. J Am Heart Assoc. 2017;6(4).
6. Viskovic K, Zidovec-Lepej S, Gorenec L, Grgic I, Lukas D, Zekan S, et al. Cardiovascular markers of inflammation and serum lipid levels in HIV-infected patients with undetectable viraemia. J Int AIDS Soc. 2014;17(4 Suppl 3):19548.
7. Kim JY, Hyun YJ, Jang Y, Lee BK, Chae JS, Kim SE, et al. Lipoprotein-associated phospholipase A2 activity is associated with coronary artery disease and markers of oxidative stress: a case-control study. Am J Clin Nutr. 2008;88(3):630-7.
8. Goncalves I, Edsfeldt A, Ko NY, Grufman H, Berg K, Bjorkbacka H, et al. Evidence supporting a key role of Lp-PLA2-generated lysophosphatidylcholine in human atherosclerotic plaque inflammation. Arterioscler Thromb Vasc Biol. 2012;32(6):1505-12.
9. Jellinger PS, Handelsman Y, Rosenblit PD, Bloomgarden ZT, Fonseca VA, Garber AJ, et al. AMERICAN ASSOCIATION OF CLINICAL ENDOCRINOLOGISTS AND AMERICAN COLLEGE OF ENDOCRINOLOGY GUIDELINES FOR MANAGEMENT OF DYSLIPIDEMIA AND PREVENTION OF CARDIOVASCULAR DISEASE - EXECUTIVE SUMMARYComplete Appendix to Guidelines available at http://journals.aace.com. Endocr Pract. 2017;23(4):479-97.
10. Eckard AR, Jiang Y, Debanne SM, Funderburg NT, McComsey GA. Effect of 24 weeks of statin therapy on systemic and vascular inflammation in HIV-infected subjects receiving antiretroviral therapy. J Infect Dis. 2014;209(8):1156-64.
11. Ng N, Van Minh H, Tesfaye F, Bonita R, Byass P, Stenlund H, et al. Combining risk factors and demographic surveillance: potentials of WHO STEPS and INDEPTH methodologies for assessing epidemiological transition. Scand J Public Health. 2006;34(2):199-208.

531207-L-bw-Vos
Processed on: 20-5-2019

CHAPTER

5

HIV and risk of cardiovascular disease in sub-Saharan Africa. Rationale and design of the Ndlovu Cohort Study

A.G. Vos, H. Tempelman, W. Devillé, R. Barth, A. Wensing, M. Kretzschmar, K. Klipstein-Grobusch, A. Hoepelman, K. Tesselaar, S. Aitken, M. Madzivhandila, C. Uiterwaal, F. Venter, R. Coutinho, D. E. Grobbee

Eur J Prev Cardiol. 2017 Jul;24(10)

Abstract

Background The largest proportion of people living with HIV resides in sub-Saharan Africa (SSA). Evidence from developed countries suggests that HIV infection increases the relative risk of cardiovascular disease (CVD) up to 50%. Differences in lifestyle, gender distribution, routes of HIV transmission and HIV virus subtype preclude generalisation of data from Western countries to the SSA situation. The Ndlovu Cohort Study aims to provide insight in the burden of cardiovascular risk factors and disease, mechanisms driving CVD risk, and the contribution of HIV infection and its treatment to the development of CVD in a rural area in SSA.

Design The Ndlovu Cohort Study is a prospective study in the Moutse area, Limpopo Province, South Africa.

Methods 1,000 HIV-positive and 1,000 HIV-negative participants aged 18 years and older with a male to female ratio of 1:1 will be recruited. Measurements on CVD risk factors and HIV related characteristics are performed at baseline, and participants are followed-up over time at six month intervals. Burden of CVD is assessed with repeated carotid intima-media thickness (CIMT) - and pulse wave velocity (PWV) measurements, and by recording clinical cardiovascular events that occur during the follow-up period.

Conclusion This project will contribute to the understanding of the epidemiology and pathogenesis of CVD in the context of HIV infection in a rural SSA area. The ultimate goal is to improve cardiovascular risk prediction and to indicate preventive approaches in the HIV-infected population and, potentially, for non-infected high-risk populations in a low resource setting.

Background and rationale

Currently more than 35 million people are estimated to be living with human immunodeficiency virus (HIV). Nearly 71% of the global total, 24.7 million, reside in sub-Saharan Africa (SSA), where the majority of people living with HIV are women (58% of all people living with a HIV infection)[1]. Globally, combination antiretroviral treatment (cART) has been initiated in approximately 16 million people living with HIV[2] and, as a result, HIV has become a chronic disease that for many patients will span several decades of their lives[3]. Persistent immune activation and inflammation in treated HIV infection, however, appear to be associated with an 'accentuated' increased relative risk of cardiovascular disease (CVD) of up to 50% and an earlier 'accelerated' onset of CVD compared to HIV-negative individuals[4-7].

Yet our understanding of the mechanisms driving CVD in HIV infection remains limited. The exact role of HIV infection, HIV treatment, and their relation with conventional risk factors still have to be determined[8-10]. In addition, it is unclear whether and how differential levels of viremia, immune activation and cardiovascular disease are related with the HIV-1 subtype[11]. Several factors are hypothesised to contribute to an accelerated development of CVD amongst HIV-infected individuals. Those include: 1) the host response to HIV-infection, which results in chronic immune activation and inflammation; 2) conventional CVD risk factors, potentially amplified by adverse effects of HIV treatment, 3) HIV treatment, with some antiretroviral drugs particularly being linked to an increased risk of CVD, and 4) HIV-induced metabolic effects. A summary of potential mechanisms is given in figure 1[6].

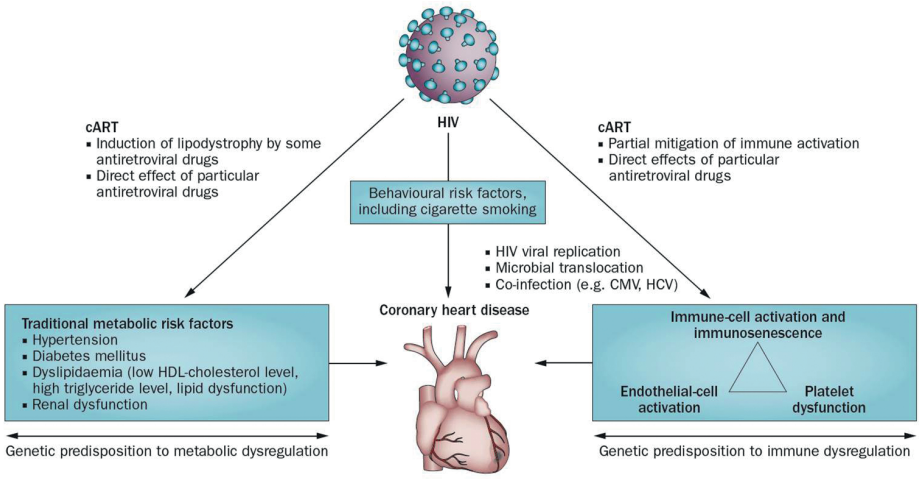


Figure 1. Mechanisms and risk factors postulated to be involved with an increased risk of coronary heart disease risk in patients with HIV.

Legend: HIV: human immune deficiency virus, cART: combination antiretroviral therapy, CMV: cytomegaly virus, HCV: herpes simplex virus. (Reproduced with permission from Zanni MV *et al.* (2014) Risk of coronary heart disease in patients with HIV infection *Nat. Rev. Cardiol.* doi:10.1038/nrcardio.2014.167).

As data from the SSA continent is scarce, especially longitudinal data, it is currently not clear whether an amplification of the CVD epidemic due to HIV infection is to be expected. Several cross-sectional studies in SSA addressing cardiovascular (CV) risk in HIV infected individuals did not indicate an increase in CV risk profile as a result of HIV[12,13], although available studies have mainly included HIV-positive patients and not added an HIV-negative control group for proper comparison[4].

The Ndlovu Cohort Study has been set-up in a rural area in South Africa to address the above mentioned issues for the SSA region. This prospective study will include both HIV-positive and HIV-negative individuals. As the aim is to provide a comprehensive understanding of the interaction between HIV and CVD, the problem will be approached in a multidisciplinary manner. The impact of an HIV infection on social and mental health in both HIV infected participants and the community will be evaluated and virological characteristics like resistance and therapy failure will be identified. The ultimate aim is to improve cardiovascular risk prediction and identify potential novel, cost effective, preventive approaches in the HIV-infected population. These may also be applicable for non-infected high risk populations in a low resource setting.

Methods

The Ndlovu Cohort Study is an initiative of the Ndlovu Research Consortium, formed by the Wits Reproductive Health and HIV Institute (Wits RHI),University of the Witwatersrand, South Africa, Utrecht University, the Netherlands, including the Faculty of Social Sciences and the University Medical Center Utrecht (department of Infectious Diseases, Immunology, Clinical Epidemiology, Public Health), and the Ndlovu Care Group,Limpopo, South Africa

Study setting

The Ndlovu Cohort study is conducted in Elandsdoorn, a rural township in the Moutse area, Limpopo Province, South Africa. Dedicated research facilities are based at the Ndlovu Care Group (NCG, www.Ndlovucaregroup.com), a non-governmental organisation advancing rural communities with Healthcare, Childcare, Community Development and Research Programs.

Study population

This prospective cohort study will recruit 1,000 HIV uninfected and 1,000 HIV infected participants and intends to include an equal proportion of men and women. Within the HIV infected group, the aim is to include 20% antiretroviral therapy naïve individuals with a CD4 count >100 cells/mm^3. Eligible individuals are 1) aged 18 years or over, 2) living within a range of 30 km around the NMC, 3) able to provide written, informed consent, 4) committed to long-term follow-up. Individuals unable to undergo the study procedures for any reason will be excluded.

Inclusion

The study aims to include a population that represents a typical rural South African district. Participants will be recruited through a community liaison officer and a team of counsellors at the Ndlovu Medical Centre HIV clinic, local events, in shopping areas and through community campaigns. Based on literature, it is estimated that 3.9% of HIV negative participants will become infected with HIV and start cART within a follow-up period of three years[14].

HIV testing

HIV negative participants or participants with an unknown status will undergo HIV testing at enrolment in the study and at yearly follow-up visits. Testing is performed with an antibody-based point of care test (ADVANCED QUALITY™ Rapid HIV Test (InTec Products, Xiamen, China)), which has a sensitivity of 98.8% and a specificity of 100%[15]. Specimens that test positive will be retested with a second point-of-care test (ABON™ HIV 1/2/O Tri-Line HIV Rapid Test Device (ABON Biopharm Hangzhou, China)), which has a sensitivity of 100% and specificity of 97.7%[16]. An enzyme-linked immunosorbent

assay (ELISA) will be performed to clarify any indeterminate results and confirm positive results.

Participants with a confirmed HIV-positive status will be recruited from the Ndlovu Medical Centre or from outreach testing programs. Documentation of a positive HIV test result is needed for all patients to be eligible for enrolment as HIV-positive participants in the study. HIV testing will be conducted to confirm HIV status according to the procedure described above in case of any doubt.

Ethics approval

Study approval was obtained from the Human Research Ethics Committee at the University of Pretoria, Pretoria, South Africa, and the Limpopo Department of Health Ethics Committee and written informed consent is obtained from all participants prior to study participation.

Sample size

Calculations are based on carotid intima media thickness (CIMT) using a mixed model approach. A simplified approach was used to evaluate power to detect very small differences between groups as we did not have reasonable estimations for CIMT progression over time. Power was evaluated for a given sample size of 1000 HIV-positive and 1000 HIV- patients for a significance level of 0.05, a constant difference in mean CIMT over time of 0.006, 0.012, 0.018 and 0.024mm for an increasing correlation between measurements in time from 0.00 to 0.75, and a standard deviation of 0.09mm. A minor difference of 0.012 is still detectable with 95% power and a correlation of 0.60. For larger differences, the power exceeds 0.95 even with a correlation of 0.75. This sample size can also detect meaningful differences in other outcomes as prevalence of cardiovascular risk factors and pulse wave velocity (PWV).

Data analysis

Prevalence of the cardiovascular risk factors will be described and compared between various (sub)groups within the study population (HIV infected versus – uninfected, whether or not on ART, various CD4 levels and ART regimens). Continuous variables will be summarised by medians with interquartile range, or means with standard deviations. Categorical variables will be summarised by frequency counts and percentages. 95% confidence intervals will be calculated for incidence rates as applicable. Continuous data will be compared with the Student's t-test, Wilcoxon rank sum test or the Mann-Whitney U test. The distribution differences of categorical variables will be compared with the chi-square test. The CVD risk factor burden will be calculated using the Framingham risk equation, D:A:D score and Reynold score. The prognosis (expressed as a percentage) of having a CVD event in the next 10 years will be reported. The distribution of CIMT and PWV among the whole population will be determined. The progression of CIMT and

PWV over time will be analyzed with a linear mixed model with a random intercept, a random effect for time and other covariates such as age, gender, cardiovascular risk factors and baseline CIMT or PWV. Multiple imputations will be used for missing data, presuming a random distribution.

Data collection

General Characteristics

In-/exclusion criteria will be checked and a signed Informed Consent will be obtained. Information will be collected on age, gender, demographics, general health and HIV-testing behaviour. A full medical history, current medical condition(s) and chronic medication use is obtained. Detailed information on HIV treatment (time between diagnosis and treatment initiation, and specific medication prescribed) and response to treatment (latest plasma HIV-1 viremia, latest CD4+ T-cell count) will be recorded. Information on cardiovascular risk factors, family history, smoking and alcohol use is obtained with a modified version of the WHO STEPs instrument[17]. Physical activity is assessed with the International Physical Activity Questionnaire (IPAQ)[18]. The Patient Health Questionnaire-9 (PHQ-9) is used as a screening tool on depression and anxiety[19]. (Table 1. Schedule of assessments at baseline and follow-up).

Table 1. Schedule of assessments at base line and follow-up.

Baseline	Every 6 month follow-up	Every 12 month follow-up
Baseline questionnaire: - Demographics - Income - Civil status - Mental health - Physical activity - Smoking, alcohol - Food security	Telephone questionnaire - Civil status - Employment - Mental health - Smoking, alcohol	Follow-up questionnaire - Civil status - Employment - Mental health - Smoking, alcohol
Physical examination: - Blood pressure - Weight, height - Waist & hip circumference - CIMT ultrasound		Physical examination - Blood pressure - Weight, height - Waist & hip circumference - PWV or CIMT ultrasound (alternating)
Medical history: - Physical health - Family history - Chronic medication - Tuberculosis questionnaire - HIV-status and cART	Medical history: - Physical health - Chronic medicatio	Medical history: - Physical health - Chronic medication - Tuberculosis questionnaire - HIV-status and cART
Biological examination: - Full blood count, - CD4 cells, viral load - Lipids - Blood glucose, HbA1C - CRP - Urine microalbumin & creatinine		Biological examination: - HIV test (in case of a HIV-negative participant) - Full blood count - CD4 cells, viral load - Lipids - Blood glucose, HbA1C - CRP - Urine microalbumin & creatinine
Blood sample collection for storage: - 1 EDTA tube and 1 Heparin tube (plasma stored at -80^{o}C) - 2 extra Heparin tubes for PBMC isolation (only for HIV+ not on cART; every 3 months for 2yrs) (PBMCs are stored at -120^{o}C)	- Blood sample collection for storage only for HIV+ not on cART: 1 EDTA tube (plasma stored at -80^{o}C) - 2 extra Heparin tubes for PBMC isolation (every 3 months for 2yrs) (PBMCs are stored at -120^{o})	Blood sample collection for storage: - 1 EDTA tube and 1 Heparin tube (plasma stored at -80^{o}C -) - 2 extra Heparin tubes for PBMC isolation (only for HIV+ not on cART; every 3 months for 2yrs) (PBMCs are stored at -120^{o}C)

Legend: CIMT, carotid intima media thickness, PWV, pulse wave velocity, HIV, human immunodeficiency virus, cART, combination antiretroviral therapy, CRP, c-reactive protein, PBMC, peripheral blood mononuclear cell

Social and psychological aspects

The following topics are covered in (validated) questionnaires: employment, income position and household support (NIDS Wave 3 2012 Adults Questionnaire), food security and diet (SANHANES), impact of stigma[20], opinions about HIV testing[21], sexual history in the past 12 months and social support and quality of life (WHO quality of life, brief version). Data on adherence is collected with a structured questionnaire in case of HIV infection.

Physical measurements

Information is collected on height, weight, hip and waist circumference. Hip circumference is measured in centimetres at maximum posterior extension of the buttocks, waist circumference halfway between the lower rib and the iliac crest during expiration, both in standing position. Blood pressure is measured in seated position after five minutes of rest, with a sphygmomanometric device

Biological material

Blood and urine samples

Approximately 50 ml of blood will be collected during the baseline visit. This will comprise 2 EDTA tubes, 1 SST tube, 1 fluoride oxalate tube and 1 heparin tube. Full blood count (white cell count, red cell count, haemoglobin, haematocrit, mean cell volume, platelets), total cholesterol, high density lipoprotein cholesterol (HDL), low density lipoprotein cholesterol (LDL), triglycerides, random glucose, HbA1c and C-reactive protein (CRP) are measured in all individuals. Viral load and CD4 cell count are measured in case of HIV infection. In a subgroup consisting of all newly diagnosed HIV-positive, ART-naïve patients (n = 200) EDTA plasma and peripheral blood mononuclear cells (PBMCs) are stored for ultrasensitive HIV-RNA and DNA analysis, co-receptor expression, drug resistance analysis and drug level measurements. A urine sample will be collected during the baseline visit for measurement of urine creatinine, microalbumin and albumin/creatinine ratio.

Blood storage and biobanking

Plasma from blood collected in one of the EDTA tubes and in the heparin tube will be stored in a -80°C freezer at NMC for future research purposes. From the sub-group of 200 HIV-positive but therapy naïve patients at baseline, two extra sodium heparin tubes (10 ml each) are drawn for isolation of PBMCs for viro-immunological studies. PBMCs are isolated in the NMC laboratory and stored at -120˚ C.

Cardiovascular measurements

Carotid Intima-Media Thickness

To estimate the presence of subclinical atherosclerotic disease the thickness of the carotid artery intima-media is measured using a standardised ultrasonographic protocol (Details in Appendix 1). CIMT is a well-established, continuous, quantitative measure of atherosclerosis, and is able to identify increased cardiovascular risk in both black and white populations[22-24]. Ultrasound measurements of the CIMT are performed by trained nurses at the Ndlovu Research Unit. Quality assurance processes include regular performance reviews and training.

All images will be read in a batch fashion after completion of scanning per study period (e.g. after completion of baseline scans, 2nd year follow up scans). The reader will be blinded for the HIV status of the participant. Mean and maximum thickness will be measured semi-automatically with the Artery Measurement System software (Chalmers University, Götenburg, Sweden) with a uniform reading protocol that ensures standardised settings across reading stations.

Pulse wave velocity

To get insight in central vascular function, a PWV measurement will be taken by trained nurses using a SphygmoCor device (AtCor Medical), starting from the first year follow-up visit (Details in appendix 2). PWV is regarded as the gold standard for measuring aortic stiffness; an increase was found to be an independent risk factor for cardiovascular disease[25,26]. Carotid-femoral PWV is calculated by dividing travelled distance by transit time. The direct carotid-femoral distance will be multiplied by 0.8, and a cut off value of 10m/s will be used to define abnormal PWV[27].

Repeat measurements and follow-up

Data will be obtained on mortality, prevalent and incident cases of CVD morbidity, and hospitalisations. Clinical manifestations of CVD considered in the study include acute myocardial infarction, revascularizations, symptomatic heart failure and stroke. Comorbidity and non-cardiovascular outcomes are measured by recording non-cardiovascular related hospitalisations and mortality. All participants with abnormal findings (for example hypertension) will be referred to the Ndlovu Medical Center or to a primary healthcare clinic for further analysis. Information on treatment will be updated at every follow up visit (Table 1).

Telephone follow up

Six months after the baseline visit and yearly thereafter (month 6, month 18, month 30 and so on), information on the current/ongoing medical condition(s), medical conditions

during the past six months and medication use, lifestyle factors (e.g., alcohol usage and smoking habits, and cART adherence for HIV-positives on cART) is collected during phone interviews with participants.

Follow up visit at the research center

Participants are invited for annual (month 12, month 24, and month 36 and yearly thereafter following baseline) visits to the research center for follow-up questionnaires, measurements, and blood sampling in line with baseline assessment. (See Table 1 for a listing of data collected during follow-up). Blood drawing is intensified for the group of 200 HIV positive, ART naïve participants at baseline; one EDTA tube and two sodium heparin tubes (10 ml blood each) will be drawn quarterly during the first two years after enrolment for storage and isolation of PBMCs. Both CIMT measurement and PWV measurement will be performed bi-annually, in alternating sequence.

Intended analysis on stored blood samples

Future analysis will focus on prospective analysis of immune markers representing different pathways of immune dysfunction and immune activation, in relation to CIMT, PWV and overt cardiovascular outcomes. Examples are interleukin-6, CD163, d-dimer and sVCAM-1[28,29].

Current state of the art

Enrolment started in November 2014 and will be finished by December 2016. The intended follow up duration is 10 years.

Discussion

Life expectancy is rising in the HIV infected population as a result of increasing ART coverage. Consequently, the risk of experiencing non-communicable diseases, such as CVD, leading to substantial morbidity and mortality amongst HIV infected people increases.

So far, data on CVD risk in HIV-infected populations in SSA is scarce. Existing studies are limited by size, retrospective character or a single focus on risk factors, without any observations on the prevalence of cardiovascular end points or disease. Moreover, an HIV-negative control group is generally lacking. Current literature suggests a different cardiovascular risk profile in HIV-infected individuals compared to non-HIV infected individuals in SSA, but does not clarify to which extent HIV or HIV-related factors influence the cardiovascular risk profile and the occurrence of CVD[4].

Strengths

The Ndlovu Cohort Study is designed to address CVD risk in relation to HIV infection in a rural SSA population. The Moutse area is an ideal setting to perform a cohort study as it is a typical rural area. Lifestyle, environmental characteristics, gender distribution (more females than males infected) and the economic status with extreme poverty, are representative for the situation in resource limited settings, making the results generalizable to other rural districts in Sub-Sahara Africa.

The affiliation with Ndlovu Care Group (NGC) provides an excellent setting to establish and follow a cohort given its long-term relation with the local community since 1994. A major advantage of the cohort is the inclusion of both HIV-positive and HIV-negative individuals, making it possible to investigate the cardiovascular risk profile of the rural population in general, as well as the additional effects of HIV and HIV treatment. As the intended duration of follow up is several years, the study will not only provide data on prevalence of cardiovascular risk factors, but also data on cardiovascular endpoints. In the meantime, CIMT and PWV will be used as surrogate markers to get insight into the risk of CVD. The multidisciplinary approach of the Ndlovu Cohort Study integrating clinical and translational medicine, (clinical) epidemiology, infectious diseases, virology, immunology, pharmacology, social sciences, mathematical modelling and public health will combine efforts to gain new insights into pathogenesis, prevention and risk behaviour for both HIV and CVD.

Limitations

Unemployment rates in the Moutse area are up to 50%, which may result in loss to follow up, especially for men, due to labour migration. However, drop out is expected to be randomly distributed and multiple imputation will be used to account for missing data. We used a simplified approach to estimate the power given the number of patients to be included. Even though the analysis planned will require additional degrees of freedom compared to the power analysis, we offset this by analyzing power for a very small difference for increasing correlations between time measurements of CIMT. Another limitation is the relatively young age of the population, limiting the number of expected CVD events. Moreover, survival of endpoints may hamper further examinations (for example disability after a stroke, which makes a visit to the clinic impossible). Effort will be made to trace all participants to keep follow up rates as high as possible, and to document reasons in case of loss to follow up. This will be done by telephone calls, home visits when needed and community engagement.

Conclusion

The Ndlovu Cohort Study is designed to investigate cardiovascular risk profiles and the occurrence of CVD in a rural population in SSA where HIV infection is prevalent, more females than males are infected and the predominant HIV subtype is subtype C. The multidisciplinary approach will foster new insights into prevalence of risk factors, risk behaviour, the role of HIV and ART in the pathogenesis of CVD and the occurrence of cardiovascular endpoints. The ultimate goal is improvement of risk prediction and development of targeted prevention and treatment strategies to provide integrated care for HIV and CVD.

Author contributions

HT, WD, RB, AW, MK, KKG, AH, KT, CU, FV, RC and DG contributed to the conception or design of the work. AV, SA, MM contributed to the acquisition, analysis, or interpretation of data for the work. AV, WD, KKG and DG drafted the manuscript. HT, RB, AW, MK, AH, FV and RC critically revised the manuscript. All gave final approval and agree to be accountable for all aspects of work ensuring integrity and accuracy.

Acknowledgements

Acknowledgments go to the community liaison officer, Bhuti Nyathi; counsellors Stephina Rakghoto, Neo Mojapelo, Jotham Dhladhla, and nurses Maphoko Molepo and Elizabeth Moima Peter Zuithoff, statistician, the funders (Dioraphte Foundation, AIDS Fund, De Grote Onderneming, University Medical Center Utrecht, University of Utrecht), and all the study participants.

Funding acknowledgments

The Ndlovu Cohort Study is funded by grants from the Dutch AIDS Foundation, Dioraphte Foundation, De Grote Onderneming and the University Medical Center Utrecht (UMCU).

Declaration of Conflicting Interests

The Authors declare that there is no conflict of interest.

References

1. UNAIDS. The Gap Report. . 2014. Available: http://www.unaids.org/en/resources/documents/2014/20140716_UNAIDS_gap_report.
2. World Health Organisation. Estimated numbers of people receiving antiretroviral therapy globally and by WHO region and percentage coverage globally, 2000-2015. . 2015: https://www.who.int/hiv/data/art_2003_2015.png.
3. Deeks SG, Lewin SR, Havlir DV. The end of AIDS: HIV infection as a chronic disease. Lancet. 2013;382: 1525-1533. doi: 10.1016/S0140-6736(13)61809-7 [doi].
4. Dillon DG, Gurdasani D, Riha J, Ekoru K, Asiki G, Mayanja BN, et al. Association of HIV and ART with cardiometabolic traits in sub-Saharan Africa: a systematic review and meta-analysis. Int J Epidemiol. 2013;42: 1754-1771. doi: 10.1093/ije/dyt198 [doi].
5. Freiberg MS, Chang CC, Kuller LH, Skanderson M, Lowy E, Kraemer KL, et al. HIV infection and the risk of acute myocardial infarction. JAMA Intern Med. 2013;173: 614-622. doi: 10.1001/jamainternmed.2013.3728 [doi].
6. Zanni MV, Schouten J, Grinspoon SK, Reiss P. Risk of coronary heart disease in patients with HIV infection. Nat Rev Cardiol. 2014;11: 728-741. doi: 10.1038/nrcardio.2014.167 [doi].
7. 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;92: 2506-2512. doi: jc.2006-2190 [pii].
8. Boccara F, Lang S, Meuleman C, Ederhy S, Mary-Krause M, Costagliola D, et al. HIV and coronary heart disease: time for a better understanding. J Am Coll Cardiol. 2013;61: 511-523. doi: 10.1016/j.jacc.2012.06.063 [doi].
9. Baker JV, Lundgren JD. Cardiovascular implications from untreated human immunodeficiency virus infection. Eur Heart J. 2011;32: 945-951. doi: 10.1093/eurheartj/ehq483 [doi].
10. Lang S, Mary-Krause M, Simon A, Partisani M, Gilquin J, Cotte L, et al. HIV replication and immune status are independent predictors of the risk of myocardial infarction in HIV-infected individuals. Clin Infect Dis. 2012;55: 600-607. doi: cis489 [pii].
11. Buonaguro L, Tornesello ML, Buonaguro FM. Human immunodeficiency virus type 1 subtype distribution in the worldwide epidemic: pathogenetic and therapeutic implications. J Virol. 2007;81: 10209-10219. doi: JVI.00872-07 [pii].
12. Schoffelen AF, de Groot E, Tempelman HA, Visseren FL, Hoepelman AI, Barth RE. Carotid Intima Media Thickness in Mainly Female HIV-Infected Subjects in Rural South Africa: Association With Cardiovascular but Not HIV-Related Factors. Clin Infect Dis. 2015;61: 1606-1614. doi: 10.1093/cid/civ586 [doi].

13. Clark SJ, Gomez-Olive FX, Houle B, Thorogood M, Klipstein-Grobusch K, Angotti N, et al. Cardiometabolic disease risk and HIV status in rural South Africa: establishing a baseline. BMC Public Health. 2015;15: 135-015-1467-1. doi: 10.1186/s12889-015-1467-1 [doi].
14. Rehle TM, Hallett TB, Shisana O, Pillay-van Wyk V, Zuma K, Carrara H, et al. A decline in new HIV infections in South Africa: estimating HIV incidence from three national HIV surveys in 2002, 2005 and 2008. PLoS One. 2010;5: e11094. doi: 10.1371/journal.pone.0011094 [doi].
15. World Health Organisation. HIV assays: operational characteristics (phase I). Report 12. Simple/rapid tests whole blood specimens. . : 17 June 2016.
16. World Health Organisation. Prequalification of Diagnostics Programme PUBLIC REPORT. Geneva, September 2014, version 2.0. 17 June 2016.
17. World Health Organisation. WHO STEPS Instrument (Core and Expanded). Version 3.2.
18. Craig CL, Marshall AL, Sjostrom M, Bauman AE, Booth ML, Ainsworth BE, et al. International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exerc. 2003;35: 1381-1395. doi: 10.1249/01.MSS.0000078924.61453.FB [doi].
19. Kroenke K, Spitzer RL, Williams JB. The PHQ-9: validity of a brief depression severity measure. J Gen Intern Med. 2001;16: 606-613. doi: jgi01114 [pii].
20. Kalichman SC, Simbayi LC. HIV testing attitudes, AIDS stigma, and voluntary HIV counselling and testing in a black township in Cape Town, South Africa. Sex Transm Infect. 2003;79: 442-447.
21. Creel AH, Rimal RN. Factors related to HIV-testing behavior and interest in testing in Namibia. AIDS Care. 2011;23: 901-907. doi: 10.1080/09540121.2010.540227 [doi].
22. Bots ML, Hoes AW, Koudstaal PJ, Hofman A, Grobbee DE. Common carotid intima-media thickness and risk of stroke and myocardial infarction: the Rotterdam Study. Circulation. 1997;96: 1432-1437.
23. Den Ruijter HM, Peters SA, Anderson TJ, Britton AR, Dekker JM, Eijkemans MJ, et al. Common carotid intima-media thickness measurements in cardiovascular risk prediction: a meta-analysis. JAMA. 2012;308: 796-803. doi: 10.1001/jama.2012.9630 [doi].
24. Owolabi MO, Agunloye AM, Umeh EO, Akpa OM. Can common carotid intima media thickness serve as an indicator of both cardiovascular phenotype and risk among black Africans? Eur J Prev Cardiol. 2015;22: 1442-1451. doi: 10.1177/2047487314547656 [doi].
25. Laurent S, Cockcroft J, Van Bortel L, Boutouyrie P, Giannattasio C, Hayoz D, et al. Expert consensus document on arterial stiffness: methodological issues and clinical applications. Eur Heart J. 2006;27: 2588-2605. doi: ehl254 [pii].
26. Vlachopoulos C, Aznaouridis K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. J Am Coll Cardiol. 2010;55: 1318-1327. doi: 10.1016/j.jacc.2009.10.061 [doi].

27. Van Bortel LM, Laurent S, Boutouyrie P, Chowienczyk P, Cruickshank JK, De Backer T, et al. Expert consensus document on the measurement of aortic stiffness in daily practice using carotid-femoral pulse wave velocity. J Hypertens. 2012;30: 445-448. doi: 10.1097/HJH.0b013e32834fa8b0 [doi].

28. Vos AG, Idris NS, Barth RE, Klipstein-Grobusch K, Grobbee DE. Pro-Inflammatory Markers in Relation to Cardiovascular Disease in HIV Infection. A Systematic Review. PLoS One. 2016;11: e0147484. doi: 10.1371/journal.pone.0147484 [doi].

29. Nordell AD, McKenna M, Borges AH, Duprez D, Neuhaus J, Neaton JD, et al. Severity of Cardiovascular Disease Outcomes Among Patients With HIV Is Related to Markers of Inflammation and Coagulation. J Am Heart Assoc. 2014;3: 10.1161/JAHA.114.000844. doi: 10.1161/JAHA.114.000844 [doi].

Appendix 1 – CIMT protocol

Every two years a CIMT measurement will be performed on every participant according the following procedure:

1. Sonographer Scan Protocol: Use the Meijer Arc. Start with the transverse scan at the right side at the level of the clavicle and scan at an angle of 45 degrees at the 135 degrees angle. Optimize the boundaries during the scan and locate the orientation of the bifurcation to obtain the "Y" for vessel identification in the longitudinal images later on. Store one image clip at least 5 seconds in duration to illustrate the anatomy.

2. Start of the 4 Selections

RT 150 degrees - CCA both walls: Start the scan at 150 degrees. Place the text indicating the angle of interrogation on the screen. Position the tip of the flow divider approximately 15mm from the left side and keep this position during all selections. Horizontalise the vessel optimizing both boundaries. When both boundaries are clearly seen, freeze, scroll back in the cine-loop, identify the clearest boundaries on the R-wave of the ECG and store the image.

RT 120 Degrees - Move the transducer to the next angle on the arc (120 degrees) and repeat the procedure as described above.

RT 90 Degrees - Move the transducer to the next angle on the arc (90 degrees) and repeat the procedure as described for the CCA above.

Bulb far wall 120 degrees or optimal angle - Change annotations including the angle, then optimize the boundary, check the tip position, freeze, scroll back in the cine-loop and store the clearest still frame out of the cine-loop using the R-wave of the ECG.

Longitudinal scan of the CCA (optimal angle): Place the text 'right' in the lower left corner on the screen. Position the tip of the flow divider approximately 15mm from the left side and keep this position. Horizontalize the vessel optimizing both boundaries. When both boundaries are clearly seen, stabilize the probe and store a 5-second clip.

Left side

- Repeat the same procedure on the left side at angles 210, 240 and 270 for the CCA segment and 240 degrees (or optimal angle) for bifurcation segment

- Repeat the same procedure to obtain a 5-second clip of the CCA left.

Appendix 2 – PWV protocol

Every two years a PWV measurement will be performed on every participant according the following procedure:

1. *Preparations*: make sure that the patient is relaxed and that a stable ECG trace with positive R-waves is obtained. Record information on use of caffeine containing drinks or cigarettes in the 3 hours preceding the measurement. Check eligibility criteria (no history of carotid stenosis, no history of or visible arrhythmia's).

2. The carotid and femoral pulse at the right side will be identified. The distance between both pulses is measured and multiplied by 0.8.

3. The Tonometer is used to obtain a steady pulse waveform on the Carotid artery and subsequently on the Femoral artery, both at the right side.

4. Data are captured in case the following criteria are met:

    a. Standard deviation is less than 10% of the PWV

    b. Operator index is ≥85%

    c. All ECG indicators are within the expected range

CHAPTER

6

Cardiovascular disease burden in rural Africa: does HIV and antiretroviral treatment play a role? Baseline analysis of the Ndlovu Cohort Study

A.G. Vos, R.E. Barth, K.Klipstein-Grobusch, H.A. Tempelman, W.L.J. Devillé, R.A. Coutinho, D.E. Grobbee

Submitted

Abstract

Background HIV is associated with an increased risk of cardiovascular disease (CVD) in high income countries. Little is known about the CVD burden in sub-Saharan Africa (SSA), where 70% of the world's HIV-positive population lives. This study aims to provide insight into the burden of CVD risk in a rural setting in SSA considering HIV infection and antiretroviral treatment (ART).

Methods A cross-sectional analysis was conducted of the baseline of the Ndlovu Cohort study including HIV-negative and HIV-positive participants in South Africa between 2014 and 2017. Information was collected on demographics, socio-economic status and CVD risk factors. Carotid intima-media thickness (CIMT) measurement was performed. The influence of HIV and ART on the burden of CVD was determined by comparing HIV-positive participants who were ART-naïve, on first-line ART or on second-line ART to HIV-negative participants.

Results 1927 participants were included; 887 (46%) were HIV-positive, 54% were women. The median age was 38 years. Overall, 690 participants (79%) were on ART; 613 (89%) on first-line and 77 (11%) on second-line. Participants with HIV had lower values for most of the CVD risk factors, but higher CRP levels than HIV-negatives. Overall, ART-naïve HIV-positive participants had similar CIMT compared to HIV-negatives but CIMT was increased for participants on ART aged 30 years and over compared to HIV-negative participants.

Conclusion HIV-positive participants presented with a favourable CVD risk profile compared to HIV-negative participants. However, CIMT was increased in HIV-positive participants on ART, indicating a higher burden of subclinical CVD for the HIV-positive population.

Background

Almost seventy percent of all HIV infected people reside in Sub-Saharan Africa (SSA) [1]. The successful roll-out of antiretroviral therapy (ART) has changed HIV from a life-threatening illness to a chronic condition. Life expectancy for people living with HIV has increased substantially[2]. As a result, the health care system will be faced with an aging HIV population, and hence with an increasing number of HIV infected people with co-morbidities[3, 4].

Meanwhile the African continent is facing an increasing burden of non-communicable diseases[5]. Cerebrovascular - and ischemic heart disease were the fourth and fifth leading causes of life years lost in South Africa in 2015[6]. Simultaneously, a high prevalence of classic cardiovascular risk factors like hypertension, obesity and smoking is observed[7, 8].

Research from high-income countries (HIC) indicated that HIV infection and ART are independent risk factors for cardiovascular disease (CVD)[9]. The situation for SSA is less clear. Conventional CVD risk factor levels appear to be lower for people living with HIV (PLHIV) compared to the general population[8, 10, 11]. On the other hand, HIV infection and treatment with ART result in ongoing low-grade inflammation and elevation of markers of endothelial damage, which are known contributors to CVD risk[12, 13].

So far, there are no longitudinal studies addressing CVD risk in HIV in SSA, but there are some cross-sectional studies which all show that HIV is associated with a higher risk of CVD or stroke compared to the non-HIV infected population[14-16]. The role of ART is even less clear than the role of HIV[13, 17]. To gain insight into the burden of CVD in HIV infection, surrogate markers for CVD risk have been used, among which is the well-established carotid intima-media thickness (CIMT) measurement[18]. HIV has been associated with an increase in CIMT in HIC[19-22]; however, smaller studies in SSA did not find a relation between HIV and CIMT[13, 23, 24].

The Ndlovu Cohort Study (NCS) was set up to investigate the role of HIV and ART on the burden of cardiovascular risk factors and CVD in a rural African population. This study presents the cardiovascular risk factor profile at baseline and assesses the burden of subclinical CVD using carotid intima-media thickness in PLHIV, whether or not on treatment, in comparison to people without HIV.

Methods

The NCS is located in a rural area in Limpopo, South Africa, and included 1040 HIV-negative participants and 887 HIV-positive participants from November 2014 to August 2017. The design and the methods have been described previously[25]. Briefly, participants were ≥18 years and they were recruited through community campaigns, at local events and shopping centers as well as at the Ndlovu Medical Center HIV clinic. Following informed consent, participants underwent HIV testing unless they were on HIV treatment. Information was collected on demographics, socio-economic status, medical history and medication use (both HIV related as well as for other medical conditions) using standardized questionnaires. ART treatment status was assessed by self-report and complemented with data from an electronic HIV registry (TIER.net). A participant who was diagnosed with HIV at maximum eight weeks prior to inclusion was considered to be newly diagnosed and a participant who initiated ART at maximum eight weeks before enrolment was considered to be ART- naive. Date of HIV diagnosis and ART use were set to the first of July if only the year was known. If the date of the first ART prescription in TIER.net was prior to the self-reported date of HIV diagnosis, the date of the first prescription was assumed to also be the date of HIV diagnosis. Smoking, alcohol use and other cardiovascular risk factors were assessed with a modified version of the WHO STEPs instrument[26]. Family history was considered positive for CVD when a history of stroke and/or heart attack was reported in a first-degree family member (parent or sibling) before the age of 60. Physical activity was assessed with the International Physical Activity Questionnaire[27]. Anthropometric measurements included height, weight, and waist and hip circumference. Three blood pressure measurements were obtained after a five minute rest. The average of the second and the third measurement were used for the analysis. Blood was drawn for analysis of lipids, glucose and HIV viral load and CD4+ cell count for all HIV-positive participants. HbA1c was added to the analysis some months after the start of the study; results were available for 1494 (77.5%) of the participants. In addition, a urine sample was taken for analysis of urine-albumin and -creatinine. All blood samples were spun the same day and analysed the next day at an accredited laboratory (TogaLabs, South Africa).

CIMT measurement

CIMT was measured in all participants after a 15 minute rest using a Siemens Acuson p300 ultrasound (Siemens Healthcare (Pty) Ltd, South Africa). Scans were obtained in B-mode with a ≥7.0 MHz linear probe. The near wall and far wall of the common carotid artery (CCA) were measured at three standardized angles at both the right and left side using the Meijer's Arc[28]. The far walls of the carotid bulb on the right and left sides were captured at the best visible angle. CIMT was measured semi-automatically with

the Artery Measurement System software (Chalmers University, Gothenburg, Sweden) and adjusted manually if needed. Analyses were done in batch with a uniform reading protocol by three readers who were blinded to the HIV status of the participant. The inter-reading agreement for the readers was excellent for mean CCA-IMT and good for max CCA-IMT (0.93 and 0.87 respectively)[29]. CIMT reading included mean and maximum (max) thickness of the intima-media layer of the near and far wall across all six angles of the CCA (mean CCA intima-media thickness (IMT) and max CCA-IMT), and the max IMT at the carotid bulb left and right (max bulb-IMT). A mean CCA-IMT of >1.0 mm at any of the measured angles was considered as a plaque[30].

Statistical analysis

Descriptive data were presented as mean with standard deviation, median with interquartile range or count with percentage, as appropriate. Baseline characteristics and CIMT outcomes were presented by HIV and ART status. A total of 43.3% of the blood pressure readings were regarded as missing data as these measurements were taken with a non-validated blood pressure device (all blood pressure data obtained in 2016 and 2017). Multiple imputations were used according to a Markov chain Monte Carlo (MCMC) method to estimate the missing values while stratifying the data on HIV and treatment status. Imputation was repeated 20 times. A single imputed data set was created by selecting a random draw from the 20 data sets for the final imputed blood pressure values. Cardiovascular risk factors were compared across groups (HIV-negative, HIV-positive ART naïve, or on first-line or second line ART) using the HIV-negative group as the reference group whilst adjusting for sex and age.

As previous research suggested that the effect of HIV on CIMT could be age dependent[31], we first tested if there was an interaction between age and HIV on CIMT in our data. This interaction turned out to be positive, and therefore the analysis was stratified in three age categories: 18-29 years, 30-49 years and 50+ years. Participants on first-line and second-line ART were regrouped to 'HIV-positive on ART' as the relatively small number of participants on second-line ART (n=77) did not allow a separate analysis on second-line ART in the different age strata. The influence of HIV and ART on mean CCA-IMT, max CCA-IMT and max bulb-IMT values were analysed in a linear regression analysis whilst using the HIV-negative group as a reference group. The first model was unadjusted, the second model was adjusted for age, the third model for age and sex and the fourth model was additionally adjusted for known contributors to CIMT; namely male sex, age, smoking, systolic blood pressure, body mass index, high density lipoprotein (HDL) -cholesterol, low density lipoprotein (LDL) -cholesterol and glucose[28].

We repeated all these steps for HIV-positive participants only, using the ART naïve group as the reference group. The final model was additionally adjusted for CD4-cell count, viral load, known duration of HIV infection and time on ART. The influence of viremia (either between 50-1000 copies or >1000 cp/ml) on viral load was tested in a linear regression while using the group with undetectable viral load (<50cp/ml) as reference group. Finally, we analysed the influence of HIV and ART on mean CCA-outcomes for men and women separately.

Results

A total of 1927 participants were recruited, 1056 (55%) women and 887 (46%) HIV-positive. The median age of the total population was 38 years. HIV-negative participants were significantly younger than the HIV-positive participants (32 years versus 41 years, $p < 0.001$). The majority of the population was unemployed and lived under the poverty line, defined as a monthly income of less than 648 South African Rand (approximately $46) [32]. Sixty-one percent of the HIV-negative group versus 55% of the HIV-positive group was in a stable relationship ($p = 0.004$) (Table 1).

Table 1a. Baseline description.

		HIV-negative (n=1040)	ART-naïve (n=197)	HIV-positive First-line ART (n=613)	Second-line ART (n=77)
Demographics and socio-economic background					
Age in years *(median, IQR)*		32.0 (24.0 – 48.0)	35.0 (28.0 – 45.0)	41.0 (36.0 – 49.0)	43.0 (37.5 – 49.5)
Gender, female		527 (50.7)	124 (62.9)	362 (59.1)	43 (55.8)
Highest level of education	None	42 (4.0)	5 (2.5)	31 (5.1)	4 (5.2)
	Primary	179 (17.2)	48 (24.4)	130 (21.2)	21 (27.3)
	Secondary & Matric	711 (68.4)	125 (63.5)	419 (68.4)	45 (58.4)
	College & University	108 (10.4)	19 (9.6)	33 (5.4)	7 (9.1)
Employment	Unemployed	696 (66.9)	150 (76.1)	408 (66.6)	53 (68.8)
	(Self) employed	159 (15.3)	34 (17.3)	185 (30.2)	22 (28.6)
	Other *(student, retired, volunteer)*	185 (17.8)	13 (6.6)	20 (3.3)	2 (2.6)
Income per person per month in rands[1] (n=1824)	<648	621 (62.8)	125 (66.8)	349 (60.7)	48 (65.8)
	648-992	79 (8.0)	12 (6.4)	49 (8.5)	4 (5.5)
	>992	289 (29.2)	50 (26.7)	177 (30.8)	21 (28.8)
Stable relationship *(married, life partner, cohabiting)*		638 (61.3)	91 (46.2)	351 (57.3)	44 (57.1)
Cardiovascular risk factors					
Alcohol use, ever		777 (74.7)	154 (78.2)	378 (61.7)	49 (63.6)
Alcohol use in the past 30 days		406 (39.0)	69 (35.0)	154 (25.1)	23 (29.9)
Smoker (n=1923)	Ever	459 (44.1)	83 (42.1)	214 (35.0)	30 (39.0)
	Current	334 (32.1)	58 (29.4)	128 (20.9)	18 (23.4)
Cigarettes/cigars per day (n) *(median, IQR)*		6.0 (4.0-10.0)	6.0 (4.0 – 11.5)	6.0 (4.0 -10.0)	4.5 (4.0 – 9.3)
Positive family history for CVD		35 (3.4)	4 (2.0)	8 (1.3)	0
Physical activity (MET-minutes/wk)	Moderate	401 (38.6)	73 (37.1)	187 (30.5)	26 (33.8)
	High	302 (29.0)	48 (24.4)	124 (20.2)	18 (23.4)

Data in mean with standard deviation or count with percentage, unless otherwise specified.
ART; antiretroviral therapy, CVD; cardiovascular disease, MET; metabolic equivalent task, wk; week.
1. <648: lower bound poverty line, >992 upper bound poverty line.

Table 1b. Baseline description

	HIV-negative (n=1040)	ART-naïve (n=197)	HIV-positive First-line ART (n=613)	Second-line ART (n=77)
Physical examination				
Average systolic blood pressure (mmHg)	120.1 (24.1)	115.5 (21.5)	114.1 (20.5)	116.8 (17.8)
Average diastolic blood pressure (mmHg)	74.8 (14.2)	74.5 (12.7)	73.3 (13.1)	74.0 (12.5)
Body Mass Index (kg/m2) *(median, IQR)*	23.1 (19.8-28.3)	22.5 (19.3 – 26.9)	22.8 (19.6 – 26.5)	22.1 (19.1 – 26.9)
Waist circumference (cm)	82.7 (13.9)	82.2 (12.8)	85.5 (12.5)	83.6 (12.3)
Hip circumference (cm)	99.4 (14.0)	99.6 (14.1)	99.9 (13.3)	98.5 (15.6)
Laboratory analysis				
Fasting glucose (mmol/L) (n=1912)	5.02 (2.65)	4.73 (1.34)	4.89 (1.16)	4.89 (1.39)
HbA1c (%) (n=1495)	5.58 (0.88)	5.52 (0.38)	5.62 (0.66)	5.49 (0.66)
Total cholesterol (mmol/L) (n=1909)	4.19 (1.01)	3.88 (0.91)	4.38 (0.99)	4.31 (1.09)
HDL-C (mmol/L) (n=1909)	1.38 (0.34)	1.26 (0.37)	1.49 (0.42)	1.44 (0.51)
LDL-C (mmol/L) (n=1904)	2.32 (0.89)	2.18 (0.77)	2.35 (0.86)	2.26 (0.83)
Triglycerides (mmol/L) *(median, IQR)*	0.90 (0.60-1.30)	0.90 (0.65 – 1.20)	1.00 (0.80 – 1.50)	1.10 (0.73 – 1.70)
CRP (mg/L) *(median, IQR)*	3.0 (2.0-6.0)	3.0 (2.0 – 13.0)	5.0 (2.0 – 11.0)	4.0 (2.0 – 9.8)
Urine albumin/creatinine ratio (mg/mmol) *(median, IQR)*	0.65 (0.43-1.26)	0.85 (0.55 – 1.55)	1.05 (0.59 – 2.26)	0.82 (0.55 – 1.64)
Carotid intima-media thickness outcomes				
Mean CCA-IMT, mm (n=1774) *(median, IQR)*	0.565 (0.510 – 0.660)	0.555 (0.509 – 0.629)	0.610 (0.541 – 0.696)	0.630 (0.547 – 0.694)
Max CCA-IMT, mm (n=1774) *(median, IQR)*	0.645 (0.571 – 0.759)	0.637 (0.573 – 0.722)	0.693 (0.613 – 0.800)	0.712 (0.636 – 0.800)
Max bulb-IMT, mm (n=1595) *(median, IQR)*	0.781 (0.649 – 0.942)	0.773 (0.636 – 0.942)	0.848 (0.719 – 1.009)	0.852 (0.723 – 1.026)
Plaque (mean CCA-IMT >1mm)	44 (4.2)	3 (1.5)	34 (5.5)	6 (7.8)

Data in mean with standard deviation or count with percentage, unless otherwise specified.
ART; antiretroviral therapy, CCA; common carotid artery, CRP; C-reactive protein, HDL-C; high density lipoprotein cholesterol, IMT; intima-media thickness, IQR; interquartile range, LDL-C; low-density lipoprotein cholesterol.

People with HIV knew their diagnosis for about five years, ranging from zero weeks for newly diagnosed participants to more than 10 years for some participants on second-line ART. Only about 65% of all participants were virally suppressed, including 16% of the ART naïve participants (Table 2).

Table 2. HIV related characteristics

		ART-naïve n=197	First-line ART n=612	Second-line ART n=77
Time since HIV diagnosis (months) (n=881)		0.0 (0.0 – 7.0)	67.0 (30.0 – 102.0)	99.0 (70.5 – 126.5)
Newly diagnosed upon enrolment* (n=881) (n,%)		139 (72.4)	0	0
Time on ART (months)		-	59.0 (21.0 – 97.0)	97.0 (59.0 – 122.5)
Of which time on second-line ART		-	-	42.0 (15.5 – 54.8)
CD4+ cell-count (cells/mm^3) (n=873)		399 (275 – 553)	494 (338 – 679)	467 (330 – 647)
CD4+ <200 (cells/mm^3) (n,%)		36 (18.6)	51 (8.3)	8 (10.5)
Viral load (cp/ml) (n=872) (n,%)	<50	30 (15.5)	492 (81.7)	45 (59.2)
	50-1000	26 (13.4)	47 (7.8)	14 (18.4)
	>1000	138 (71.1)	63 (10.5)	17 (22.4)

Data in median with interquartile range or count with percentage. *diagnosed within 8 weeks prior to enrolment. ART; antiretroviral therapy

Table 3. Distribution of CVD risk factors according to HIV and ART corrected for sex and age.

HIV-negative				**HIV-positive**			
		ART-naïve n = 197		**First-line ART n = 613**		**Second-line ART n = 77**	
		β, 95% CI	*p*	β, 95% CI	*p*	β, 95% CI	*p*
Systolic blood pressure (mmHg)	REF	-4.54 (-7.64 - -1.43)	0.004	-10.73 (-12.82 - -8.64)	<0.001	-8.77 (-13.50 - -4.03)	<0.001
Diastolic blood pressure (mmHg)	REF	-0.46 (-2.39 – 1.48)	0.644	-4.10 (-5.41 - -2.80)	<0.001	-3.81 (-6.76 - -0.86)	0.011
BMI (kg/m^2)	REF	-1.49 (-2.33 - -0.65)	0.001	-1.95 (-2.51 - -1.38)	<0.001	-1.93 (-3.21 - -0.65)	0.003
Fasting glucose (mmol/L)	REF	-0.272 (-0.555 – 0.011)	0.059	-0.197 (-0.384 - -0.010)	0.039	-0.212 (-0.615 – 0.191)	0.302
HbA1c (%)	REF	-0.113 (-0.243 – 0.017))	0.089	-0.120 (-0.206 - -0.034)	0.006	-0.264 (-0.450 - -0.079)	0.005
Total cholesterol (mmol/L)	REF	-0.359 (-0.501 - -0.217)	<0.001	-0.001 (-0.097 – 0.095)	0.988	-0.090 (-0.309 – 0.130)	0.423
HDL-C (mmol/L)	REF	-0.118 (-0.175 - -0.061)	<0.001	0.106 (0.067 – 0.144)	<0.001	0.013 -0.076 – 0.101)	0.780
LDL-C (mmol/L)	REF	-0.194 (-0.320 - -0.068)	0.003	-0.115 (-0.200 - -0.030)	0.008	-0.214 (-0.408 - -0.019)	0.031
Log-TG (mmol/L)	REF	-0.043 (-0.122 – 0.036)	0.287	0.025 (-0.029 – 0.078)	0.366	0.133 (0.011 – 0.254)	0.032
Log CRP	REF	0.332 (0.179 – 0.484)	<0.001	0.410 (0.308 – 0.513)	<0.001	0.210 (-0.025 - 0.446)	0.080
Current smoking (OR)	REF	1.071 (0.628 – 1.828)	0.801	0.696 (0.486 – 0.997)	0.048	0.708 (0.323 – 1.551)	0.388

ART; antiretroviral therapy, BMI; body mass index, CRP; C-reactive protein, HDL-C; high density lipoprotein cholesterol, LDL-C; low density lipoprotein cholesterol, OR; odds ratio, REF; reference, TG; triglycerides.

More than 90% of participants on first-line ART were using the recommended first-line ART regimen tenofovir, emtricitabine, efavirenz. The majority of participants on second-line ART were using ritonavir-boosted lopinavir.

Systolic and diastolic blood pressure, BMI, glucose, HbA1c, total cholesterol and LDL cholesterol were lower in HIV-positive participants compared to HIV-negative participants following adjustment for age and sex (see table 3 for a comparison between the treatment groups). On the contrary, CRP was significantly higher for PLHIV compared to the HIV-negative group (p<0.001).

Table 4. HIV and ART status on mean CCA intima-media thickness (n=1775)

		HIV-negative	HIV-positive ART-naïve	*P*	HIV-positive on ART	*p*
Model 1	Age 18-29 (n=500)	REF	-0.007 (-0.024 – 0.010)	0.431	0.004 (-0.013 – 0.021)	0.657
	Age 30-49 (n=840)		-0.023 (-0.043 – -0.003)	0.023	0.005 (-0.007 – 0.018)	0.401
	Age ≥ 50 (n=435)		-0.046 (-0.100 – 0.009)	0.099	0.011 (-0.018 – 0.040)	0.463
Model 2	Age 18-29 (n=500)	REF	-0.009 (-0.026 – 0.008)	0.311	0.000 (-0.017 – 0.018)	0.965
	Age 30-49 (n=840)		-0.014 (-0.033 – 0.004)	0.118	-0.001 (-0.012 – 0.011)	0.905
	Age ≥ 50 (n=435)		-0.033 (-0.085 – 0.019)	0.212	0.024 (-0.004 – 0.052)	0.097
Model 3	Age 18-29 (n=500)	REF	-0.004 (-0.021 – 0.013)	0.655	0.006 (-0.012 – 0.024)	0.508
	Age 30-49 (n=840)		-0.013 (-0.032 – 0.005)	0.145	0.000 (-0.011 – 0.012)	0.938
	Age ≥ 50 (n=435)		-0.034 (-0.086 – 0.017)	0.189	0.019 (-0.009 – 0.047)	0.183
Model 4	Age 18-29 (n=492)	REF	-0.004 (-0.021 – 0.013)	0.629	0.011 (-0.007 – 0.029)	0.237
	Age 30-49 (n=834)		-0.001 (-0.019 – 0.016)	0.870	0.018 (0.007 – 0.030)	0.002
	Age ≥ 50 (n=429)		-0.014 (-0.063 – 0.035)	0.571	0.050 (0.022 – 0.078)	<0.001

Age in years. ART: antiretroviral therapy, CCA: common carotid artery, HIV: human immunodeficiency virus, LDL; low density lipoprotein, REF: reference.

Model 1 unadjusted

Model 2 adjusted for age

Model 3 adjusted for age and sex

Model 4 adjusted for age, sex, current smoking, systolic blood pressure, BMI, HDL cholesterol, LDL cholesterol and fasting glucose

Mean and max CCA-IMT was available for 1775 (92%), and max bulb-IMT for 1596 (83%) of the participants. Plaques were present in 87 (4.5%) of the participants, and this prevalence was not different between HIV-positive and HIV-negative participants following correction for age and sex (p=0.46).

The unadjusted mean CCA-IMT was lower in ART-naive participants in the age group 30-49 years (Table 4). After adjustment for age, mean CCA-IMT did not differ anymore between the groups.

Following adjustment for conventional CVD risk factors mean CCA-IMT was higher in HIV-positive participants on ART aged 30 and over, and this effect increased with age (β 0.018 p=0.002 in the age group 30-49 year versus β 0.050, p<0.001 in the age group of 50+ years).

Exclusion of all HIV-positive ART-naïve participants with a suppressed viral load from the analysis did not change the direction or magnitude of the findings. The contribution of CVD risk factors to mean CCA-IMT increased with age (Table 5).

Table 5. Influence of CVD risk factors on CCA-IMT.

	18-29 years		30-49 year		≥50 years	
	β (95% CI)	*p*	β (95% CI)	*p*	β (95% CI)	*p*
Age (years)	0.001 (-0.001 – 0.002)	0.368	**0.006 (0.005 – 0.007)**	**<0.001**	**0.007 (0.005 – 0.009)**	**<0.001**
Male sex	**0.024 (0.012 – 0.037)**	**<0.001**	**0.023 (0.010 – 0.036)**	**0.001**	**0.080 (0.052 – 0.109)**	**<0.001**
Current smoking	-0.001 (-0.014 – 0.012)	0.904	-0.002 (-0.016 – 0.011)	0.752	0.002 (-0.028 – 0.032)	0.886
Systolic blood pressure (mmHg)	**+0.000 (0.000 – 0.001)**	**0.014**	**0.001 (0.001 – 0.001)**	**<0.001**	**0.001 (0.000 – 0.001)**	**0.002**
Body mass index (kg/m2)	0.003 (0.001 – 0.004)	<0.001	**0.002 (0.001 – 0.003)**	**<0.001**	**0.003 (0.000 – 0.005)**	**0.023**
HDL cholesterol (mmol/L)	-0.007 (-0.023 – 0.008)	0.355	-0.001 (-0.014 – 0.012)	0.879	**-0.039 (-0.072 - -0.006)**	**0.020**
LDL cholesterol (mmol/L)	0.000 (-0.007 - 0.007)	0.969	**0.009 (0.003 – 0.015)**	**0.004**	**0.033 (0.020 – 0.046)**	**<0.001**
Glucose (mmol/L)	**0.005 (0.001 – 0.010)**	**0.028**	**0.004 (0.000 – 0.007)**	**0.030**	**0.005 (0.001 – 0.010)**	**0.010**

CI; confidence interval, HDL; high density lipoprotein, LDL; low density lipoprotein

The effects of HIV, ART and CVD risk factors on max CCA-IMT had the same direction and magnitude (data not shown). Max-bulb IMT did not differ by HIV and ART status in any of the age strata, and adjustment for age, sex and CVD risk factors did not change this finding (data not shown).

To investigate the influence of HIV-characteristics on mean CCA-IMT HIV-positive participants were analysed separately. Time on ART was associated with a higher CCA-IMT in the age group 30-49 (β=0.006 per year of use, p<0.001), while years since HIV diagnosis was associated with lower CCA-IMT (β=-0.005 per year since diagnosis, p=0.001). Viral load was not associated with mean CCA-IMT, but an increase in CD4+ cell count was associated with a lower mean CCA-IMT (β=-0.004 per increase with 100cells/mm^3, p=0.01). Using max CCA-IMT as outcome, the same trends were seen in the age group 30-49 years. None of the HIV-related variables were associated with mean or max CCA-IMT in the age group 50+ years.

Finally, mean CCA-IMT results were analysed for men and women separately. Following adjustment for CVD risk factors the same trends were observed with a higher CCA-IMT for participants on ART compared to HIV-negative participants in the age category 30-49 years and, for men only, 50 years and over (data not shown).

Discussion

In this large study comparing PLHIV whether or not on ART to HIV-negative participants, PLHIV had favourable levels of most conventional CVD risk factors compared to HIV-negative participants. HIV itself seemed not to be associated with increased CCA-IMT, but treatment with ART was associated with an increase in CCA-IMT in people aged 30 years and over, and this effect increased with age. The influence of conventional CVD risk factors on CCA-IMT also increased with age.

Lower levels of conventional CVD risk factors in PLHIV compared to the HIV-negative group is in contrast to studies from HIC reporting a higher burden of CVD risk factors in the HIV-positive compared to the HIV negative population[33, 34]. Our findings are, however, in line with two meta-analyses including studies from SSA only [10, 35] as well as with more recent, population based surveys in South-Africa[7, 13]. This likely reflects the differences in sex distribution and lifestyle between the HIV positive population in HIC compared to SSA. The fact that a large proportion of HIV positive subjects in SSA are relatively young may obscure any adverse effects on cardiovascular risk with advancing age.

The influence of HIV on CIMT was observed to vary across the lifespan, with a higher CCA-IMT for people on ART from the age of 30 years compared to HIV-negative

individuals. This effect seems to be driven by ART rather than by HIV as time since HIV diagnosis was associated with a decrease in CCA-IMT, but the time on ART with an increase in CCA-IMT. The age-dependency of the influence of HIV and ART on CIMT was also described in a meta-analysis by Hanna et al.[31]. They found higher CIMT values for the HIV-positive participants aged six to 29 years compared to HIV-negative participants, and, in the age category 30 years and over, similar CIMT for HIV-positive participants on ART compared to HIV-negative controls. In contrast to these findings we observed similar CIMT values between PLHIV and HIV-negative participants in the young age category, and a higher CIMT in participants on ART aged 30 years and over compared to HIV negative controls.

Our results are in line with several studies conducted in HIC, which all reported higher CIMT values for PLHIV on ART compared to HIV-negative controls[21, 22, 36]. However, studies conducted in SSA all found equal or lower CIMT values in PLHIV compared to HIV-negative participants[13, 23, 24, 37]. It is challenging to explain why our findings differ from these studies. The average age of participants in these studies was comparable to our study, but these studies were smaller and only one study included participants on second-line ART[23]. Other reasons to consider are differences in time since HIV diagnosis, time on ART, exposure to older ART regimens, as well as differences in the extent of immune dysregulation. In our cohort, 35% of PLHIV had detectable viremia, and the accompanying immune activation was reflected by the higher CRP levels for PLHIV compared to the HIV-negative participants. Both viremia and immune activation are known risk factors for CVD[38-40]. However, this might not explain everything as most of the studies in SSA also included HIV-positive, ART naïve participants, and our analysis suggests that CIMT is mainly driven by ART and not by HIV. Given the large sample size and the inclusion of a representative HIV-negative control group in the current study, we believe that the current results reliably reflect the effect of HIV and ART on CIMT in this urban, African setting.

Some limitations of this study need to be mentioned. A material proportion of the blood pressure values were imputed as we could not use the original data. We assumed data to be missing completely at random so it is unlikely that this affects the comparison between the groups, but it limits the ability to state something about the prevalence of hypertension in our population. There is a remarkably high percentage of HIV-positive, ART-naive participants with undetectable viral load. This may reflect non-disclosure about HIV status and use of ART, and this may have diminished differences between the ART-naïve group and participants on ART. However, excluding these participants from the analysis did not change the findings. Of concern is the high percentage of PLHIV with detectable viremia (18% of participants on first-line ART and 41% of participants on second-line ART). Apart from the clinical implications, it might limit the generalizability

of our results to settings with higher rates of viral suppression. Finally, we could present CVD risk profile by ART line (first or second-line), but upon stratification the number of participants on second-line ART per group was too small to include this group separately in the analysis of CIMT.

In conclusion, our data suggests that the older HIV-positive population on ART has a higher risk of CVD than the HIV-negative population as estimated from the carotid artery wall thickness. Results from prospective studies addressing CVD endpoints are needed to confirm this finding. The Ndlovu Cohort Study will contribute to understanding the effects of HIV on the burden of CVD in the long term. The first participants in our cohort have now completed four years of follow-up. In future publications we will address change in CIMT over time between the HIV-positive and HIV-negative participants, as well as CVD endpoints. In the meantime, HIV care should incorporate screening for and treatment of risk factors for CVD, and treatment thresholds might need to be stricter as PLHIV seem to have an increased risk of CVD despite a lower level of conventional CVD risk factors compared to the HIV-negative population.

Funding

This work was supported by a grant from the Dutch AIDS Foundation [Amsterdam Diner 2014/2014, dossiernummer 20133053]; Dioraphte Foundation [project number 13041500 RV-UC/NDLOVU research Consortium 2014-2016]; De Grote Onderneming; Hofstee Stichting and the University Medical Center Utrecht.

Acknowledgements

We would like to acknowledge the Ndlovu Cohort Study team; Ms M. Malepo, Ms B. Mahlangu, Ms S. Rakghoto and Sir B. Nyathi for collecting all the data. We thank Ms. N. Godijk, Ms. V. Jongen and Ms. L. Ketelaar who analysed the CIMT data. Finally, we would like to thank all study participants without whom this work would not be possible.

References

1. Joint United Nations Programme on HIV/AIDS (UNAIDS). Unaids data 2017. UNAIDS/JC2910E, 2017.
2. Antiretroviral Therapy Cohort Collaboration. Survival of HIV-positive patients starting antiretroviral therapy between 1996 and 2013: A collaborative analysis of cohort studies. Lancet HIV, 2017; 4: e349-56.
3. Deeks SG. HIV infection, inflammation, immunosenescence, and aging. Annu Rev Med, 2011; 62: 141-55.
4. Smit M, Brinkman K, Geerlings S, et al. Future challenges for clinical care of an ageing population infected with HIV: A modelling study. Lancet Infect Dis, 2015; 15: 810-8.
5. GBD 2016 Causes of Death Collaborators. Global, regional, and national age-sex specific mortality for 264 causes of death, 1980-2016: A systematic analysis for the global burden of disease study 2016. Lancet, 2017; 390: 1151-210.
6. Massyn N, Padarath A, Peer N, Day C. District health barometer 2016/17. Health Systems Trust, November 2017.
7. Clark SJ, Gomez-Olive FX, Houle B, et al. Cardiometabolic disease risk and HIV status in rural South Africa: Establishing a baseline. BMC Public Health, 2015; 15: 135,015-1467-1.
8. Gaziano TA, Abrahams-Gessel S, Gomez-Olive FX, et al. Cardiometabolic risk in a population of older adults with multiple co-morbidities in rural South Africa: The HAALSI (health and aging in Africa: Longitudinal studies of INDEPTH communities) study. BMC Public Health, 2017; 17: 206,017-4117-y.
9. 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; 13: 453-68.
10. Dillon DG, Gurdasani D, Riha J, et al. Association of HIV and ART with cardiometabolic traits in sub-Saharan Africa: A systematic review and meta-analysis. Int J Epidemiol, 2013; 42: 1754-71.
11. Clark SJ, Gomez-Olive FX, Houle B, et al. Cardiometabolic disease risk and HIV status in rural South Africa: Establishing a baseline. BMC Public Health, 2015; 15: 135,015-1467-1.
12. Zanni MV, Schouten J, Grinspoon SK, Reiss P. Risk of coronary heart disease in patients with HIV infection. Nat Rev Cardiol, 2014; 11: 728-41.
13. Fourie CM, Schutte AE, Smith W, Kruger A, van Rooyen JM. Endothelial activation and cardiometabolic profiles of treated and never-treated HIV infected Africans. Atherosclerosis, 2015; 240: 154-60.
14. Wester CW, Koethe JR, Shepherd BE, et al. Non-AIDS-defining events among HIV-1-infected adults receiving combination antiretroviral therapy in resource-replete versus resource-limited urban setting. AIDS, 2011; 25: 1471-9.

15. Benjamin LA, Corbett EL, Connor MD, et al. HIV, antiretroviral treatment, hypertension, and stroke in Malawian adults: A case-control study. Neurology, 2016; 86: 324-33.
16. Stroke risk factors in an incident population in urban and rural Tanzania: A prospective, community-based, case-control study. Lancet Glob Health, 2013; 1: e282-8.
17. Stein JH, Ribaudo HJ, Hodis HN, et al. A prospective, randomized clinical trial of antiretroviral therapies on carotid wall thickness. AIDS, 2015; 29: 1775-83.
18. Stein JH, Korcarz CE, Hurst RT, et al. Use of carotid ultrasound to identify subclinical vascular disease and evaluate cardiovascular disease risk: A consensus statement from the American society of echocardiography carotid intima-media thickness task force. Endorsed by the society for vascular medicine. J Am Soc Echocardiogr, 2008; 21: 93,111; quiz 189-90.
19. Lorenz MW, Stephan C, Harmjanz A, et al. Both long-term HIV infection and highly active antiretroviral therapy are independent risk factors for early carotid atherosclerosis. Atherosclerosis, 2008; 196: 720-6.
20. van Vonderen MG, Smulders YM, Stehouwer CD, et al. Carotid intima-media thickness and arterial stiffness in HIV-infected patients: The role of HIV, antiretroviral therapy, and lipodystrophy. J Acquir Immune Defic Syndr, 2009; 50: 153-61.
21. Hsue PY, Ordovas K, Lee T, et al. Carotid intima-media thickness among human immunodeficiency virus-infected patients without coronary calcium. Am J Cardiol, 2012; 109: 742-7.
22. Gupta PK, Gupta M, Lal AK, Taneja A, Taneja RS, Rewari BB. Markers of subclinical atherosclerotic disease in HIV-infected individuals. J Virus Erad, 2018; 4: 21-5.
23. Gleason RL, Jr, Caulk AW, Seifu D, et al. Current efavirenz (EFV) or ritonavir-boosted lopinavir (LPV/r) use correlates with elevate markers of atherosclerosis in HIV-infected subjects in Addis Ababa, Ethiopia. PLoS One, 2015; 10: e0117125.
24. Mosepele M, Hemphill LC, Moloi W, et al. Pre-clinical carotid atherosclerosis and sCD163 among virally suppressed HIV patients in Botswana compared with uninfected controls. PLoS One, 2017; 12: e0179994.
25. Vos A, Tempelman H, Deville W, et al. HIV and risk of cardiovascular disease in sub-Saharan Africa: Rationale and design of the Ndlovu cohort study. Eur J Prev Cardiol, 2017; 24: 1043-50.
26. . WHO STEPS instrument (core and expanded). version 3.2. Available at: https://www.who.int/ncds/surveillance/steps/instrument/en/.
27. Craig CL, Marshall AL, Sjostrom M, et al. International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exerc, 2003; 35: 1381-95.
28. Bots ML, Evans GW, Tegeler CH, Meijer R. Carotid intima-media thickness measurements: Relations with atherosclerosis, risk of cardiovascular disease and application in randomized controlled trials. Chin Med J (Engl), 2016; 129: 215-26.

29. Koo TK, Li MY. A guideline of selecting and reporting intraclass correlation coefficients for reliability research. J Chiropr Med, 2016; 15: 155-63.
30. Boulos NM, Gardin JM, Malik S, Postley J, Wong ND. Carotid plaque characterization, stenosis, and intima-media thickness according to age and gender in a large registry cohort. Am J Cardiol, 2016; 117: 1185-91.
31. Hanna DB, Guo M, Buzkova P, et al. HIV infection and carotid artery intima-media thickness: Pooled analyses across 5 cohorts of the NHLBI HIV-CVD collaborative. Clin Infect Dis, 2016; 63: 249-56.
32. STATISTICAL RELEASE P0309.3. Embargoed until: 27 March 2018.
33. 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; 92: 2506-12.
34. Mdodo R, Frazier EL, Dube SR, et al. Cigarette smoking prevalence among adults with HIV compared with the general adult population in the United States: Cross-sectional surveys. Ann Intern Med, 2015; 162: 335-44.
35. Ekoru K, Young EH, Dillon DG, et al. HIV treatment is associated with a two-fold higher probability of raised triglycerides: Pooled analyses in 21 023 individuals in sub-Saharan Africa. Glob Health Epidemiol Genom, 2018; 3: 10.1017/gheg.2018.7. Epub 2018 May 8.
36. Grunfeld C, Delaney JA, Wanke C, et al. Preclinical atherosclerosis due to HIV infection: Carotid intima-medial thickness measurements from the FRAM study. AIDS, 2009; 23: 1841-9.
37. Vos AG, Hoeve K, Barth RE, Peper J, Moorhouse M, Crowther NJ, Venter WDF, Grobbee DE, Bots ML, Klipstein-Grobusch K. Cardiovascular disease risk in an urban African population: What is the role of HIV and antiretroviral treatment? submitted, 2019.
38. Lang S, Mary-Krause M, Simon A, et al. HIV replication and immune status are independent predictors of the risk of myocardial infarction in HIV-infected individuals. Clin Infect Dis, 2012; 55: 600-7.
39. Triant VA, Meigs JB, Grinspoon SK. Association of C-reactive protein and HIV infection with acute myocardial infarction. J Acquir Immune Defic Syndr, 2009; 51: 268-73.
40. Nou E, Lo J, Grinspoon SK. Inflammation, immune activation, and cardiovascular disease in HIV. AIDS, 2016; 30: 1495-509.

CHAPTER

7

Heart rate variability, HIV and the risk of cardiovascular diseases in rural South Africa

N.G. Godijk, A.G. Vos, V.W. Jongen, R. Moraba, H.A. Tempelman, D.E. Grobbee, R.A. Coutinho, W.L.J. Devillé, K. Klipstein-Grobusch

Submitted

Abstract

Background Antiretroviral therapy transformed human immunodeficiency virus (HIV) infection into a chronic disease. Possible HIV-associated complications have emerged including cardiovascular diseases (CVD). This study aims to determine the distribution of heart rate variability (HRV) and the association between HRV and HIV in a rural African population.

Setting This cross-sectional study included participants of the Ndlovu Cohort Study, South Africa.

Methods HRV was measured using a standardized 5-minute resting ECG and assessed the standard deviation of the normal RR intervals (SDNN), the root of the mean squares of successive RR differences (RMSSD), the percentage of RR intervals greater than fifty milliseconds different from its predecessor (pNN50), total-, low- and high-frequency power. CVD risk factors were assessed using measurements (blood pressure, anthropometry) and questionnaires (e.g. socio-demographics, alcohol, smoking, physical activity). We used a Wilcoxon rank test to assess the difference in medians between HIV-infected and HIV-uninfected participants and multivariable linear regression to investigate the association between HRV and HIV.

Results The study included 325 participants, of whom 202 (62.2%) were HIV-infected. HIV-infected participants drank less alcohol, were more physically active and had lower educational attainment and systolic blood pressure. The medians of all HRV parameters were lower for the HIV-infected compared to HIV-uninfected participants. A significant inverse association was found between HIV and SDNN, RMSSD and pNN50.

Conclusion Although HIV-infected participants presented with less CVD risk factors they had a lower HRV, indicating an increased risk of CVD and underlining the importance of embedding CVD prevention in HIV-care.

Introduction

In 2017, an estimated 60% of all people with human immunodeficiency virus (HIV) globally received antiretroviral treatment (ART)[1]. Long-term treatment with ART increases the life expectancy of HIV-patients and has transformed HIV into a chronic disease[2]. With the ageing of the HIV-infected population, possible HIV-associated complications have emerged, most notably cardiovascular diseases (CVD)[3, 4]. Apart from conventional CVD risk factors, HIV-infected individuals may be at an increased risk of CVD due to HIV-infection, possibly related to immune activation while certain antivirals may also play a role[4, 5]. A recent meta-analysis reported that people living with HIV have a twofold increased risk of cardiovascular disease[5].

While approximately 25.5 million HIV-infected individuals live in sub-Saharan Africa (SSA), that is nearly 70% of all people infected with HIV, research on the association between HIV and CVD in this area is scarce[6]. The differences in lifestyle, socio-economic factors and HIV subtype preclude generalization of data from Western countries to the SSA region.

One way of estimating CVD risk is by using surrogate markers[7]. Heart rate variability (HRV) has been shown to be an independent predictor of CVD[8-10]. High HRV is a sign of good adaptation and efficient autonomic mechanisms. Conversely, low HRV is often an indicator of inadequate adaptation of the autonomic nervous system (ANS) [11]. Autonomic dysfunction was commonly detected in HIV and AIDS patients prior to the advent of ART, suggesting an autonomic neuropathological effect of HIV[10]. However, the effect of HIV on HRV in the current era of widespread ART availability is more ambiguous[10, 12]. Research on a small group of HIV-infected subjects on ART demonstrated a decrease in all parameters using 24-hour HRV, while another study did not find a difference in parasympathetic activity but only in total HRV[13]. Research on the distribution of HRV in HIV-infected and HIV-uninfected populations in SSA which takes into account classical CVD risk factors and ART allows to further assess the link between HIV, ART, CVD risk and autonomic function[14].

The aim of this study is twofold. First, we assessed differences between CVD risk factors of HIV-infected compared to HIV-uninfected subjects. Second, we investigated the distribution of HRV and the difference between HRV of HIV-infected compared to HIV-uninfected subjects while taking CVD risk factors into account.

Methods

Study design

This cross-sectional study was embedded in the Ndlovu Cohort study (NCS)[15]. All measurements, including HRV, were taken on the day the participant came for a baseline or follow-up visit in the period August 2017 to December 2017.

Recruitment of participants

The NCS is a prospective study in the Moutse area, Limpopo Province, South Africa and aims to provide a comprehensive understanding of the interaction between HIV and CVD in the black SSA population. From November 2014 until August 2017 this study recruited 1,040 HIV-uninfected and 887 HIV-infected participants. Criteria for eligibility were age 18 years and older, being able to provide written informed consent and be committed to long-term follow-up. Routine physical examination was used to assess CVD risk factors. The methods have been described in details in another publication[15].

All NCS participants who came to the research site were approached for additional HRV assessment. We excluded pregnant women and individuals unable to undergo the study procedures for any reason. Ethics approval was obtained from the Review Ethical Committee of the University of Pretoria (ref. number 227_2017).

Heart Rate Variability

HRV was measured in laying position with the upper body at a slight upward angle during 5-minutes using a 12-lead computer-based ECG Sampling box (SE-1515 DP12, EDAN, 4204 Jutland Dr Suite B, San Diego, CA 92117, United States) and complementary software. All measurements were performed according to standardized procedures by trained two investigators who were unaware of the subjects' HIV-status at the time the measurements were taken.

Time and frequency domain parameters were measured. Time-domain parameters address the magnitude of variability and provide information about the vagal (parasympathetic) modulation of the heart, with higher variability generally reflecting greater parasympathetic modulation[14]. Time-domain measures include the standard deviation of the normal RR intervals (SDNN), the root of the mean squares of successive RR differences (RMSSD) and the percentage of RR intervals greater than fifty milliseconds different from its predecessor (pNN50). Overall HRV is reflected by SDNN and RMSSD measures, with SDNN being the most representative parameter of HRV, while pNN50 measures HRV's short-term components[14, 16].

Frequency parameters use power spectral analysis of the beat-to-beat variations of the heart rate (R–R interval)[17]. This method divides total variance ("power") of a continuous series of heartbeats into frequency components[16, 17]. Of the frequency

parameters, three spectral components were measured: low-frequency (LF), high-frequency (HF) and total-frequency (TF) power. The influence of LF power on the autonomic nervous system is controversial, some consider LF power (LF; 0.04 to 0.15 Hertz) to represent sympathetic activity[18], while others claim is represents parasympathetic and sympathetic activity[19], HF power (HF; 0.15 to 0.40 Hertz) reflects sympathetic activity[14, 16]. TF power reflects total autonomic activity[16].

Other measurements

Information was collected on date of birth, sex, smoking status, alcohol use, chronic medication use and physical activity using standardized questionnaires[20]. Physical activity was categorized as inactive and active according to the WHO definition[21]. Validated questionnaires were used for information on education, employment, income and household support[22]. Education was classified as low (non or primary), middle (secondary or matric) and high (college or university). Information was collected on height, weight and blood pressure (BP). BP was measured in a seated position after a five-minute rest. BP was taken at both wrists and repeated at the wrist with the highest values. The average of the second and third reading was used for the analysis. Fasting glucose, triglycerides, total, HDL and LDL cholesterol were measured by Togalabs, South-Africa. In HIV-uninfected subjects, HIV status was measured according to the NCS protocol[15].

Statistical analysis

Data were analysed using R version 3.4.0.[23]. Due to technical complications during the HRV measurement 31 participants were excluded. Missing data for medication use, BMI, systolic and diastolic BP were less than one percentage. A t-test and a Wilcoxon rank test were used to test for differences in continuous variables. Chi-square test was used to test for a difference in the categorical variables; smoking, alcohol use, income and educational level. The difference in the distributions of median HRV between HIV-positive and HIV-negative participants was assessed using the Wilcoxon rank test.

HRV parameters were log-transformed to reach a normal distribution. Linear regression analysis investigated the association of HIV and HRV parameters. Model 1 included HIV, age and sex. Model 2 included HIV, age, sex and all CVD risk factors with a p-value <0.2 in the univariable analysis. Model 3 was additionally adjusted for socio-economic variables with a p-value <0.2.

Results

The study population comprised 325 participants, of whom 202 (62.2%) were HIV-infected and almost all (n=195 97.0%) were on ART (table 1). The HIV-infected participants were more often women, were on average about five years older and were

Table 1. Baseline characteristics and descriptive statistics of study participants.

		HIV-negative	HIV-positive	*p*
Characteristics (n, %)				
Age (mean, SD)		38.85 (12.79)	43.77 (9.36)	<0.001
Gender	Woman	43 (35%)	111 (55%)	<0.001
Education	Low	17 (14%)	54 (27%)	<0.001
	Middle	85 (69%)	140 (69%)	
	High	21 (17%)	8 (4%)	
Income	≤ R600	90 (73%)	127 (64%)	<0.001
	R600-3100	11(9%)	19 (10%)	
	R3100-11000	15 (12%)	47 (24%)	
	≥R11000	7 (6%)	6 (3%)	
Smoking	Never	63 (51%)	130 (64%)	0.06
	Past	19 (15%)	20 (10%)	
	Current	41 (33%)	52 (26%)	
Alcohol	Never	43 (35%)	103 (51%)	<0.001
	Past	20 (16%)	34 (17%)	
	Current	60 (49%)	65 (32%)	
Physical Activity	Active	34 (27%)	94 (47%)	<0.001
Diabetes		4 (3%)	7 (3%)	1
Medication for	Diabetes	2 (2%)	2 (1%)	0.64
	HB pressure	5 (4%)	12 (6%)	0.61
Physical measurements				
BMI kg/m^2 (median, IQR)		22.7 (20.8-27.7)	23.74 (20.4- 27.8)	0.79
Systolic BP (mmHg; mean, SD)		139.6 (15.7)	134.5 (15.4)	0.01
Diastolic BP (mmHg; mean, SD)		97.6(15.7)	96.6 (14.7)	0.37
Glucose (median, IQR)		4.5 (4.1-4.9)	4.7 (4.4-5.2)	0.01
Total cholesterol (median, IQR)		4.0 (3.5-4.9)	4.0 (3.6-4.9)	0.99
HDL cholesterol (median, IQR)		1.4 (1.2-1.6)	1.5(1.2-1.7)	0.11
LDL cholesterol (median, IQR)		2.2 (1.7-2.9)	2.0 (1.7-2.7)	0.21
Triglycerides (median, IQR)		0.9 (0.7-1.3)	1 (0.7-1.5)	0.11

Abbreviations: SD: standard deviation, IQR: inter quartile range, BP: blood pressure, HDL: high-density lipoprotein, LDL: low-density lipoprotein

Table 2. Distribution of heart rate variability.

Variable	HIV status	Mean	Median (IQR)	*p*	Min.	Max.	Spread
TF Power	HIV-	1874.10	1478.50 (1025.5-2278.2)	<0.01	126.88	6792.50	4918.40
	HIV+	1492.25	1191.72 (647.6-2110.9)		4.90	5264.60	5259.70
LF Power	HIV-	504.10	356.20 (203.9-673.8)	<0.001	43.20	1968.90	1925.70
	HIV+	363.50	263.62 (127.2-497.2)		4.90	1885.68	1880.78
HF Power	HIV-	576.36	424.46 (192.6-765.3)	<0.01	12.93	3381.97	3369.04
	HIV+	424.74	265.81 (131.6-536.6)		6.90	2666.84	2659.94
SDNN	HIV-	45.49	43.19 (31.7-54.3)	<0.001	8.37	158.57	150.20
	HIV+	38.03	34.24 (24.8-47.1)		5.57	121.73	121.16
RMSSD	HIV-	49.158	43.30 (28.1-64.2)	<0.001	4.27	231.91	227.64
	HIV+	38.26	32.14 (20.4-47.1)		4.52	154.72	150.20
PNN50	HIV-	25.28	20.40 (3.6-41.0)	<0.001	0.00	73.30	73.30
	HIV+	15.24	7.32 (0.9-24.2)		0.00	76.03	76.03

Abbreviations: IQR: interquartile range, Min.: minimum, Max.: maximum, SDNN: standard deviation of the normal RR intervals, RMSSD: the root of the mean squares of successive RR differences, pNN50: the percentage of RR intervals greater than fifty milliseconds different from its predecessor, TF: total-frequency, LF: low-frequency and HF: high-frequency power.

less educated than the HIV-uninfected participants (p<0.001). HIV-infected participants had lower systolic blood pressure, higher glucose, used significantly less alcohol and were more physically active than HIV-uninfected participants (p<0.01). The medians of both time and frequency domain parameters were significantly lower for HIV-infected participants compared to the HIV-uninfected participants (table 2). The distribution of all HRV parameters was skewed to the right (figure 1).

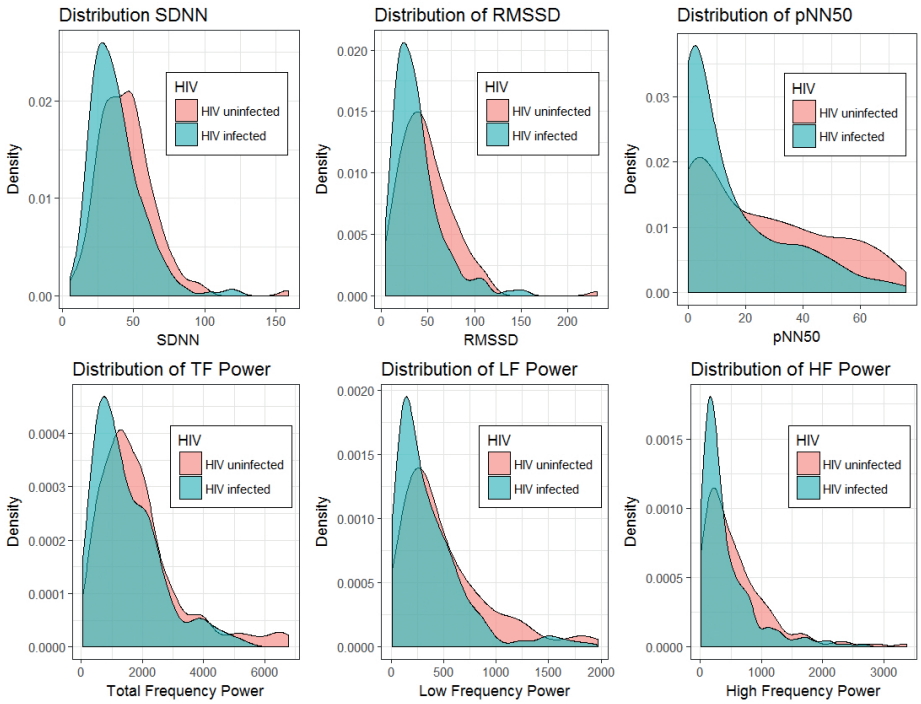


Figure 1. Distribution of heart rate variability. Abbreviations: SDNN: standard deviation of the normal RR intervals, RMSSD: the root of the mean squares of successive RR differences, pNN50: the percentage of RR intervals greater than fifty milliseconds different from its predecessor, TF: total-frequency, LF: low-frequency and HF: high-frequency power.

The multivariable associations with all HRV outcomes are presented in table 3. Model 2 includes age, sex, systolic blood pressure, smoking status, alcohol use, physical activity, total cholesterol and glucose. In addition to these variables, model 3 includes education and income. TG, HDL and LDL cholesterol were excluded due to multicollinearity with each other and/or total cholesterol. Diabetes was excluded due to multicollinearity with glucose. The multivariable models showed a significant inverse association between HIV and log SDNN, log RMSSD and log pNN50. A trend towards an inverse association was observed for HF power.

531207-L-bw-Vos
Processed on: 20-5-2019

Table 3. Association between HRV parameters and HIV.

Model	HIV		Model 1; HIV + Age & Gender		Model 2; Model 1 + classic CVD risk factors		Model 3; Model 2 + education & income	
	HIV		HIV		HIV		HIV	
	Coef.	*p*	Coef.	*p*	Coef.	*p*	Coef.	*p*
log SDNN	-0.19	<0.001	-0.11	0.04	-0.12	0.03	-0.12	0.04
log RMSSD	-0.27	<0.001	-0.15	0.048	-0.16	0.04	-0.16	0.04
log pNN50	-0.91	<0.001	-0.52	0.02	-0.60	<0.01	-0.60	0.01
log TF power	-0.26	<0.01	-0.10	0.23	-0.11	0.20	-0.10	0.25
log LF power	-0.38	<0.001	-0.16	0.12	-0.14	0.19	-0.15	0.17
log HF power	-0.38	<0.01	-0.19	0.10	-0.22	0.06	-0.18	0.13

Model 2 includes physical activity, smoking status, alcohol use, systolic blood pressure, glucose and total cholesterol. HDL, LDL and TG were excluded due to multicollinearity with each other and/or total cholesterol. Diabetes was excluded due to multicollinearity with glucose. Reference category: HIV-uninfected woman with low education, income <600R, non-smoker, no alcohol use and not physical active. Abbreviations: Coef.: coefficient, SDNN: standard deviation of the normal RR intervals, RMSSD: the root of the mean squares of successive RR differences, pNN50: the percentage of RR intervals greater than fifty milliseconds different from its predecessor, TF: total-frequency, LF: low-frequency and HF: high-frequency.

Discussion

HIV-infected participants had a favourable CVD risk profile compared to HIV-uninfected participants, but showed significantly lower median values for all HRV parameters. HIV was an independent risk factor for lower variability on log SDNN, log RMSSD and log pNN50, indicating a decreased functionality of the parasympathetic nervous system. The frequency domain measuring HF and LF showed a trend towards significance; the latter pointing towards a role of HIV affecting the sympathetic nervous system, though representation of the sympathetic nervous system by LF power is debated.(18, 19) Overall, our findings indicate lower HRV and thus a higher risk of CVD for those infected with HIV. In addition, age was found to be associated with a decrease in the adaption of the sympathetic and parasympathetic nervous system.

In contrast to most studies from high-income countries (HIC), we found a lower prevalence of conventional CVD risk factors in the HIV infected population compared to the HIV-uninfected population[4]. These findings are in line with other publications on CVD risk in SSA[24, 25]. The difference in CVD risk profile between the HIV infected population in HIC and low-middle income countries (LMIC) most likely reflects the differences in the HIV epidemic between HIC and LMIC. Whereas in HIC HIV infection is mainly seen in sub-populations like males having sex with males and injecting drug

users, the epidemic in LMIC is affecting the general population. The lifestyle in HIC and LMIC also seem to differ, with a higher prevalence of smoking and obesity in the HIV-infected population in HIC whereas the HIV infected populations in SSA are less often smokers and have a lower BMI compared to the non-infected population[4, 25].

The increased risk of CVD in the HIV infected population that we observed is in line with a large body of literature suggesting that HIV infection increases the risk of CVD[4, 5]. HRV has previously been shown to be related to CVD. In the Framingham Heart Study, a one–standard deviation decrement in log SDNN was associated with a hazard ratio of 1.47 for new cardiac events (95% confidence interval of 1.16 to 1.86)[26]. In the Atherosclerosis Risk in Communities Study, lifetime CVD risk was significantly increased for participants in the lowest compared to the highest tertile of the HRV outcomes LF/HF in men and SDDN, LF and LF/HF in women[27]. Our findings of a decreased HRV in the HIV-infected individuals indicate a higher risk for CVD in the HIV-infected population in SSA, which needs to be confirmed by longitudinal studies assessing overt cardiovascular disease in SSA.

Limitations of this study are related to self-reported socio-demographic and lifestyle information which are potentially subject to social desirability and recall bias[28]. Besides, blood pressure readings were taken with a wrist device, and not with a recommended arm-cuff device[29]. However, the same method was used for HIV-infected versus HIV-uninfected participants and therefore, this does not interfere with the comparison between both groups. A further limitation has been the use of 5-minute HRV measurements. While 24-hour HRV measurements are the golden standard, short 5-minute HRV measurements have been considered methodologically adequate[30].

Major strengths of this study are the presence of an HIV-uninfected control group, allowing to gain insight into the role of HIV on HRV and the taking into consideration of classic CVD risk for assessment of the effect of HIV on CVD risk. As the study was undertaken in a general black rural sub-Saharan African population, the results may be generalizable to other rural SSA populations.

To conclude, we observed a favourable CVD risk profile based on traditional CVD risk factors in the HIV-infected cohort population. However, HIV was negatively associated with HRV, denoting a decreased functioning of the parasympathetic and sympathetic nervous system and thus a higher risk of CVD. Our findings underline the importance of embedding CVD prevention in HIV-care.

Acknowledgements

We gratefully acknowledge the support of the Ndlovu Research Center staff without whom the present study could not have been completed. We would like to thank all the participants of this study.

Authors contributions

NG, AV, and KKG conceptualized the study. NG and VJ undertook data collection. NG analysed the data and wrote the first manuscript draft with support from AV, WV, and KKG. All authors commented on the manuscript, gave final approval and agreed to be accountable for all aspects of work ensuring integrity and accuracy.

Funding

The Ndlovu cohort study is funded by grants from the Dutch AIDS Foundation, Dioraphte Foundation, De Grote Onderneming and the University Medical Center Utrecht. This research received no further specific grants.

Conflict of interest

The authors declare that there is no conflict of interest.

References

1. World Health Organization. Antiretroviral therapy coverage. Data and estimates by WHO region. 2018 [cited 2018 3rd of November]; Available from: http://apps.who.int/gho/data/view.main.23300REGION?lang=en.
2. World Health Organisation. Prevent HIV, test and treat all - WHO support for country impact. 2016 Contract No.: WHO/HIV/2016.24.
3. Deeks SG, Lewin SR, Havlir DV. The End of AIDS: HIV Infection as a Chronic Disease. Lancet. 2013;382(9903):1525-33.
4. Aberg JA. Cardiovascular complications in HIV management: past, present, and future. J Acquir Immune Defic Syndr. 2009;50(1):54-64.
5. Shah ASV, Stelzle D, Lee KK, Beck EJ, Alam S, Clifford S, et al. Global Burden of Atherosclerotic Cardiovascular Disease in People Living With HIV. Circulation. 2018;138(11):1100-12.
6. UNAIDS. The Gap Report. Geneva: 2014. Assesed on the 10th of March 2018.
7. Temple R. Are surrogate markers adequate to assess cardiovascular disease drugs? JAMA. 1999;282(8):790-5.
8. Kleiger RE, Miller JP, Bigger JT, Jr., Moss AJ. Decreased heart rate variability and its association with increased mortality after acute myocardial infarction. The American journal of cardiology. 1987;59(4):256-62. Epub 1987/02/01.
9. Sassi R, Cerutti S, Lombardi F, Malik M, Huikuri HV, Peng CK, et al. Advances in heart rate variability signal analysis: joint position statement by the e-Cardiology ESC Working Group and the European Heart Rhythm Association co-endorsed by the Asia Pacific Heart Rhythm Society. Europace : European pacing, arrhythmias, and cardiac electrophysiology : journal of the working groups on cardiac pacing, arrhythmias, and cardiac cellular electrophysiology of the European Society of Cardiology. 2015;17(9):1341-53. Epub 2015/07/17.
10. McIntosh RC. A meta-analysis of HIV and heart rate variability in the era of antiretroviral therapy. Clin Auton Res. 2016;26(4):287-94.
11. Vanderlei LCM, Pastre CM, Hoshi RA, Carvalho TDd, Godoy MFd. Noções básicas de variabilidade da frequência cardíaca e sua aplicabilidade clínica. Brazilian Journal of Cardiovascular Surgery. 2009;24:205-17.
12. Askgaard G, Kristoffersen US, Mehlsen J, Kronborg G, Kjaer A, Lebech AM. Decreased heart rate variability in HIV positive patients receiving antiretroviral therapy: importance of blood glucose and cholesterol. PLoS One. 2011;6(5):31.
13. Wongcharoen W, Khienprasit K, Phrommintikul A, Sukonthasarn A, Chattipakorn N. Heart rate variability and heart rate turbulence in HIV-infected patients receiving combination antiretroviral therapy. Ann Noninvasive Electrocardiol. 2013;18(5):450-6.

14. Kocher MH, Hetzler RK, Shikuma CM, Kimura IF, Stickley CD, Lindsey RA, et al. Autonomic Function is Associated with Fitness Level in HIV-Infected Individuals. Jacobs J AIDS HIV. 2015;1(1):005.
15. Vos A, Tempelman H, Deville W, Barth R, Wensing A, Kretzschmar M, et al. HIV and risk of cardiovascular disease in sub-Saharan Africa: Rationale and design of the Ndlovu Cohort Study. European Journal of Preventive Cardiology. 2017;24(10):1043-50.
16. Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Circulation. 1996;93(5):1043-65.
17. Billman GE. Heart rate variability - a historical perspective. Front Physiol. 2011;2:86. Epub 2011/12/07.
18. Montano N, Ruscone TG, Porta A, Lombardi F, Pagani M, Malliani A. Power spectrum analysis of heart rate variability to assess the changes in sympathovagal balance during graded orthostatic tilt. Circulation. 1994;90(4):1826-31.
19. Valenza G, Citi L, Saul JP, Barbieri R. Measures of Sympathetic and Parasympathetic Autonomic Outflow from Heartbeat Dynamics. J Appl Physiol. 1985;15(10).
20. World Health Organization. The STEPS Instrument and Support Materials. 2018 [10th of March 2018]; Available at: http://www.who.int/ncds/surveillance/steps/instrument/en/.
21. World Health Organization. Physical Activity. 2018 [cited 2018 10th of March]; Available from: http://www.who.int/mediacentre/factsheets/fs385/en/.
22. Study NID. Wave 3 Adult Questionnaire. 2012 [cited 2018 28th of June]; Available from: http://www.nids.uct.ac.za/documents/wave-3-documents-and-questionnaires/wave-3-questionnaires/158-wave-3-adult-questionnaire.
23. Gaziano TA, Abrahams-Gessel S, Gomez-Olive FX, Wade A, Crowther NJ, Alam S, et al. Cardiometabolic risk in a population of older adults with multiple co-morbidities in rural south africa: the HAALSI (Health and Aging in Africa: longitudinal studies of INDEPTH communities) study. BMC Public Health. 2017;17(1):017-4117.
24. Dillon DG, Gurdasani D, Riha J, Ekoru K, Asiki G, Mayanja BN, et al. Association of HIV and ART with cardiometabolic traits in sub-Saharan Africa: a systematic review and meta-analysis. International journal of epidemiology. 2013;42(6):1754-71. Epub 2014/01/08.
25. Tsuji H, Larson MG, Venditti FJ, Jr., Manders ES, Evans JC, Feldman CL, et al. Impact of reduced heart rate variability on risk for cardiac events. The Framingham Heart Study. Circulation. 1996;94(11):2850-5.
26. Kubota Y, Chen LY, Whitsel EA, Folsom AR. Heart rate variability and lifetime risk of cardiovascular disease: the Atherosclerosis Risk in Communities Study. Ann Epidemiol. 2017;27(10):619-25.

27. Fan X, Miller BC, Park K-E, Winward BW, Christensen M, Grotevant HD, et al. An Exploratory Study about Inaccuracy and Invalidity in Adolescent Self-Report Surveys. Field Methods. 2006;18(3):223-44.

28. Kikuya M, Chonan K, Imai Y, Goto E, Ishii M. Accuracy and reliability of wrist-cuff devices for self-measurement of blood pressure. J Hypertens. 2002;20(4):629-38.

29. Nussinovitch U, Elishkevitz KP, Katz K, Nussinovitch M, Segev S, Volovitz B, et al. Reliability of Ultra-Short ECG Indices for Heart Rate Variability. Ann Noninvasive Electrocardiol. 2011;16(2):117-22.

531207-L-bw-Vos
Processed on: 20-5-2019

CHAPTER

8

Cardiovascular disease risk in an urban African population: what is the role of HIV and antiretroviral treatment?

A.G.Vos, K. Hoeve, R.E. Barth, J. Peper, M. Moorhouse,
N.J. Crowther, W.D.F. Venter, D.E. Grobbee, M.L. Bots,
K.Klipstein-Grobusch

Submitted

Abstract

Introduction Life expectancy is increasing in the HIV-positive population and age-related non-communicable diseases, such as cardiovascular disease, (CVD) are seen more frequently. This study investigated to what extent HIV and antiretroviral therapy (ART) is associated with CVD risk in an urban African population.

Methods A cross-sectional study was performed in Johannesburg, South Africa, between July 2016 and November 2017. Both HIV-positive adults (ART-naïve, or on first- or second-line ART), as well as age and sex matched HIV-negative controls who were family or friends of the HIV-positive participants were included. Data were collected on demographics, cardiovascular risk factors, HIV-related characteristics, carotid intima-media thickness (CIMT) and carotid distensibility. The association between HIV, ART and CIMT and distensibility was analysed with linear regression models, adjusting for age, gender and CVD risk factors.

Results The study included 548 participants, 337 (62%) females, age 38.3 ± 9.5 years of whom 104 (19.0%) were HIV-positive, ART-naïve; 94 (17.2%) were on first-line ART; 197 (35.9%) were on second-line ART; and 153 (27.9%) were HIV-negative. Participants on second-line ART had higher CIMT and lower distensibility compared to the other groups ($p < 0.001$). After adjustment for age, these outcomes were similar between groups. Further adjustment for CVD and HIV-related factors did not alter the findings.

Conclusion Neither HIV nor ART was associated with CIMT or carotid distensibility in this urban African population. Longitudinal studies are needed to fully understand the relationship between HIV and CVD across different populations.

Introduction

Life expectancy has increased for the human immunodeficiency virus (HIV) infected population due to the successful roll out of antiretroviral therapy (ART)[1, 2]. As a result, HIV-positive populations will increasingly experience ageing-related non-communicable diseases (NCDs) such as cardiovascular disease (CVD).

The incidence of NCDs is high in sub-Saharan Africa (SSA). In South Africa, NCDs accounted for 54.7% of all deaths in 2016[3]. Cerebrovascular disease and ischaemic heart disease were ranked fourth and fifth for years of life lost in 2015 and diabetes was the second biggest killer after TB in 2016[3, 4].

In high-income countries (HIC) HIV infection is associated with an increased risk of CVD of up to 50%[5, 6]. The pathophysiology is likely multifactorial and depends on chronic inflammation[7, 8], metabolic side-effects of ART and the burden of classical risk factors for CVD that is likely higher in people with HIV compared to the general population[9].

The situation for SSA is less clear, even though 70% of the world's HIV-infected population resides in this region[10]. It is questionable whether data from HIC can be applied to SSA as the demographics of both HIV-positive populations and the general population differ substantially between these regions. Where the HIV epidemic in HIC is predominantly among white homosexual men and intravenous drug users, the majority of the HIV-infected population in SSA are black, heterosexual women[10]. Lifestyle differences are also apparent from an evaluation of the burden of classic CVD risk factors. In HIC, the percentage of people with hypertension, diabetes and smoking is higher for the HIV-positive group compared to the HIV-negative population[5, 11, 12]. In SSA these risk factors seem to be less prevalent in the HIV-positive population compared to the HIV-negative population[13-15].

The contribution made by ART to the development of CVD is a matter of debate. Although ART restores immune function, inflammatory markers do not return to normal levels, indicating ongoing low-grade inflammation, which is associated with CVD risk[16-18]. Older ART regimens evidently carry a high risk of metabolic side-effects, including lipodystrophy, altered glucose metabolism and dyslipidaemia, and have been associated with increased carotid intima-media thickness (CIMT), a marker of subclinical atherosclerosis[19]. Newer ART regimens have a lower risk of metabolic side-effects, yet markers of endothelial damage still seem to be higher in the HIV-positive population on ART compared to the HIV-negative population[20, 21]. This underlines the importance of taking ART regimens into account when evaluating CVD risk in HIV-positive individuals.

It is difficult to obtain data on the long-term effects of HIV and ART on CVD risk as many participants and years of follow-up are needed in studies using clinical endpoints

such as myocardial infarction and stroke. However, alternative markers associated with future clinical CVD events are available and can be used to estimate CVD risk. CIMT is an established proxy for CVD risk in both white and black populations[22]. Arterial distensibility is a marker of subclinical atherosclerosis and is an independent predictor of cardiovascular mortality[23]. Arterial stiffening, and hence reduced distensibility, occurs as part of normal ageing and is accelerated by a number of conditions including hypertension, diabetes mellitus and renal failure[24].

Therefore, this study's aim is to gain insight into the influence of HIV and ART on CIMT and carotid distensibility, and hence CVD risk, in an urban African population.

Methods

Recruitment

We performed a cross-sectional study including four groups of participants aged ≥ 18 years between July 2016 and November 2017 at Charlotte Maxeke Johannesburg Academic Hospital, Johannesburg, South Africa. All HIV-positive participants were diagnosed during routine HIV testing programmes in inner-city Johannesburg. The first group included newly diagnosed HIV-positive participants. Participants were recruited from an ongoing randomised controlled trial (RCT) comparing two new first-line ART regimens with the current standard of care first-line ART regimen (trial WRHI 060, ClinicalTrials.gov Identifier: NCT03122262). Participants had HIV-1 infection and plasma HIV-1 RNA ³ 500 copies/mL at screening and no exposure to ART. Participants were eligible for participation in our study any moment from enrolment in WRHI 060 to a maximum follow-up duration of 36 weeks. The second group was on a first-line tenofovir containing regimen for at least 2.5 years. Participants were recruited from an RCT completed in 2016, comparing two different first line ART-regimens and included 600 HIV-positive, ART-naïve individuals in Johannesburg, South Africa, between 2012 and 2014[25]. The third group included participants on second-line ART. They were recruited from an open label randomised study to demonstrate non-inferiority of low-dose ritonavir-boosted darunavir compared with ritonavir-boosted lopinavir-based second-line ART regimen[26]. Inclusion criteria for this trial were second-line ART for at least 48-weeks and plasma HIV-1 RNA levels < 50 copies/mL. Participants of this trial were approached for enrolment in our project during the baseline or one of the follow-up visits. The fourth group consisted of HIV-negative participants recruited from the local community. The HIV-positive participants were invited to refer a family member or friend of the same age (± 5 years) and gender who was not known to be HIV-positive. All participants without a documented HIV-positive status underwent HIV testing upon enrolment according to South African Department of Health HIV testing guideline[27]. If a participant tested HIV-positive and there was no history of ART use they were

assigned to the first group (HIV-positive, ART-naïve), and referred to a local clinic to initiate ART. If a participant that tested positive for HIV was found to be receiving ART, he/she was included in the group on first- or second-line ART, depending on their current ART regimen.

Data collection

Data were collected on demographics, medical history and medication use, including HIV status and ART use, cardiovascular risk factors using a modified version of WHO-STEPs questionnaire[28] and physical activity in Metabolic Equivalent for Task (MET) minutes per week using the International Physical Activity Questionnaire[29]. Students, retirees, disabled and volunteers were considered unemployed. Being married or cohabitating was considered a stable relationship. A positive family history of CVD was defined as a history of stroke and/or heart attack of a first-degree family member (parent or sibling) before the age of 60 years. A physical examination included measurement of height, weight, waist and hip circumference. Blood pressure was measured in a seated position after a 5-minute rest on the left arm and the right arm, and repeated on the arm with the highest value. The average of the second and third measurement was used for analysis. Blood was drawn for the analysis of creatinine, random glucose and lipids, and from HIV-positive participants also for HIV viral load. Urine was collected for analysis of albumin to creatinine ratio. An accredited laboratory analysed blood and urine samples. For the HIV-positive, ART-naïve participants who were recruited from the WRHI 060 trial and for participants on second-line ART, laboratory data of the clinical trial visit closest to the visit at our study site was used. For participants on first-line ART, HIV viral load and CD4+ cell count were retrieved from the last RCT study visit.

Outcome measurement

CIMT was measured in all participants after a 15 minute rest. We used a Siemens Acuson p500 ultrasound (Siemens Healthcare (Pty) Ltd, South Africa) and scans were obtained in B-mode with a ≥ 7.0 MHz linear probe. The near wall and far wall of the common carotid artery (CCA) was measured at three standardised angles at both the right and left side using the Meijer's Arc[30]. The far wall of the carotid bulb on the right and left side was captured at the best visible angle. A 5-second video-clip of both arteries was made with the transducer positioned in line with the CCA one centimetre from the carotid bifurcation. CIMT was measured semi-automatically with the Artery Measurement System software (Chalmers University, Götenburg, Sweden) and adjusted manually if needed[31]. Analyses were done in batch with a uniform reading protocol. CIMT reading included mean and maximum thickness of the intima-media layer of the near and far wall across all six angles of the CCA (mean CCA intima-media thickness (IMT) and max CCA-IMT), and the maximum IMT at the carotid bulb left and right (max

bulb-IMT). A mean CCA-IMT of > 1.0 mm at any of the measured angles was considered as a plaque[32].

For carotid distensibility, approximately 100 discrete measurement points were used to obtain the maximum (LDmax) and minimum (LDmin) carotid lumen diameter in a 10 mm segment within 20mm from the bifurcation. One reader read the CIMT images and two readers analysed carotid distensibility clips. The readers were blinded to the HIV status and treatment group. Inter-reader agreement for the clips was 0.90 (95% CI 0.62 – 0.96) using the intra-class correlation coefficient with a two-way random-effects model.

Pulse pressure (PP) was calculated by subtracting the diastolic blood pressure from the systolic blood pressure. The carotid distensibility was calculated according to the following formula:

$$\text{Distensibility} = \frac{\left(\frac{2(\text{LDmin-LDmax})}{\text{LDmin}}\right)}{\text{PP}}$$

Lower values reflect a stiffer carotid artery. Distensibility was standardized according to units in 10^{-6} * New-tons^{-1} * metres^{2}[33]. Measurements of the right CCA were used for analysis[34-36].

The study was approved by the Human Ethics Research Committee of the University of the Witwatersrand (ethics clearance number M160130) and performed conform the Declaration of Helsinki. All participants provided written, informed consent prior to participation.

Statistical analysis

Baseline characteristics were presented for each of the four groups as mean (standard deviation (SD)), median (interquartile range (IQR)) or frequency count (percentage (%)) as appropriate. First, CIMT outcomes and carotid distensibility were compared across the groups using the Kruskal-Wallis test with *post-hoc* testing using a Bonferroni correction. Second, the relationship of HIV and ART status with mean and max CCA-IMT, max bulb-IMT and carotid distensibility was analysed using linear regression models.

The first model included all groups using the HIV-negative group as reference group with no adjustments; the second model was adjusted for age; the third model was adjusted for age and sex; and the fourth model was further adjusted for CVD risk factors that were shown in the literature to be related to CIMT, namely systolic blood pressure, body mass index, LDL cholesterol, HDL cholesterol, glucose and current smoking [30]. An additional analysis was performed using the same method including the HIV-

positive participants only and using the ART-naïve group as the reference to assess the contribution of CD4+ cell count, HIV viral load and duration since HIV diagnosis. Third, models were run separately for men and women, and a possible interaction between HIV status and age was investigated by adding an interaction term to the models. A two-sided $p < 0.05$ was considered as statistically significant. Statistical analyses were performed using IBM SPSS Statistics Version 25 (SPSS, Chicago, Illinois, USA).

Results

In total, 548 participants were included: 153 HIV-negative controls; 104 newly diagnosed HIV-positive ART-naïve participants; 94 participants with HIV on stable first-line therapy; and 197 participants on stable second-line ART (table 1). All except one were Black African, the majority were women (n=337, 61.5%) and the mean age was 38.3 (SD 9.5) years. Overall, 38.4% completed matric or university, and most participants were single. Employment varied significantly with the highest employment rate for participants on first-line ART (82.8%) and the lowest employment rate for the HIV-negative controls (32.9%). Participants on second-line ART were older, more likely to be women, and weighed more than the other participants. Participants on first-line ART knew their HIV diagnosis for about four years, and participants on second-line ART for approximately nine years.

Table 1. Characteristics of the study population.

	HIV-negative	HIV-positive		
		ART-naïve	First-line ART	Second-line ART
	n=153	n=104	n=94	n=197
Demographics and socio-economic status				
Age (years)	34.6 (10.6)	33.9 (8.4)	36.9 (6.4)	43.0 (8.0)
Women, *n*(%)	76 (49.7)	65 (62.5)	57 (60.6)	139 (70.6)
Highest level of education (n=542), *n*(%)				
Primary school or less	12 (7.9)	13 (12.7)	10 (10.8)	27 (13.8)
Secondary school completed	74 (48.7)	51 (50.0)	51 (54.8)	96 (49.2)
More than secondary school	66 (43.4)	38 (37.2)	32 (34.4)	72 (37.0)
Work status (n=545), *n*(%)				
Employed	50 (32.9)	55 (53.4)	77 (82.8)	132 (67.0)
Unemployed	102 (76.1)	48 (46.6)	16 (17.2)	65 (33.0)
Marital status (n=545), *n(%)*				
Married or in a stable relationship	31 (20.4)	15 (14.6)	36 (38.7)	75 (38.1)
Single	121 (79.6)	88 (85.4)	57 (61.3)	122 (61.9)
Cardiovascular risk profile				
Family history of CVD (n=547), *n*(%)	7 (4.6)	2 (1.9)	4 (4.3)	21 (10.7)
Physical activity, MET min/week	1200 (720 – 1920)	1198 (868 – 1693)	834 (445 – 2092)	984 (480 – 1688)
Current smokers (n=113), *n(%)*	56 (37.1)	27 (26.2)	13 (13.8)	17 (8.6)
Packyears for current smokers, (median, IQR)	3.6 (1.3-7.3)	3.9 (2.2-6.8)	2.2 (0.7-3.6)	7.4 (2.3-12.1)
Former smokers (n=34), *n*(%)	6 (4.0)	7 (6.8)	7 (7.4)	14 (7.1)
Never smokers (n=398), *n*(%)	89 (58.6)	69 (67.0)	74 (78.7)	166 (84.3)
Systolic blood pressure (mmHg) (n=547)	123.8 (18.5)	119.5 (15.1)	124.2 (17.2)	121.5 (18.8)
Diastolic blood pressure (mmHg) (n=547)	77.8 (12.8)	75.2 (8.8)	81.4 (10.4)	77.8 (11.5)
Body mass index (kg/m^2) (median, IQR)	24.5 (21.2 – 28.9)	23.6 (20.9 – 27.0)	24.2 (21.3 – 29.1)	26.3 (22.9 – 32.0)
<18.5 kg/m^2, *n(%)*	9 (5.9)	7 (6.7)	3 (3.2)	4 (2.0)
18.5 – 24.9 kg/m^2, *n(%)*	74 (48.4)	57 (54.8)	49 (52.1)	76 (38.6)
25.0 – 29.9 kg/m^2, *n(%)*	36 (23.5)	25 (24.0)	20 (21.3)	50 (25.4)
≥ 30.0 kg/m^2, *n(%)*	34 (22.2)	15 (14.4)	22 (23.4)	67 (34.0)
Waist-to-hip ratio (n=547)	0.85 (0.06)	0.85 (0.06)	0.85 (0.09)	0.87 (0.06)

Table 1. Continued

	HIV-negative	HIV-positive		
		ART-naïve	First-line ART	Second-line ART
	n=153	n=104	n=94	n=197
Total-C (mmol/L) (n=522)	4.1 (0.9)	3.9 (0.8)	4.4 (1.1)	4.8 (0.8)
HDL (mmol/L) (n=522)	1.4 (0.5)	1.2 (0.5)	1.4 (0.4)	1.4 (0.4)
LDL (mmol/L) (n=522)	2.2 (0.7)	2.3 (0.7)	2.5 (0.9)	3.1 (0.8)
TG (mmol/L), (n=519), median (IQR)	1.0 (0.7-1.3)	0.9 (0.7-1.2)	1.0 (0.8-1.5)	1.2 (0.9-1.7)
Total-C:HDL ratio (n=522)	3.1 (0.9)	3.4 (1.0)	3.4 (1.1)	3.7 (1.1)
Random glucose (mmol/L) (n=514)	4.9 (1.0)	4.5 (0.9)	5.0 (0.9)	4.8 (1.6)
Creatinine clearance (mL/min) (n=523)	119.7 (32.3)	122.9 (33.9)	121.9 (33.2)	120.7 (38.3)
Urine protein present (n=164), *n*(%)	N/A	7 (9.1)	2 (2.3)	N/A
Albumin-to-creatinine ratio, (n=439), median (IQR)	0.8 (0.4-1.5)	0.9 (0.5-1.9)	0.7 (0.5-1.5)	0.9 (0.5-1.8)
HIV/ART characteristics (n=395)				
CD4-cell count (cells/mm^3) (n=350), median (IQR)	-	281[†] (192-401)	414 (279-575)	619 (430-798)
Viral load (cp/mL) (n=380), *n*(%) < 50	-	12 (11.8)	81 (91.0)	180 (95.2)
50-1000	-	10 (9.8)	3 (3.4)	4 (2.1)
> 1000	-	80 (78.4)	5 (5.6)	5 (2.6)
Years since diagnosis (n=393), median (IQR)	-	0.0 (0.0-0.1)	3.9 (3.1-6.0)	9.0 (7.0-12.4)
Time on ART (n=355), months median (IQR)	-	0 (0.0 – 0.0)	39 (35 – 48)	96 (72 – 126)

Outcomes in mean with SD unless otherwise specified.

[†,] Nadir CD4-cell count . Abbreviations: AP: angina pectoris; ART: antiretroviral therapy; CVA: cerebrovascular accident, CVD: cardiovascular disease, HDL-C: high-density lipoprotein cholesterol, HIV: human immunodeficiency virus, IQR: interquartile range, LDL-C: low density lipoprotein cholesterol, MET: metabolic equivalent of Task, MI: myocardial infarction, N/A: not available, SD: standard deviation, Total-C: total cholesterol, TG: triglycerides.

CCA-IMT was available for 534 (97.4%) participants, bulb-IMT for 474 (86.5%), and carotid distensibility for 514 (93.8%) participants. Mean and max CCA-IMT and max bulb-IMT were significantly higher and carotid distensibility was significantly lower for participants on second-line ART compared to the other groups (table 2a and 2b).

Table 2a and 2b. CIMT and carotid distensibility per group.

CIMT per group (n = 534)					
	HIV-negative	HIV-positive			
		ART-naïve	First-line ART	Second-line ART	*P*
	n= 152	n=104	n=87	n= 191	
CCA mean (mm)	0.504 (0.477 – 0.562)	0.504 (0.470 – 0.560)	0.521 (0.488 – 0.564)	0.564 (0.512 – 0.655)	<0.001†
CCA max (mm)	0.575 (0.525 – 0.669)	0.575 (0.523 – 0.651)	0.594 (0.554 – 0.653)	0.667 (0.598 – 0.756)	<0.001†
Plaque¶	0	1 (1.0%)	0	5 (2.6%)	0.08
	n=133	n=98	n=80	n=163	
Bulb max (mm)	0.686 (0.583 – 0.802)	0.673 (0.542 – 0.824)	0.717 (0.579 – 0.797)	0.809 (0.698 – 0.971)	<0.001†
Carotid distensibility (10^{-6} N^{-1} m^2) per group (n = 514)					
	n =148	n = 100	n = 82	n = 184	
Pulse Pressure (mmHg)	45.5 (38.5 – 51.0)	42.5 (39.0 – 50.0)	41.5 (34.5 – 50.6)	41.5 (36.6 – 49.5)	0.06
LD min right CCA (mm)	5.765 (5.331 – 6.160)	5.813 (5.444 – 6.102)	5.699 (5.423 – 6.098)	5.706 (5.421 – 6.155)	0.932
LD diff right CCA (mm)	0.633 (0.489 – 0.784)	0.618 (0.475 – 0.769)	0.566 (0.451 – 0.709)	0.530 (0.434 – 0.632)	<0.001‡
Distensibility (10^{-6} N^{-1} m^2)	36.96 (27.03 – 48.00)	37.82 (26.75 – 50.12)	35.91 (27.45 – 45.04)	31.68 (25.93 – 40.85)	0.009§

Outcomes in median with IQR.

† p<0.001 for second-line ART vs ART-naïve, second-line ART vs first-line ART and second-line ART vs HIV-negative

‡ p=0.002 for second-line ART vs ART-naïve and p <0.001 for second-line ART vs HIV-negative

§ p=0.025 for second-line ART vs ART-naïve and p <0.036 for second-line ART vs HIV-negative

¶ mean CCA thickness >1.0mm at any angle.

Legend: ART; anti-retroviral therapy, CCA; common carotid artery, CIMT; carotid artery intima-media thickness, LD; luminal diameter, mm; millimeter. IQR; interquartile range.

The p-value is based on the Kruskal-Wallis test with post-hoc testing using a Bonferroni correction.

There were only a few participants with plaque in the CCA. When CCA-IMT and distensibility outcomes were adjusted for age, differences between the groups disappeared. Further adjustment for CVD risk factors did not change the magnitude and direction of the relation between HIV, ART and mean or max CCA-IMT or carotid distensibility (see table 3a and 3b for the models for mean CCA-IMT and carotid distensibility). Following multivariable adjustment age (β=0.006, p<0.001), systolic blood pressure (β=0.000, p=0.01) and LDL cholesterol (β=0.009, p=0.03) were associated with mean CCA-IMT. The same factors contributed to max CCA-IMT (data not shown).

Table 3a and 3b. HIV and ART status on mean CCA intima-media thickness and carotid distensibility.

HIV and ART status on mean CCA intima-media thickness (n = 534)							
	HIV-negative			HIV-positive			
		ART-naïve	*p*	First-line ART	*p*	Second-line ART	*p*
Model 1	REF	-0.015 (-0.038 – 0.007)	0.178	-0.001 (-0.025 – 0.023)	0.239	0.052 (0.033 – 0.071)	<0.001
Model 2	REF	-0.010 (-0.028 – 0.007	0.239	-0.012 (-0.031 – 0.006)	0.187	0.000 (-0.016 – 0.016)	0.963
Model 3	REF	-0.009 (-0.027 – 0.008)	0.287	-0.012 (-0.030 – 0.007)	0.219	0.002 (-0.015 – 0.018)	0.854
Model 4†	REF	-0.011 (-0.029 – 0.008)	0.253	-0.012 (-0.032 – 0.007)	0.224	-0.005 (-0.023 – 0.013)	0.557
HIV and ART status on carotid distensibility (10^{-6} N^{-1} m^2) (n = 514)							
Model 1	REF	1.314 (-2.707 – 5.335)	0.521	-1.365 (-5.641 – 2.911)	0.531	-4.315 (-7.745 - -0.885)	0.014
Model 2	REF	0.675 (-2.939 – 4.290)	0.714	0.280 (-3.573 – 4.134)	0.886	2.387 (-0.916 – 5.690)	0.156
Model 3	REF	0.470 (-3.165 – 4.104)	0.800	0.107 (-3.759 – 3.973)	0.957	2.019 (-1.353 – 5.391)	0.240
Model 4‡	REF	-1.567 (-4.891 – 1.757)	0.355	0.268 (-3.351 – 3.888)	0.884	0.278 (-3.046 – 3.601)	0.870

β-coefficient with 95% CI.

Model 1 unadjusted

Model 2 adjusted for age

Model 3 adjusted for age and sex

Model 4 adjusted for age, sex, systolic blood pressure, body mass index, LDL cholesterol, HDL cholesterol, glucose and current smoking

† n = 37 (6.9%) participants were excluded from this model due to missing data in one or more covariates

‡ n = 35 (6.8%) participants were excluded from this model due to missing data in one or more covariates

Legend: ART; antiretroviral therapy, CCA; common carotid artery, CI; confidence interval, HIV; human immunodeficiency virus, REF; reference.

Max bulb-IMT was lower in HIV-positive ART-naïve participants compared to HIV-negative participants (β=-0.070, p=0.03) following multivariable adjustment and participants on first-line ART tended to have a lower max bulb-IMT (β=-0.065, p=0.07). Max bulb-IMT did not differ between participants on second-line ART and HIV-negative controls (p=0.20) (supplementary table 1).

Table 4. ART status and common carotid artery IMT and carotid distensibility.

ART status and common carotid artery IMT (n = 382)					
	ART-naïve	First-line ART	*p*	Second-line ART	*p*
Model 1	REF	0.014 (-0.011 – 0.039)	0.259	0.067 (0.046 – 0.088)	<0.001
Model 2	REF	-0.002 (-0.022 – 0.018)	0.829	0.009 (-0.010 – 0.028)	0.341
Model 3	REF	-0.002 (-0.023 – 0.018)	0.826	0.011 (-0.008 – 0.029)	0.272
Model 4†	REF	-0.012 (-0.038 – 0.014)	0.360	-0.016 (-0.046 – 0.014)	0.291
ART status and carotid distensibility (10^{-6} N^{-1} m^2) (n = 366)					
Model 1	REF	-2.679 (-7.221 – 1.864)	0.247	-5.629 (-9.417 - -1.841)	0.004
Model 2	REF	-0.565 (-4.816 – 3.686)	0.794	1.166 (-2.755 – 5.087)	0.559
Model 3	REF	-0.562 (-4.819 – 3.694)	0.795	1.107 (-2.855 – 5.070)	0.583
Model 4‡	REF	1.090 (-3.698 – 5.879)	0.654	-0.042 (-5.646 – 5.563)	0.988

β-coefficient with 95% CI.
Model 1 unadjusted
Model 2 adjusted for age
Model 3 adjusted for age and sex
Model 4 adjusted for age, sex, systolic blood pressure, body mass index, LDL cholesterol, HDL cholesterol, glucose and current smoking
† n = 57 (14.9%) participants were excluded from this model due to missing data in one or more covariates
‡ n = 56 (15.3%) participants were excluded from this model due to missing data in one or more covariates
Legend: ART; antiretroviral therapy, IMT; intima media thickness

Finally, men and women were analysed separately and the same relations were observed (data not shown). There was no indication that the effect of HIV on CIMT or carotid distensibility differed between younger and older participants as the interaction term between age and HIV was not statistically significant.

Discussion

HIV and ART were not related to increased CIMT and reduced carotid distensibility and hence CVD risk in this well-characterised African population. Participants on second-line ART had a higher CIMT and lower carotid distensibility, but this was explained by participants being older in this group. Max bulb-IMT was lower for HIV-positive ART-naïve participants than for HIV-negative participants. Additional correction for HIV-related factors such as CD4+ cell count, HIV viral load and duration since HIV diagnosis did not change the direction or magnitude of the relationship between HIV, ART and CVD risk.

There is no consensus in the literature on the relationship between HIV and CIMT. Several studies, all conducted outside SSA, comparing HIV-positive individuals, whether or not on ART, to HIV-negative individuals found a higher CIMT for the HIV-infected group, indicating a higher CVD risk[34, 37-40]. Furthermore, HIV infection seems to be related to CIMT as the level of viraemia influences CIMT, with a lower CIMT for people with an undetectable viral load as compared to people with low-level viraemia[41]. However, other studies did not find a difference in baseline CIMT between HIV-positive and HIV-negative participants[42-44].

The association between HIV and carotid distensibility has previously been addressed in two studies conducted in HIC. The first included 77, predominantly white, males (median age 42.3 years), including both ART-naïve patients and patients on ART[34]. The second study included 2,789 participants, half of them Afro-Americans; the average age was 44.3 years[35]. Both observed HIV to be associated with a significantly lower carotid distensibility, and hence higher arterial stiffness.

The influence of ART on CIMT is also debated. Some studies showed a higher CIMT for people on ART[39, 40], whereas other studies showed no difference in CIMT between HIV-positive, both untreated and ART-treated groups[44, 45]. The class of antiretrovirals may influence the association with CIMT[46, 47]. ART use was also not consistently associated with arterial stiffness[34, 35].

Results presented in the current analysis are in line with those from other, smaller, studies conducted in SSA. Gleason et al., 2015[48], Fourie et al., 2015[45] and Mosepele et al., 2017[49] all compare HIV-positive to HIV-negative participants in Ethiopia, South Africa and Botswana respectively, and observed no increase in CIMT based on HIV or ART status. Fourie et al.,[45] even found a lower CCA-IMT for the HIV-positive compared to the HIV-negative group. We found a lower max bulb-IMT, but not CCA-IMT, for HIV-positive participants not yet on ART compared to HIV-negative participants. The reason for this finding is not known, however it is interesting to note that previous studies in

SSA have shown a lower prevalence of classic CVD risk factors in HIV-positive compared to HIV-negative subjects[13-15].Our findings should be confirmed in future research. Schoffelen et al., 2014[50] in a cross-sectional study performed in South-Africa in HIV-positive participants, showed a relationship of classic CVD risk factors to CIMT, but not for HIV-related factors. In accordance with this, none of the HIV-related factors in our study were associated with CIMT. These findings suggest that neither HIV nor ART increase CIMT in a Black African population.

To interpret the conflicting findings on the relationship between HIV and CIMT the following arguments should be considered. First, studies vary in sample size, and a small sample size is more likely to result in biased findings. Second, the duration of uncontrolled HIV infection should be taken into account as this was strongly related to CVD[51] and, to a lesser extent, CIMT[38, 40]. Third, the selection of the HIV-negative control group can lead to biased findings as controls are often matched on age and gender only and not on socio-economic status. The HIV-positive groups in HIC represent a group of people with a specific lifestyle and higher CVD risk than the general population. Although studies correct for CVD risk factors there may be unmeasured factors that result in a higher CVD risk, and hence CIMT, in the HIV-positive population.

We did not find an interaction between HIV and age on CIMT. This is in contrast to a recent pooled analysis of CIMT across five cohorts of HIV-positive and HIV-negative individuals. In this analysis the relationship between HIV and CIMT was found to be age dependent: for the age group 30-49 years no association was seen between HIV and CIMT, whereas from the age of 50 years HIV infection was associated with lower CIMT than in the HIV-negative group[52]. Traditional CVD risk factors were observed to be the main drivers of CIMT and this effect increased with age[52]. In our study the number of participants over the age of 50 years was relatively small (10.9%), which might explain why we did not find an age-dependent relation between HIV and CIMT.

In summary, our findings do not support the view that CVD risk is increased in the HIV-infected population compared to the non-infected population. The question remains whether CIMT and carotid distensibility are appropriate surrogate markers to detect a difference in CVD risk in a relatively young and otherwise healthy population. Two studies in SSA could not identify an association between CIMT and elevated markers of endothelial damage s-VCAM and s-ICAM[21, 45], indicating that there is endothelial damage even when CIMT is still normal.

To date, most research in SSA using clinical endpoints such as myocardial infarction and stroke observed an increased risk for the HIV-infected population[53-55]. A recent meta-analysis using national disability-adjusted life-year estimates for cardiovascular disease found that people with HIV are twice as likely to develop cardiovascular disease

as compared to the non-infected population, and that this burden was higher in SSA than in HIC[56], possibly indicating that CVD risk is increased in HIV infection in SSA.

Major strengths of our study are the large sample size; the use of an HIV-negative control group; the comparison between HIV-infected participants not yet on treatment, on stable first-line and stable second-line ART; and the extensive, standardised assessment of CVD risk.

However, some limitations need to be recognised. We used a full case analysis and this resulted in 15% missing data in the models assessing the influence of HIV-related characteristics. Assuming data were missing completely at random, any bias is likely to be small. HIV-positive participants were recruited from clinical trials using stringent inclusion criteria while the HIV-negative controls were recruited from the community. This may lead to an underestimation of the CVD risk in the HIV-positive participants; although impaired kidney function was the only CVD risk factor that was used as an exclusion criterion in the RCTs. The high unemployment rate in the HIV-negative group indicates that unemployed people were keener to participate than people who would have had to take leave from work. In addition, this group had slightly more men than the HIV-positive groups and a high percentage of current smokers. Therefore, it might be possible that the HIV-negative group does not precisely reflect CVD risk in the general, non-HIV infected population precisely. Finally, we could not correct for HIV severity at start of ART as we only had nadir CD4+ cell count for the newly diagnosed HIV-positive participants.

In conclusion, in this urban South African cohort, neither HIV nor ART were associated with CVD risk as assessed by CIMT and carotid distensibility. The question remains whether underlying immune activation and endothelial damage have not yet resulted in increased CIMT and reduced carotid distensibility in this young population. Future research using clinical endpoints such as stroke and myocardial infarction is needed to gain further insight into the role of HIV and ART on CVD risk in the SSA context. Routine HIV care should focus on prevention and treatment of CVD risk factors known to be major drivers of CVD risk.

Acknowledgements

We would like to thank the research nurse, Ms. E. Taylor, and all study participants without whom this study would not be possible.

Author contribution

A.G.V., R.E.B., M.M., N.J.C., W.D.F.V., D.E.G. and K.K.G. contributed to the conception or design of the work. A.G.V., K.H., J.P., and M.L.B. contributed to the acquisition, analysis or interpretation of data for the work. A.G.V., K.H., R.E.B., K.K. drafted the manuscript. K.H., R.E.B., J.P., M.M., N.J.C., W.D.F.V., D.E.G., M.L.B. and K.K.G. critically revised the manuscript. All gave final approval and agree to be accountable for all aspects of work ensuring integrity and accuracy.

Funding

A.G. Vos and N.J. Crowther received a grant from the National Health and Laboratory Sciences, South-Africa in 2015 (grant no. 94566). The laboratory analysis was paid from this grant.

Conflicts of Interest

None were declared

References

1. Legarth RA, Ahlstrom MG, Kronborg G, Larsen CS, Pedersen C, Pedersen G, et al. Long-Term Mortality in HIV-Infected Individuals 50 Years or Older: A Nationwide, Population-Based Cohort Study. J Acquir Immune Defic Syndr 2016; 71:213-218.
2. Pillay-van Wyk V, Msemburi W, Laubscher R, Dorrington RE, Groenewald P, Glass T, et al. Mortality trends and differentials in South Africa from 1997 to 2012: second National Burden of Disease Study. The Lancet Global Health 2016; 4:e642-e653.
3. Stats SA. Mortality and causes of death in South Africa, 2016: Findings from death notification. Statistical release P0309.3. 27 March 2018;.
4. Massyn N, Padarath A, Peer N, Day C. District Health Barometer 2016/17. Health Systems Trust November 2017;.
5. 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; 92:2506-2512.
6. Freiberg MS, Chang CC, Kuller LH, Skanderson M, Lowy E, Kraemer KL, et al. HIV infection and the risk of acute myocardial infarction. JAMA Intern Med 2013; 173:614-622.
7. Miedema F, Hazenberg MD, Tesselaar K, van Baarle D, de Boer RJ, Borghans JA. Immune activation and collateral damage in AIDS pathogenesis. Front Immunol 2013; 4:298.
8. Nou E, Lo J, Grinspoon SK. Inflammation, immune activation, and cardiovascular disease in HIV. AIDS 2016; 30:1495-1509.
9. Knobel H, Domingo P, Suarez-Lozano I, Gutierrez F, Estrada V, Palacios R, et al. Rate of cardiovascular, renal and bone disease and their major risks factors in HIV-infected individuals on antiretroviral therapy in Spain. Enferm Infecc Microbiol Clin 2018;.
10. Joint United Nations Programme on HIV/AIDS (UNAIDS). UNAIDS DATA 2017. UNAIDS/JC2910E 2017;.
11. Vidrine DJ. Cigarette smoking and HIV/AIDS: health implications, smoker characteristics and cessation strategies. AIDS Educ Prev 2009; 21:3-13.
12. Mdodo R, Frazier EL, Dube SR, Mattson CL, Sutton MY, Brooks JT, et al. Cigarette smoking prevalence among adults with HIV compared with the general adult population in the United States: cross-sectional surveys. Ann Intern Med 2015; 162:335-344.
13. Waweru P, Anderson R, Steel H, Venter WD, Murdoch D, Feldman C. The prevalence of smoking and the knowledge of smoking hazards and smoking cessation strategies among HIV- positive patients in Johannesburg, South Africa. S Afr Med J 2013; 103:858-860.
14. Clark SJ, Gomez-Olive FX, Houle B, Thorogood M, Klipstein-Grobusch K, Angotti N, et al. Cardiometabolic disease risk and HIV status in rural South Africa: establishing a baseline. BMC Public Health 2015; 15:135-015-1467-1.

15. Gaziano TA, Abrahams-Gessel S, Gomez-Olive FX, Wade A, Crowther NJ, Alam S, et al. Cardiometabolic risk in a population of older adults with multiple co-morbidities in rural South Africa: the HAALSI (Health and Aging in Africa: longitudinal studies of INDEPTH communities) study. BMC Public Health 2017; 17:206-017-4117-y.
16. Hattab S, Guiguet M, Carcelain G, Fourati S, Guihot A, Autran B, et al. Soluble biomarkers of immune activation and inflammation in HIV infection: impact of 2 years of effective first-line combination antiretroviral therapy. HIV Med 2015; 16:553-562.
17. Rudy BJ, Kapogiannis BG, Worrell C, Squires K, Bethel J, Li S, et al. Immune Reconstitution but Persistent Activation After 48 Weeks of Antiretroviral Therapy in Youth With Pre-Therapy CD4 >350 in ATN 061. J Acquir Immune Defic Syndr 2015; 69:52-60.
18. Mosepele M, Hemphill LC, Moloi W, Moyo S, Nkele I, Makhema J, et al. Pre-clinical carotid atherosclerosis and sCD163 among virally suppressed HIV patients in Botswana compared with uninfected controls. PLoS One 2017; 12:e0179994.
19. Gleason RL,Jr, Caulk AW, Seifu D, Rosebush JC, Shapiro AM, Schwartz MH, et al. Efavirenz and ritonavir-boosted lopinavir use exhibited elevated markers of atherosclerosis across age groups in people living with HIV in Ethiopia. J Biomech 2016; 49:2584-2592.
20. Graham SM, Rajwans N, Jaoko W, Estambale BB, McClelland RS, Overbaugh J, et al. Endothelial activation biomarkers increase after HIV-1 acquisition: plasma vascular cell adhesion molecule-1 predicts disease progression. AIDS 2013; 27:1803-1813.
21. Mosepele M, Mohammed T, Mupfumi L, Moyo S, Bennett K, Lockman S, et al. HIV disease is associated with increased biomarkers of endothelial dysfunction despite viral suppression on long-term antiretroviral therapy in Botswana. Cardiovasc J Afr 2018; 29:155-161.
22. Owolabi MO, Agunloye AM, Umeh EO, Akpa OM. Can common carotid intima media thickness serve as an indicator of both cardiovascular phenotype and risk among black Africans? Eur J Prev Cardiol 2015; 22:1442-1451.
23. Willum-Hansen T, Staessen JA, Torp-Pedersen C, Rasmussen S, Thijs L, Ibsen H, et al. Prognostic value of aortic pulse wave velocity as index of arterial stiffness in the general population. Circulation 2006; 113:664-670.
24. Townsend RR, Wilkinson IB, Schiffrin EL, Avolio AP, Chirinos JA, Cockcroft JR, et al. Recommendations for Improving and Standardizing Vascular Research on Arterial Stiffness: A Scientific Statement From the American Heart Association. Hypertension 2015; 66:698-722.
25. Venter WDF, Kambugu A, Chersich MF, Becker S, Hill A, Arulappan N, et al. Efficacy and safety of tenofovir disoproxil fumarate versus low-dose stavudine over 96 weeks: a multi-country randomised, non-inferiority trial. J Acquir Immune Defic Syndr 2018;.

26. Venter F, Moorhouse M, Sokhela S, Maharaj E, Akpomiemie G, Simmons B, et al. Non-inferior efficacy for darunavir/ritonavir 400/100 mg once daily versus lopinavir/ritonavir, for patients with HIV RNA below 50 copies/mL in South Africa: The 48-week WRHI 052 study. J Int AIDS Soc 2018; 21.
27. Department of Health South Africa. NATIONAL HIV TESTING SERVICES: POLICY 2016. 2016;:https://sahivsoc.org/Files/HTS%20Policy%2028%20July%20final%20copy.pdf.
28. World Health Organisation. WHO STEPS Instrument (Core and Expanded). Version 3.2. 2018.
29. Craig CL, Marshall AL, Sjostrom M, Bauman AE, Booth ML, Ainsworth BE, et al. International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exerc 2003; 35:1381-1395.
30. Bots ML, Evans GW, Tegeler CH, Meijer R. Carotid Intima-media Thickness Measurements: Relations with Atherosclerosis, Risk of Cardiovascular Disease and Application in Randomized Controlled Trials. Chin Med J (Engl) 2016; 129:215-226.
31. Vos A, Tempelman H, Deville W, Barth R, Wensing A, Kretzschmar M, et al. HIV and risk of cardiovascular disease in sub-Saharan Africa: Rationale and design of the Ndlovu Cohort Study. Eur J Prev Cardiol 2017; 24:1043-1050.
32. Boulos NM, Gardin JM, Malik S, Postley J, Wong ND. Carotid Plaque Characterization, Stenosis, and Intima-Media Thickness According to Age and Gender in a Large Registry Cohort. Am J Cardiol 2016; 117:1185-1191.
33. Lage SG, Kopel L, Monachini MC, Medeiros CJ, Pileggi F, Polak JF, et al. Carotid arterial compliance in patients with congestive heart failure secondary to idiopathic dilated cardiomyopathy. Am J Cardiol 1994; 74:691-695.
34. van Vonderen MG, Smulders YM, Stehouwer CD, Danner SA, Gundy CM, Vos F, et al. Carotid intima-media thickness and arterial stiffness in HIV-infected patients: the role of HIV, antiretroviral therapy, and lipodystrophy. J Acquir Immune Defic Syndr 2009; 50:153-161.
35. Seaberg EC, Benning L, Sharrett AR, Lazar JM, Hodis HN, Mack WJ, et al. Association between human immunodeficiency virus infection and stiffness of the common carotid artery. Stroke 2010; 41:2163-2170.
36. Idris NS, Grobbee DE, Burgner D, Cheung MM, Kurniati N, Uiterwaal CS. Effects of paediatric HIV infection on childhood vasculature. Eur Heart J 2016; 37:3610-3616.
37. Grunfeld C, Delaney JA, Wanke C, Currier JS, Scherzer R, Biggs ML, et al. Preclinical atherosclerosis due to HIV infection: carotid intima-medial thickness measurements from the FRAM study. AIDS 2009; 23:1841-1849.
38. Hsue PY, Ordovas K, Lee T, Reddy G, Gotway M, Schnell A, et al. Carotid intima-media thickness among human immunodeficiency virus-infected patients without coronary calcium. Am J Cardiol 2012; 109:742-747.

39. Lorenz MW, Stephan C, Harmjanz A, Staszewski S, Buehler A, Bickel M, et al. Both long-term HIV infection and highly active antiretroviral therapy are independent risk factors for early carotid atherosclerosis. Atherosclerosis 2008; 196:720-726.

40. Gupta PK, Gupta M, Lal AK, Taneja A, Taneja RS, Rewari BB. Markers of subclinical atherosclerotic disease in HIV-infected individuals. J Virus Erad 2018; 4:21-25.

41. Boyd A, Meynard JL, Morand-Joubert L, Michon A, Boccara F, Bastard JP, et al. Association of residual plasma viremia and intima-media thickness in antiretroviral-treated patients with controlled human immunodeficiency virus infection. PLoS One 2014; 9:e113876.

42. Currier JS, Kendall MA, Zackin R, Henry WK, Alston-Smith B, Torriani FJ, et al. Carotid artery intima-media thickness and HIV infection: traditional risk factors overshadow impact of protease inhibitor exposure. AIDS 2005; 19:927-933.

43. Hileman CO, Carman TL, Longenecker CT, Labbato DE, Storer NJ, White CA, et al. Rate and predictors of carotid artery intima media thickness progression in antiretroviral-naive HIV-infected and uninfected adults: a 48-week matched prospective cohort study. Antivir Ther 2013; 18:921-929.

44. Kirichenko TV, Myasoedova VA, Shimonova TE, Melnichenko AA, Sviridov D, Sobenin IA, et al. Atherosclerosis in subjects newly diagnosed with human immunodeficiency virus infection. Biosci Rep 2018; 38:10.1042/BSR20180597. Print 2018 Aug 31.

45. Fourie CM, Schutte AE, Smith W, Kruger A, van Rooyen JM. Endothelial activation and cardiometabolic profiles of treated and never-treated HIV infected Africans. Atherosclerosis 2015; 240:154-160.

46. Delaney JA, Scherzer R, Biggs ML, Shliplak MG, Polak JF, Currier JS, et al. Associations of antiretroviral drug use and HIV-specific risk factors with carotid intima-media thickness. AIDS 2010; 24:2201-2209.

47. Stein JH, Ribaudo HJ, Hodis HN, Brown TT, Tran TT, Yan M, et al. A prospective, randomized clinical trial of antiretroviral therapies on carotid wall thickness. AIDS 2015; 29:1775-1783.

48. Gleason RL,Jr, Caulk AW, Seifu D, Parker I, Vidakovic B, Getenet H, et al. Current Efavirenz (EFV) or ritonavir-boosted lopinavir (LPV/r) use correlates with elevate markers of atherosclerosis in HIV-infected subjects in Addis Ababa, Ethiopia. PLoS One 2015; 10:e0117125.

49. Mosepele M, Hemphill LC, Moloi W, Moyo S, Nkele I, Makhema J, et al. Pre-clinical carotid atherosclerosis and sCD163 among virally suppressed HIV patients in Botswana compared with uninfected controls. PLoS One 2017; 12:e0179994.

50. Schoffelen AF, de Groot E, Tempelman HA, Visseren FL, Hoepelman AI, Barth RE. Carotid Intima Media Thickness in Mainly Female HIV-Infected Subjects in Rural South Africa: Association With Cardiovascular but Not HIV-Related Factors. Clin Infect Dis 2015; 61:1606-1614.

51. Siedner MJ. START or SMART? Timing of Antiretroviral Therapy Initiation and Cardiovascular Risk for People With Human Immunodeficiency Virus Infection. Open Forum Infect Dis 2016; 3:ofw032.
52. Hanna DB, Guo M, Buzkova P, Miller TL, Post WS, Stein JH, et al. HIV Infection and Carotid Artery Intima-media Thickness: Pooled Analyses Across 5 Cohorts of the NHLBI HIV-CVD Collaborative. Clin Infect Dis 2016; 63:249-256.
53. Wester CW, Koethe JR, Shepherd BE, Stinnette SE, Rebeiro PF, Kipp AM, et al. Non-AIDS-defining events among HIV-1-infected adults receiving combination antiretroviral therapy in resource-replete versus resource-limited urban setting. AIDS 2011; 25:1471-1479.
54. Benjamin LA, Corbett EL, Connor MD, Mzinganjira H, Kampondeni S, Choko A, et al. HIV, antiretroviral treatment, hypertension, and stroke in Malawian adults: A case-control study. Neurology 2016; 86:324-333.
55. Stroke risk factors in an incident population in urban and rural Tanzania: a prospective, community-based, case-control study. Lancet Glob Health 2013; 1:e282-8.
56. Shah ASV, Stelzle D, Lee KK, Beck EJ, Alam S, Clifford S, et al. Global Burden of Atherosclerotic Cardiovascular Disease in People Living With HIV. Circulation 2018; 138:1100-1112.

Additional material

Supplementary table. HIV and ART status on max bulb intima-media thickness (n = 474)

	HIV-negative			HIV-positive			
		ART-naive	*p*	First-line ART	*p*	Second-line ART	*p*
Model 1	REF	-0.068 (-0.147 – 0.010)	0.088	-0.033 (-0.017 – 0.050)	0.433	0.114 (0.045 – 0.183)	0.001
Model 2	REF	-0.069 (-0.136 – -0.002)	0.043	-0.072 (-0.143 – 0.000)	0.049	-0.043 (-0.106 – 0.020)	0.182
Model 3	REF	-0.068 (-0.135 – 0.000)	0.049	-0.071 (-0.142 – 0.001)	0.054	-0.041 (-0.105 – 0.024)	0.216
Model 4[1]	REF	-0.071 (-0.135 – -0.007)	0.030	-0.066 (-0.135 – 0.004)	0.064	-0.044 (-0.109 – 0.022)	0.193

Model 1 unadjusted

Model 2 adjusted for age

Model 3 adjusted for age and sex

Model 4 adjusted for age, sex, systolic blood pressure, body mass index, LDL cholesterol, HDL cholesterol, glucose and current smoking

1. n = 31 (6.5%) participants were excluded from this model due to missing data in one or more covariates

531207-L-bw-Vos
Processed on: 20-5-2019

CHAPTER

9

Cardiovascular disease risk and its determinants in people living with HIV across different settings in South Africa

G.V.T. Roozen, A.G. Vos, H.A. Tempelman, D.E. Grobbee, K. Scheuermaier, K. Klipstein-Grobusch.

Submitted

Abstract

Objectives Socio-economic factors and lifestyle are known to differ across geographies and populations, which may result in distinct risk profiles for cardiovascular disease (CVD). This study assessed carotid intima-media thickness (CIMT), a proxy for CVD, and its determinants in two groups of people living with HIV (PLHIV) in two different settings in South Africa.

Methods A cross-sectional analysis was conducted comparing data from the Ndlovu Cohort Study in the Limpopo province (rural group) and from three clinical trials in Johannesburg (urban group). The association between demographics, conventional CVD risk factors, HIV-related factors and CIMT was analysed with two separate multivariable linear regression models for the rural and the urban groups.

Results The rural group consisted of 826 participants (mean age 42.2 years) and mean CIMT was 0.626±0.128 mm. In this group, conventional CVD risk factors, ART-duration were associated with higher CIMT, and HIV-duration with lower CIMT. There were positive interactions between age and HIV-duration and age and cholesterol. The urban group consisted of 382 participants (mean age 39.5 years) and mean CIMT was 0.560±0.092 mm. In the this group, only conventional CVD risk factors were associated with higher CIMT, albeit with weaker associations than in the rural group.

Conclusions Conventional CVD risk factors were the main drivers of CIMT. The impact of some of these risk factors appeared to increase with age. Differences in recruitment criteria, age and viral suppression, might explain why an effect of HIV and ART was observed in the rural group, but not in the urban group.

Introduction

Infection with the human immunodeficiency virus (HIV) has been the main cause of death in South Africa in the past two decades[1]. Current estimates indicate that approximately 7.2 million people live with HIV in South Africa of which about 5 million are being treated with combination anti-retroviral therapy (ART)[2].

Non-communicable diseases, of which a considerable part is comprised of cardiovascular disease (CVD), are now accountable for a substantial burden of disease[3]. In 2016, one in five deaths was due to CVD in South Africa[4]. ART has transformed HIV-infection into a chronic treatable condition[5]. This results in a near-normal life expectancy of people living with HIV (PLHIV), who as a result will experience more age-related diseases like CVD[6]. Research from high-income countries suggests that people living with HIV (PLHIV) are twice as likely to develop CVD and both HIV and ART have been shown to be risk factors for the development of CVD[7,8].

The mechanisms by which HIV affects CVD risk are, however, not yet fully understood. HIV-infection is associated with an activation of the immune system, even in virally suppressed PLHIV[9]. Immune activation is a key factor in the formation of atherosclerosis[10,11]. Furthermore, ART, especially the use of protease inhibitors and efavirenz, may increase CVD risks by causing alterations in lipid and glucose metabolism[12,13]. Finally, conventional CVD risk factors such as obesity, diabetes and hypertension are common in PLHIV in South Africa[14].

Lifestyle, socio-economic factors and access to healthcare services varies across different regions of South Africa, and the country is experiencing rapid economic change and urbanisation. Therefore, the contribution of conventional CVD risk factors to the occurrence of CVD may also differ across settings. Those differences are particularly pronounced when contrasting rural and urban settings. In the last decades, lifestyle and dietary patterns in sub-Saharan Africa (SSA), and particularly in South Africa have changed substantially[15]. People have become less physically active while dietary fat and sugar consumption has increased[16]. These lifestyle changes seemingly happen first in urban areas before they occur in rural areas [17]. Urban residents have been shown to have a higher body mass index (BMI), higher blood pressure and more instances of diabetes than rural residents[18]. In South Africa, considerable differences in socio-demographics exist between rural and urban populations; lower educational attainment and high rates of unemployment contribute to lower incomes in rural areas[17]. These differences in socio-economic and CVD risk factors could create distinct CVD risk profiles across the country[19]. In addition, inequity in access to healthcare may aggravate these differences[20].

Data on the occurrence of clinical CVD in the HIV infected population in South Africa is scarce. Awaiting longitudinal studies, surrogate markers like carotid intima-media thickness (CIMT) can be used to estimate CVD risk. CIMT is associated with the risk of myocardial infarction and stroke[21,22], and it has been used in Caucasian and African populations[23,24]. In studies in high-income countries, higher CIMT values have been found in PLHIV compared to HIV-negative people, even after adjusting for conventional CVD risk factors[25].

This study aimed to assess the burden of CVD risk using CIMT as a surrogate CVD marker in groups of PLHIV from two different settings in South Africa. In addition, we investigated determinants of CIMT in these two groups, focussing on conventional CVD and HIV-specific factors.

Methods

Study setting

In this cross-sectional analysis, we included ART-naïve HIV-positive and treated HIV-positive participants who were 18 years or older from a rural and an urban site in South Africa.

The first group of participants was selected from the Ndlovu Cohort Study (NCS), a longitudinal study of which the design and methodology have been described previously[26]. In brief, the NCS is conducted in Elandsdoorn, a rural township in the Limpopo province, South Africa. Between December 2014 and July 2016, 887 HIV-positive participants were recruited. Study approval was obtained from the Human Research Ethics Committee at the University of Pretoria, Pretoria, South Africa, and the Limpopo Department of Health Ethics Committee (ethics clearance 227-2014). This group will be referred to as the rural group and its participants as rural participants.

The second group of participants was selected from three randomised controlled trials (RCTs) that recruited participants from public HIV treatment centres in the inner city of Johannesburg, South Africa. This group will be referred to as the urban group and its participants as urban participants.

Urban HIV-positive, ART-naïve participants (n=104) were recruited from an open label RCT comparing the efficacy of two dolutegravir containing regimens with the current standard of care first-line in ART-naïve participants [ClinicalTrials.gov identifier NCT03122262]. Participants were eligible for enrolment in our study before the initiation of ART or at latest within three months after initiation of ART.

Urban participants on stable first-line ART (n=94) were selected from an RCT that aimed to demonstrate non-inferiority of low-dose stavudine compared to tenofovir disoproxil

fumarate in the period 2012 to 2016[27]. Participants were ART-naïve upon enrolment in the RCT. Upon completion of the RCT all participants were switched to the standard first-line ART regimen consisting of emtricitabine, tenofovir and efavirenz. To be eligible for inclusion in our study participants had to be on a tenofovir containing regimen for at least two and half years before enrolment in our study.

Urban participants on second-line ART (n=197) were selected from an RCT that aimed to demonstrate non-inferiority of ritonavir-boosted low-dose darunavir compared with boosted lopinavir-based second-line therapy[28]. Participants were virally suppressed on second-line ART for at least six months prior to enrolment in the RCT. They were eligible for inclusion in our study at any moment of follow-up in the RCT. All participants who attended the RCT site in the time frame of our study were approached for participation. Between July 2016 and November 2017, 395 HIV-positive participants were recruited.

Study approval was obtained from the Medical Human Research Ethics Committee of the University of Witwatersrand, Johannesburg, South Africa (M160130).

Data collection

Data was collected in identical ways at the rural and the urban sites unless stated otherwise. Counsellors or nurses collected information on participants' lifestyle, medical history and medication use (both HIV-related as well as for other medical conditions). Information on demographics (including employment status and education level), smoking, alcohol use and medical history was assessed with a modified version of the WHO STEPs instrument[29]. Participants who reported having quit smoking less than one month ago were considered current smokers. Students, retirees, disabled people and volunteers were considered unemployed. Information on physical activity was assessed using the International Physical Activity Questionnaire and accordingly categorised in high, intermediate and low level of activity[30]. Family history was considered positive for a cardiovascular event when participants reported a history of stroke and/or heart attack of a first-degree family member (parent or sibling) before the age of 60. ART-duration was the time between the last initiation of ART and the inclusion date. Participants were considered ART-naïve when they did not use ART or when they had initiated ART less than three months prior to inclusion.

Blood pressure was measured with an electronic blood pressure device, in a seated position after five minutes at rest. Blood pressure was measured at both arms and a third measurement was taken on the side with the highest value; subsequently the average of the last two measurements was used. Waist circumference was measured halfway between the lower rib and the iliac crest during expiration in standing position.

Blood was taken for measurement of total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, random glucose, viral load and CD4 cell count. For urban participants, laboratory data of the last RCT visit were used. Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald formula[31].

Hypertension, abdominal obesity, dyslipidaemia and metabolic syndrome were defined according to the National Cholesterol Education Program, Adult Treatment Panel III (NCEP ATP III)[32]. Accordingly, hypertension was defined as systolic blood pressure (SBP) of >130 mmHg, diastolic blood pressure (DBP) of >85 mmHg or use of antihypertensive medication. Abdominal obesity was defined as a waist circumference ≥102 cm for men and ≥88 cm for women. Diabetes mellitus was defined as random glucose of >11 mmol/L or the use of blood glucose lowering medication. Dyslipidaemia was defined as elevated triglycerides (≥1.7 mmol/L) and/or reduced HDL cholesterol (<1.0 mmol/L for men and <1.3 mmol/L for women). Metabolic syndrome was defined as ≥ 3 out of: diabetes, hypertension, elevated triglycerides, lowered HDL cholesterol or abdominal obesity.

For all participants, the Framingham 10-year CVD risk score[33], and the 5-year CVD risk score from the Data-collection on Adverse Effects of Anti-HIV Drugs Study (D:A:D) [34] were calculated. Due to their calibration populations, the Framingham score was only calculated for participants aged 30 or older. The D:A:D was only been calculated for patients with an ART-duration of 10 years or less. The likelihood of a CVD event occurring in the next 10 or five years, respectively, was reported in percentages.

Ultrasound measurements of the CIMT were performed by trained research staff using a Siemens-Acuson system (P300 for the rural site and P500 for the urban site) with a linear probe of ≥7.0 MHz. End-diastolic images were collected of the right common carotid segment at angles 90°, 120° and 150° and of the left common carotid segment at angles 210°, 240° and 270° using a Meijer Carotid Arc[35]. Both the near wall and the far wall were measured. For the carotid bifurcation, similar approaches were used at the best visible angle on both sides while focusing on the far wall only. Performance reviews were carried out to ensure quality of measurements.

Common carotid artery (CCA) and bifurcation (BIF) intima-media thickness (IMT) were measured semi-automatically with the Artery Measurement System software (Chalmers University of Technology, Göteborg, Sweden). A uniform reading protocol was used to ensure standardized settings across reading stations. Images were read in batch fashion by trained readers who were blinded to the participants HIV-status.

The following CIMT measurements were reported: the mean IMT of the near and far wall across all angles of the CCA (mean CCA-IMT), the mean of the maximum IMT of

the near and the far wall of the CCA across all angles of the CCA (max CCA-IMT), the mean of the maximum IMT at the far wall of the bifurcation at both sided (max BIF-IMT). Mean CCA-IMT was used as the outcome variable in the multivariable linear regression models.

A max IMT >1.0 mm anywhere in one of the measured angles in the far wall of the CCA was considered a plaque[36].

Statistical Analysis

The urban and the rural group were not compared directly in the statistical analysis as they represent different populations. The rural group had been recruited from the general population, whereas the urban group had been recruited from RCTs. Hence, in the urban group the proportion of ART-naïve participants, participants on first-line ART and on second-line ART are not a representation of the ART-coverage and treatment regimens in the general HIV-positive population. In addition, the different recruitment strategies may have led to unmeasured confounding between groups. Hence, our statistical analyses were performed for the two groups in two separate models.

Demographics, CVD risk factors and CIMT of the urban and rural groups are reported as means and standard deviation (SD) for normally distributed continuous variables, medians and interquartile range (IQR) for non-normally distributed continuous variables and frequency counts with percentages for categorical variables.

For the rural group, 52% of the blood pressure readings were regarded as missing data as these measurements had been taken with a non-validated blood pressure device. This data was missing completely at random therefore we decided to not exclude those observations from the analysis and instead we imputed the missing data. Observations were stratified by HIV and treatment status and multiple imputations were used, following a Markov chain Monte Carlo method to estimate the missing values. Imputations were repeated 20 times generating 20 different datasets. Subsequently, a singly imputed dataset was created by selecting a random draw from the 20 datasets for the final imputed blood pressure values.

To assess associations between conventional CVD and HIV-related risk factors and mean CCA-IMT, a multivariable linear regression model was created for both groups separately. First, we tested the association of all socio-demographic, CVD and HIV-related factors to CIMT in a univariable linear regression model. The association between ART-status (i.e. ART-naïve, on first-line ART or on second-line ART) and mean CCA-IMT was tested using the ART-naïve participants as the reference group. Second, all variables with a p-value <0.20 in univariable regression and variables that are known determinants of CIMT (i.e. sex, age, smoking, BMI, SBP, total cholesterol, HDL, LDL and

triglycerides [35] were entered in a multivariable linear regression model. Variables were excluded from the multivariable model if multicollinearity occurred (defined as a variance inflation factor >10).

Third, possible interactions between age and HIV-duration (per five years), age and ART-duration (per five years), and age and conventional CVD risk factors (smoking, BMI, SBP, blood lipids and glucose) were tested in relation to mean CCA-IMT[37]. First, an analysis restricted to the main effects and the interaction term was tested in a multivariable linear regression. Second, the interaction terms with a p-value <0.20 were added all at once to the multivariable model.

A two-sided p-value <0.05 was considered statistically significant. All statistical analyses were performed using IBM SPSS Statistics software, version 25 (IBM, Armonk, New York, United States).

Results

Study characteristics

From the 887 HIV-positive participants of the NCS, CIMT data was available for 826 (93.1%) participants.

In the rural group, 39.7% (n=328) were men and the mean age was 42.2±10.2 years. The majority of the participants were in a relationship. Approximately 70% completed at least secondary school. The unemployment rate was over 70%. More than 30% of the participants were overweight/obese, had hypertension or dyslipidemia. The HIV diagnosis was known for about five years, and 77% of the participants were on ART, of which 11% on second-line ART. The median Framingham 10-year CVD risk for patients aged 30 or older was 2.9% (IQR: 1.6-6.1) and the median D:A:D: 5-year CVD risk for patients with an ART-duration of less than 10 years was 0.7% (IQR: 0.3-1.5).

For the urban group, of the 395 HIV-positive participants, CIMT data was available for 382 (96.7%) participants. In total, 34% (n=130) were men and the mean age was 39.5±8.8 years. More than 85% completed at least secondary school. About 50% of the participants were overweight or obese. Thirty one percent of the participants had hypertension and 50% had dyslipidemia. On average, participants knew their HIV diagnosis for six years and 72% were on ART, of which 69% on second-line ART. The median Framingham 10-year CVD risk for patients aged 30 or older was 2.5% (IQR: 1.5-4.3) and the median D:A:D: 5-year CVD risk for patients with an ART-duration of less than 10 years was 0.6% (IQR: 0.3-1.1) (Table 1).

Table 1. Baseline characteristics – rural and urban group

	Rural n = 826	Urban n = 382
Demographics, n (%)		
Male sex	328 (39.7)	130 (34.0)
Age, mean (SD), years	42.2 (10.3)	39.5 (8.8)
Age in categories, years		
18-29	100 (12.1)	51 (13.4)
30-49	530 (64.2)	282 (73.8)
≥50	196 (23.7)	49 (12.8)
Partnership status: single	369 (45.0)	140 (36.8)
Highest level of completed education		
None or primary school	232 (28.1)	50 (13.3)
Secondary school or matric	542 (65.7)	298 (79.0)
College or university	52 (6.3)	29 (7.7)
Employment status: unemployed[a]	604 (73.1)	126 (33.2)
Lifestyle, n (%)		
Physical activity[b]		
Low	382 (46.2)	161 (42.3)
Moderate	267 (32.3)	159 (41.7)
High	177 (21.5)	61 (16.0)
Smoking		
Current smoker[c]	194 (23.5)	55 (14.4)
Previous smoker	108 (13.1)	25 (6.5)
Never smoked	524 (63.4)	302 (79.1)
Heavy alcohol drinker[d]	20 (2.7)	5 (1.3)
Chronic medication use		
Antihypertensive medication	30 (3.6)	29 (7.6)
Blood glucose lowering medication	8 (1.0)	6 (1.6)
Cholesterol lowering medication	0 (0.0)	10 (2.6)

Table 1. Continued

	Rural n = 826	Urban n = 382
HIV-related factors, n (%)		
Known duration of HIV-infection, median [IQR], years	4.9 [1.2-8.3]	6.0 [2.0-10.0]
ART-status		
ART-naïve[e]	192 (23.2)	107 (28.0)
On first-line ART	564 (68.3)	84 (22.0)
On second-line ART	70 (8.5)	191 (50.0)
Total ART-duration, median [IQR], years	5.3 [2.4-8.3]	6.0 [3.5-9.0]
Duration of 1st line ART, median [IQR], years	4.9 [2.0-8.1]	4.0 [3.0-7.0]
Duration of 2nd line ART, median [IQR], years	3.4 [1.3-4.6]	1.0 [0.3-4.0]
Last CD4 cell count, median [IQR], cells/mm3	472 [323-658]	458 [294-693]
Last CD4 cell count, <200 cells/mm3	86 (10.6)	34 (10.1)
Last viral load of patients on ART in categories, copies/mL		
<50	509 (80.3)	246 (93.5)
50-1000	52 (8.2)	7 (2.7)
>1000	73 (11.5)	10 (3.8)
Anthropometric measurements, n (%)		
BMI, mean (SD), kg/m2	23.5 (5.7)	26.6 (6.2)
BMI in categories, kg/m2		
Underweight: <18.5	133 (16.1)	12 (3.1)
Normal: 18.5–25	437 (53.0)	176 (46.1)
Overweight: >25–30	145 (17.6)	94 (24.6)
Obesity: >30	110 (13.3)	100 (26.2)
Abdominal obesity[f]	251 (30.4)	176 (46.2)
Cardiovascular measurements, mean (SD)		
Systolic blood pressure, mmHg	119 (22)	122 (18)
Diastolic blood pressure, mmHg	75 (14)	78 (11)
Heart rate, beats/minute	75 (13)	71 (11)
Biochemical measurements, median [IQR]		
Total cholesterol, mmol/L	4.2 [3.6-4.9]	4.4 [3.7-5.1]
HDL cholesterol, mmol/L	1.4 [1.2-1.6]	1.3 [1.0-1.5]
LDL cholesterol, mmol/L	2.2 [1.7-2.8]	2.6 [2.1-3.2]
Triglycerides, mmol/L	1.0 [0.7-1.4]	1.1 [0.8-1.6]
Glucose, mmol/L	4.7 [4.3-5.1]	4.6 [4.3-5.0]

Table 1. Continued

	Rural n = 826	Urban n = 382
Cardiovascular risk factors, n (%)		
Hypertension[g]	274 (33.2)	118 (31.0)
Diabetes[h]	11 (1.3)	7 (2.0)
Dyslipidaemia[i]	304 (37.0)	180 (49.5)
Metabolic syndromej	83 (10.1)	62 (17.1)
Positive family history[k]	11 (1.3)	27 (7.1)
CVD prediction models, median [IQR]		
Framinghaml, 10-year CVD risk, %	2.9 [1.6-6.1]	2.5 [1.5-4.3]
D:A:D[m], 5-year CVD risk, %	0.7 [0.3-1.5]	0.6 [0.3-1.1]

[a] Also includes students, retirees, disabled people and volunteers (n rural = 29, n urban = 6).

[b] Based on the International Physical Activity Questionnaire (IPAQ).

[c] Participants who quit smoking less than one month ago were also considered current smokers.

[d] Heavy alcohol drinker: ≥5 days of drinking a week in the past month.

[e] Participants who initiated ART within three months before inclusion where also considered ART-naïve.

[f] Abdominal obesity: waist circumference ≥102 cm for men and ≥88 cm for women.

[g] Hypertension: systolic blood pressure >130 mmHg, diastolic blood pressure >85 mmHg and/or use of antihypertensive medication.

[h] Diabetes mellitus: random glucose >11 mmol/L and/or using blood glucose lowering medication.

[i] Dyslipidaemia: elevated triglycerides (≥1.7 mmol/L) and/or reduced HDL cholesterol (<1.0 mmol/L for men and <1.3 mmol/L for women).

[j] Metabolic syndrome: ≥ 3 out of: diabetes, hypertension, elevated triglycerides, lowered HDL cholesterol or abdominal obesity.

[k] Positive family history: self-reported stroke and/or heart attack of parent and/or sibling before the age of 60.

[l] Calculated for participants aged 30 or older.

[m] Calculated for participants with an ART-duration with a maximum of 10 years.

Abbreviations: ART: antiretroviral therapy, BMI: body mass index, CVD: cardiovascular disease, D:A:D: Data Collection of Adverse Events on Anti-HIV Drugs, HDL: high-density lipoprotein, HIV: human immune deficiency virus, IQR: interquartile range, LDL: low-density lipoprotein, SD: standard deviation.

CIMT

Rural participants had a mean CCA-IMT of 0.626±0.128 mm and 130 participants (16%) had plaque in the common carotid artery. In the urban group the mean CCA-IMT was 0.527±0.092 mm and 24 participants (6%) had a plaque in the CCA (Table 2).

Table 2. Carotid intima-media thickness – rural and urban group

Intima-media thickness mean (SD)	**Rural**	**Urban**
Mean CCA (mm)	0.626 (0.128)	0.560 (0.092)
Max CCA (mm)	0.712 (0.157)	0.644 (0.113)
Max BIF (mm)	0.870 (0.249)	0.797 (0.287)
Plaque[a], n (%)	130 (15.7)	24 (6.3)

[a]Plaque: IMT >1.0 mm anywhere in one of the measured angles in the far wall of the CCA.
Abbreviations: BIF: carotid artery bifurcation, CCA: common carotid artery, CIMT: carotid artery intima-media thickness, IMT: intima-media thickness, mm: millimeters, SD: standard deviation.

In the rural group, a higher mean CCA-IMT was associated with male sex, age, ART-duration, BMI, and total cholesterol following multivariable analysis. HDL cholesterol and HIV-duration were inversely associated (Table 3).

Table 3. Factors associated with mean CCA-IMT – rural group

	Univariable β (95% CI), mm	***p***	**Multivariable[a] β (95% CI), mm**	***p***
Demographic factors				
Male sex	0.057 (0.039, 0.074)	<0.001	0.044 (0.027, 0.063)	<0.001
Age, per year	0.008 (0.007, 0.009)	<0.001	0.007 (0.006, 0.008)	<0.001
Single (vs. partner)	-0.019 (-0.036, -0.001)	0.04	-0.007 (-0.021, 0.006)	0.28
No/primary education (vs. secondary/higher education)	0.085 (0.067, 0.104)	<0.001	0.001 (-0.016, 0.018)	0.89
Employed (vs. unemployed)	0.001 (-0.019, 0.021)	0.90		
HIV-related factors				
Known duration of HIV-infection, per year	0.005 (0.003, 0.007)	<0.001	-0.003 (-0.006, 0.000)	0.04
On 1st line ART (vs. ART-naïve)	0.055 (0.034, 0.076)	<0.001	0.006 (-0.018, 0.029)	0.62
On 2nd line ART (vs. ART-naïve)	0.053 (0.018, 0.088)	<0.01	-0.003 (-0.035, 0.029)	0.85
Total ART-duration, per year	0.009 (0.006, 0.011)	<0.001	0.005 (0.002, 0.009)	<0.01

Table 3. Continued

	Univariable β (95% CI), mm	*p*	Multivariable[a] β (95% CI), mm	*p*
Last CD4 cell count, per 100/ mm3	0.000 (-0.003, 0.004)	0.82		
Last viral load, copies/mL, per log10	-0.012 (-0.18, -0.006)	<0.001	0.001 (-0.005, 0.008)	0.67
Cardiovascular risk factors				
Low physical activity *(vs. moderate/high)*	0.025 (0.007, 0.042)	0.06	0.007 (-0.006, 0.021)	0.28
Ever smoked	0.014 (-0.004, 0.032)	0.14	-0.014 (-0.030, 0.001)	0.07
Heavy alcohol drinker	-0.023 (-0.079, 0.034)	0.43		
Positive family history:	-0.052 (-0.129, 0.024)	0.18	-0.017 (-0.074, 0.040)	0.56
BMI, per kg/m2	0.003 (0.001, 0.004)	<0.01	0.004 (0.002, 0.005)	<0.001
Abdominal obesity	0.012 (-0.007, 0.031)	0.22	-0.011 (-0.030, 0.008)	0.27
SBP, per mmHg	0.001 (0.000, 0.001)	<0.001	0.000 (0.000, 0.001)	0.71
DBP, per mmHg	0.001 (0.000, 0.002)	0.01	0.000 (-0.001, 0.001)	0.76
Heart rate, per beat/minute	0.000 (-0.001, 0.000)	0.20		
Total cholesterol, per mmol/L	0.030 (0.022, 0.038)	<0.001	0.019 (0.011, 0.028)	<0.001
HDL cholesterol, per mmol/L	-0.011 (0.032, 0.010)	0.30	-0.035 (-0.054, -0.017)	<0.001
LDL cholesterol, per mmol/L	0.030 (0.020, 0.040)	<0.001	&	
Triglycerides, per mmol/L	0.033 (0.022, 0.044)	<0.001	-0.004 (-0.014, 0.005)	0.39
Glucose, per mmol/L	0.017 (0.010, 0.024)	<0.001	0.005 (-0.001, 0.010)	0.09

&: Excluded from multivariable regression due to collinearity.

[a]788 participants (95.4%) were included.

Abbreviations: ART: antiretroviral therapy, BMI: body mass index, CCA-IMT: common carotid artery intima-media thickness, CI: confidence interval, DBP: diastolic blood pressure, HDL: high-density lipoprotein, HIV, human immune deficiency virus, LDL: low-density lipoprotein, SBP: systolic blood pressure.

In the urban group, age, BMI and total cholesterol were associated with a higher mean CCA-IMT in multivariable analysis (Table 4).

Table 4. Factors associated with mean CCA-IMT – urban group

	Univariable β (95% CI), mm	p	Multivariable[a] β (95% CI), mm	p
Demographic factors				
Male sex	0.020 (0.001, 0.040)	0.04	0.005 (-0.017, 0.028)	0.63
Age, per year	0.007 (0.006, 0.008)	<0.001	0.006 (0.005, 0.007)	<0.001
Single (vs. partner)	-0.010 (-0.030, 0.009)	0.29		

Table 4. Continued

	Univariable β (95% CI), mm	p	Multivariable[a] β (95% CI), mm	p
No/primary education (vs. secondary/higher education)	0.046 (0.019, 0.073)	<0.1	0.024 (0.000, 0.049)	0.05
Employed (vs. unemployed)	-0.005 (-0.024, 0.015)	0.78		
HIV-related factors				
Known duration of HIV-infection, per year	0.006 (0.004, 0.007)	<0.001	0.001 (-0.001, 0.004)	0.38
On 1st line ART (vs. ART-naïve)*	0.015 (-0.010, -0.040)	0.24	-0.016 (-0.055, 0.023)	0.43
On 2nd line ART (vs. ART-naïve)*	0.067 (0.047, 0.088)	<0.001	-0.016 (-0.073, 0.042)	0.59
Total ART-duration, per year	0.007 (0.005, 0.009)	<0.001	-0.002 (-0.006, 0.002)	0.39
Last CD4 cell count, per 100/mm3	0.007 (0.003, 0.010)	<0.001	0.000 (0.000, 0.00)	0.27
Last viral load, copies/mL, per log10	-0.016 (-0.021, -0.010)	<0.001	-0.002 (-0.013, 0.010)	0.79
Cardiovascular risk factors				
Low physical activity *(vs. moderate/high)*	0.017 (-0.002, 0.036)	0.07	0.008 (-0.008, 0.025)	0.34
Ever smoked	-0.006 (-0.029, 0.016)	0.58	-0.012 (-0.034, 0.009)	0.27
Heavy alcohol drinker	-0.006 (-0.149, 0.076)	0.88		
Positive family history	0.013 (-0.014, 0.049)	0.49		
BMI, per kg/m2	0.003 (0.001, 0.004)	<0.01	0.002 (0.000, 0.004)	0.03
Abdominal obesity: yes (vs. no)	0.023 (0.005, 0.042)	0.02	-0.020 (-0.043, 0.004)	0.10
SBP, per mmHg	0.001 (0.000, 0.001)	<0.01	0.000 (0.000, 0.001)	0.49
DBP, per mmHg	0.001 (0.000, 0.002)	0.01	0.000 (-0.001, 0.001)	0.98
Heart rate, per beat/minute	-0.001 (-0.002, 0.000)	0.04	0.000 (-0.001, 0.001)	0.63
Total cholesterol, per mmol/L	0.031 (0.021, 0.040)	<0.001	0.0011 (0.001, 0.021)	0.04
HDL cholesterol, per mmol/L	0.028 (0.004, 0.052)	0.02	-0.002 (-0.027, 0.023)	0.89
LDL cholesterol, per mmol/L	0.029 (0.018, 0.040)	<0.001	&	
Triglycerides, per mmol/L	0.021 (0.009, 0.034)	<0.01	0.000 (-0.012, 0.012)	0.99
Glucose, per mmol/l	0.007 (0.000, 0.014)	0.07	0.000 (-0.006, 0.006)	0.90

& Excluded from multivariable regression due to collinearity.

[a]318 participants (83.2%) were included.

Abbreviations: ART: antiretroviral therapy, BMI: body mass index, CCA-IMT: common carotid artery intima-media thickness, CI: confidence interval, DBP: diastolic blood pressure, HDL: high-density lipoprotein, HIV, human immune deficiency virus, LDL: low-density lipoprotein, SBP: systolic blood pressure.

Table 5 shows the contribution of the interaction terms that were added to the multivariable models. In the rural group there was a significant interaction between age and HIV-duration (per five years) (β 0.001 mm, p <0.001), age and total cholesterol (β 0.002 mm, p <0.001), and age and HDL cholesterol (β -0.003 mm, p <0.001) in relation to mean CCA-IMT. HIV-duration was associated with a lower CCA-IMT in multivariable analysis (as shown in Table 3), so the positive interaction with age implies that the 'protective effect' of HIV duration on CIMT was attenuated with aging. Conversely, the effect of total cholesterol on CCA-IMT was accentuated with age, as was the protective effect of HDL cholesterol. In the urban group, the only significant interaction was between age and total cholesterol (β 0.001 mm, p = 0.01), whereby the effect of total cholesterol on CCA-IMT was accentuated with age.

Table 5. Interactions associated with mean CCA-IMT

Interaction terms	Multivariable model with interactions β (95% CI), mm	*p*
Rural group		
Age x HIV-duration (per five years)	0.001 (0.000, 0.001)	<0.001
Age x BMI	0.000 (0.000, 0.000)	0.42
Age x total cholesterol	0.002 (0.001, 0.003)	<0.001
Age x HDL cholesterol	-0.003 (-0.005, -0.001)	<0.001
Age x triglycerides	0.000 (-0.001, 0.001)	0.45
Age x glucose	0.001 (0.000, 0.001)	0.09
Urban group		
Age x BMI	0.000 (0.000, 0.000)	0.53
Age x total cholesterol	0.001 (0.000, 0.002)	<0.01
Age x triglycerides	0.001 (0.000, 0.002)	0.15

Abbreviations: BMI: body mass index, CCA-IMT: common carotid artery intima-media thickness, CI: confidence interval, HDL: high-density lipoprotein, HIV: human immune deficiency virus, LDL: low-density lipoprotein.

Discussion

In this analysis of PLHIV in South Africa, a substantial burden of subclinical CVD was observed with 16% of the rural population and 6% of the urban population having a plaque in the common carotid artery. The main drivers of mean CCA-IMT in both the rural and the urban group were conventional CVD risk factors and the effect of these conventional risk factors increased with age, especially in rural participants. In the rural group, HIV-duration was associated with lower CCA-IMT, but this effect was attenuated with age while ART-duration was associated with an increase in mean CCA-IMT. No HIV-related factors were associated with mean CCA-IMT in the urban group.

The finding that conventional CVD risk factors contribute significantly to mean CCA-IMT is in line with research from both high-income countries and from countries in SSA [25,38]. It is surprising that we did not find a correlation between blood pressure and CIMT. Blood pressure is known to be lower in PLHIV compared to the HIV-negative population[39]. It could be that the contribution of CVD risk factors to CIMT differs between HIV-positive and HIV-negative populations. However, a substantial proportion of the rural participants had imputed data for blood pressure, what might have obscured a real association between blood pressure and CCA-IMT in this group.

We observed that the effects of total cholesterol and HDL cholesterol on CCA-IMT increased with age, in line with recent results from a meta-analysis by Hanna et al., on determinants of CIMT in an HIV-positive population. In addition, Hannah et al., reported that the influence of SBP on CIMT also increased with age[37], a finding that we could not confirm in our analysis.

In the rural group, HIV-duration was inversely associated with mean CCA-IMT in an adjusted analysis while ART duration was associated with an increase in CIMT. There was also a positive interaction between age and HIV-duration in association with mean CCA-IMT which implies that HIV-duration might be associated with lower mean CCA-IMT in the younger population, but that this effect was attenuated with age. The use of ART was associated with a higher mean CCA-IMT but the influence of ART on CCA-IMT did not seem to increase with aging. There is no consensus in the literature yet on the role of HIV and ART on CIMT with some studies reporting that HIV and ART are associated with a higher CIMT[40–42], where other studies did not find an effect of HIV and ART on CIMT [38,43–45]. Possibly, HIV-related increase in CIMT only occurs after years of living with HIV. This is supported by the study of Fourie et al.[46], who reported that CIMT was similar between HIV-positive and HIV-negative participants, despite the fact that HIV-positive participants had higher levels of sVCAM-1, a strong indicator of endothelial damage. It might also be that CIMT does not fully summarize the influence of HIV on the arterial wall. HIV likely influences CVD risk through mechanisms beyond the regular process of atherosclerosis. CVD in HIV infection has also been found to be related to disturbances in the coagulation system and direct viral infiltration of the endothelium[46].

The uncertainty on the effects of HIV on CIMT was observed in our results as well: we did not find any association between HIV-duration or ART-duration and mean CCA-IMT in the urban group, in contrast to our findings in the rural group. Since there is no reason to expect that the influence of HIV-related factors on mean CCA-IMT would differ between the two settings, we presume that the following differences between the two groups are at the cause of these findings.

First, the rural population was substantially older than the urban population with a larger proportion of participants aged 50 years and over (23.7% in the rural group versus 12.8% in the urban group). The urban group might not have been old enough to reflect the influence of HIV on CIMT in an aging population. Another explanation to consider is that the rural group had been longer on first-line ART than the urban group (median duration 4.9 years versus 4.0 years); with a greater spread (the 75% percentile of ART duration was 8.1 years in the rural group versus 7.0 year in the urban group). Possibly, rural participants were exposed for longer to older stavudine containing ART regimens. Stavudine was part of South African first-line regimens until health care professional started tot switch patients to new regimens after the guidelines recommended against stavudine in 2010, which has been associated with enhanced CVD risk [47]. A third possibility was that the virologic control differed between sites: at the rural site 80.3% of participants on ART were virally suppressed, whereas at the urban site 93.5% of the participants on ART showed viral suppression. Ongoing viremia is associated with immune activation[48], higher CIMT[49], and an increased risk of CVD [50].

To summarize, the main drivers for CIMT in an HIV-positive population in SSA remain conventional CVD risk factors. HIV duration and ART were associated with CIMT, but only in the group sampled from a rural setting, which had a substantial number of participants over the age of 50 years and had suboptimal virologic control. This suggests that immune related mechanisms may add onto CVD risk in an older treated HIV positive population.

To our knowledge, this is the first study that reports CIMT and its determinants including a rural and urban group of PLHIV in SSA. Some limitations need to be considered. Our ability to investigate whether location of residence (a more rural setting vs. a more urban setting) influences CVD risk was limited as we could not directly compare the burden of subclinical CVD according to CIMT between the rural and the urban site due to differences in population characteristics and recruitment methods. The rural population had a relatively high number of participants without viral suppression. This might impact the generalizability of our findings to populations with an adequate virologic control.

Future research including an older urban SSA HIV-positive population and a rural HIV-positive population with better virologic control is recommended to further elucidate the association between HIV, ART-use and CVD risk. Ideally, studies with a prospective design including HIV-negative controls, could provide more insight into causal relationships between HIV, conventional CVD risk factors, and CVD. Our results suggest that CVD prevention in people living with HIV should be directed at conventional CVD risk factors alongside optimizing HIV care.

Acknowledgements

We thank the participants and staff from the Ndlovu Medical Centre as well as from the Johannesburg site for their contributions.

Conflicts of Interest

None declared.

Source of Funding

The Ndlovu Cohort study was supported by a grant from the Dutch AIDS Foundation; Dioraphte Foundation; De Grote Onderneming and the University Medical Center Utrecht. The study in Johannesburg was supported by the Julius Center, University Medical Center Utrecht, Utrecht, The Netherlands, Wits Reproductive Health and HIV Institute, Johannes, South Africa and a grant from the National Health and Laboratory Sciences, South-Africa (grant no. 94566). This specific analysis was performed without financial support.

References

1. Pillay-van Wyk V, Msemburi W, Laubscher R, Dorrington RE, Groenewald P, Glass T, et al. Mortality trends and differentials in South Africa from 1997 to 2012: second National Burden of Disease Study. Lancet Glob Heal. 2016;4:e642-53.
2. Joint United Nations Programme on HIV/AIDS (UNAIDS). UNAIDS DATA 2018. 2018. Accessed December 21, 2018 at: http://www.unaids.org/sites/default/files/media_asset/unaids-data-2018_en.pdf
3. Tollman SM, Kahn K, Sartorius B, Collinson MA, Clark SJ, Garenne ML. Implications of mortality transition for primary health care in rural South Africa: a population-based surveillance study. Lancet. 2008;372:893–901.
4. World Health Organization (WHO). Non-Communicable Disease, Country profiles: South Africa. 2016. Accesed December 6, 2018 at: http://www.who.int/nmah/countries/en/
5. Johnson LF, Mossong J, Dorrington RE, Schomaker M, Hoffmann CJ, Keiser O, et al. Life Expectancies of South African Adults Starting Antiretroviral Treatment: Collaborative Analysis of Cohort Studies. PLoS Med. 2013;10:e1001418.
6. Hontelez JAC, de Vlas SJ, Baltussen R, Newell M-L, Bakker R, Tanser F, et al. The impact of antiretroviral treatment on the age composition of the HIV epidemic in sub-Saharan Africa AIDS. 2012;26:S19–30.
7. Ballocca F, D'Ascenzo F, Gili S, Grosso Marra W, Gaita F. Cardiovascular disease in patients with HIV. Trends Cardiovasc Med. 2017;27:558–63.
8. Hyle EP, Mayosi BM, Middelkoop K, Mosepele M, Martey EB, Walensky RP, et al. The association between HIV and atherosclerotic cardiovascular disease in sub-Saharan Africa: a systematic review. BMC Public Health. 2017;17:954.
9. INSIGHT START Study Group, Lundgren JD, Babiker AG, Gordin F, Emery S, Grund B, et al. Initiation of Antiretroviral Therapy in Early Asymptomatic HIV Infection. N Engl J Med. 2015;373:795–807.
10. D'Ascenzo F, Cerrato E, Appleton D, Moretti C, Calcagno A, Abouzaki N, et al. Prognostic indicators for recurrent thrombotic events in HIV-infected patients with acute coronary syndromes: use of registry data from 12 sites in Europe, South Africa and the United States Thromb Res. 2014;134:558–64.
11. Lang S, Mary-Krause M, Simon A, Partisani M, Gilquin J, Cotte L, et al. HIV replication and immune status are independent predictors of the risk of myocardial infarction in HIV-infected individuals. Clin Infect Dis. 2012;55:600–7.
12. Dimala CA, Blencowe H. Association between highly active antiretroviral therapy and selected cardiovascular disease risk factors in sub-Saharan Africa: a systematic review and meta-analysis protocol. BMJ Open. 2017;7:e013353.

13. Worm SW, Sabin C, Weber R, Reiss P, El-Sadr W, Dabis F, et al. Risk of myocardial infarction in patients with HIV infection exposed to specific individual antiretroviral drugs from the 3 major drug classes: the data collection on adverse events of anti-HIV drugs (D:A:D) study. J Infect Dis. 2010;201:318–30.
14. Clark SJ, Gómez-Olivé FX, Houle B, Thorogood M, Klipstein-Grobusch K, Angotti N, et al. Cardiometabolic disease risk and HIV status in rural South Africa: establishing a baseline. BMC Public Health. 2015;15:135.
15. Bourne LT, Lambert E V, Steyn K. Where does the black population of South Africa stand n the nutrition transition? Public Health Nutr. 2002;5:157–62.
16. Steyn NP, McHiza ZJ. Obesity and the nutrition transition in Sub-Saharan Africa. Ann N Y Acad Sci. 2014;1311:88–101.
17. THUSA study, Vorster HH, Kruger A, Venter CS, Margetts BM, Macintyre UE. Cardiovascular disease risk factors and socio-economic position of Africans in transition: the THUSA study. Cardiovasc J Afr. 2007;18:282–9.
18. Vorster HH, Venter CS, Wissing MP, Margetts BM. The nutrition and health transition in the North West Province of South Africa: a review of the THUSA (Transition and Health during Urbanisation of South Africans) study. Public Health Nutr. 2005;8:480–90.
19. Pisa PT, Behanan R, Vorster HH, Kruger A. Social drift of cardiovascular disease risk factors in Africans from the North West Province of South Africa: the PURE study. Cardiovasc J Afr. 2012;23:371–8, e379-88.
20. Harris B, Goudge J, Ataguba JE, McIntyre D, Nxumalo N, Jikwana S, et al. Inequities in access to health care in South Africa. J Public Health Policy. 2011;32 Suppl 1:S102-23.
21. Bots ML, Hoes AW, Koudstaal PJ, Hofman A, Grobbee DE. Common carotid intima-media thickness and risk of stroke and myocardial infarction: the Rotterdam Study. Circulation. 1997;96:1432–7.
22. O'Leary DH, Polak JF, Kronmal RA, Manolio TA, Burke GL, Wolfson SK. Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. N Engl J Med. 1999;340:14–22
23. Owolabi MO, Agunloye AM, Umeh EO, Akpa OM. Can common carotid intima media thickness serve as an indicator of both cardiovascular phenotype and risk among black Africans? Eur J Prev Cardiol. 2015;22:1442–51.
24. D'Agostino RB, Burke G, O'Leary D, Rewers M, Selby J, Savage PJ, et al. Ethnic differences in carotid wall thickness. The Insulin Resistance Atherosclerosis Study. Stroke. 1996;27:1744–9.
25. Hulten E, Mitchell J, Scally J, Gibbs B, Villines TC. HIV positivity, protease inhibitor exposure and subclinical atherosclerosis: a systematic review and meta-analysis of observational studies. Heart. 2009;95:1826–35.

26. Vos A, Tempelman H, Devillé W, Barth R, Wensing A, Kretzschmar M, et al. HIV and risk of cardiovascular disease in sub-Saharan Africa: Rationale and design of the Ndlovu Cohort Study. Eur J Prev Cardiol. 2017;24:1043–50.
27. Venter WDF, Kambugu A, Chersich MF, Becker S, Hill A, Arulappan N, et al. Efficacy and safety of tenofovir disoproxil fumarate versus low-dose stavudine over 96 weeks: a multi- country randomised, non-inferiority trial. J Acquir Immune Defic Syndr. 2018;80:224–33.
28. Venter W, Moorhouse M, Sokhela S, Marahaj E, Akpomiemie G, Simmons B. Non-inferior efficacy for darunavir/ritonavir 400/100 mg once daily versus lopinavir/ritonavir, for patients with HIV RNA below 50 copies/mL in South Africa: The 48-week WRHI 052 study. J Int AIDS Soc. 2018;21:156–7..
29. World Health Organization (WHO). The STEPS Instrument and Support Materials v.3.2. Accessed October 25, 2018 at:http://www.who.int/chp/steps/instrument/en/index.html
30. International Physical Activity Questionnaire (IPAQ). IPAQ scoring protocol. 2005./ Accessed October 25, 2018 at: https://sites.google.com/site/theipaq/scoring-protocol
31. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972;18:499–502.
32. Alberti KGMM, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International. Circulation. 2009;120:1640–5. 2009;120(16):1640–5.
33. D'Agostino RB, 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;117:743–53.
34. Friis-Møller N, Ryom L, Smith C, Weber R, Reiss P, Dabis F, et al. An updated prediction model of the global risk of cardiovascular disease in HIV-positive persons: The Data- collection on Adverse Effects of Anti-HIV Drugs (D:A:D) study. Eur J Prev Cardiol. 2016;23:214–23.
35. Bots ML, Evans GW, Tegeler CH, Meijer R. Carotid Intima-media Thickness Measurements: Relations with Atherosclerosis, Risk of Cardiovascular Disease and Application in Randomized Controlled Trials. Chin Med J (Engl). 2016;129:215–26.
36. Boulos NM, Gardin JM, Malik S, Postley J, Wong ND. Carotid Plaque Characterization, Stenosis, and Intima-Media Thickness According to Age and Gender in a Large Registry Cohort. Am J Cardiol. 2016;117:1185–91.
37. Hanna DB, Guo M, Bůžková P, Miller TL, Post WS, Stein JH, et al. HIV Infection and Carotid Artery Intima-media Thickness: Pooled Analyses Across 5 Cohorts of the NHLBI HIV- CVD Collaborative. Clin Infect Dis. 2016;63:249–56.

38. Schoffelen AF, de Groot E, Tempelman HA, Visseren FLJ, Hoepelman AIM, Barth RE. Carotid Intima Media Thickness in Mainly Female HIV-Infected Subjects in Rural South Africa: Association With Cardiovascular but Not HIV-Related Factors. Clin Infect Dis. 2015;61:1606–14

39. Dillon DG, Gurdasani D, Riha J, Ekoru K, Asiki G, Mayanja BN, et al. Association of HIV and ART with cardiometabolic traits in sub-Saharan Africa: A systematic review and meta- analysis. Int J Epidemiol. 2013;42:1754–71.

40. Lorenz MW, Stephan C, Harmjanz A, Staszewski S, Buehler A, Bickel M, et al. Both long- term HIV infection and highly active antiretroviral therapy are independent risk factors for early carotid atherosclerosis. Atherosclerosis. 2008;196:720–6.

41. Krikke M, Arends JE, Van Lelyveld S, Hoepelman A, Visseren F. Greater carotid intima media thickness at a younger age in HIV-infected patients compared with reference values for an uninfected cohort. HIV Med. 2017;18:275–83.

42. Gupta PK, Gupta M, Lal AK, Taneja A, Taneja RS, Rewari BB. Markers of subclinical atherosclerotic disease in HIV-infected individuals. J virus Erad. 2018;4:21–5.

43. Mosepele M, Mohammed T, Mupfumi L, Moyo S, Bennett K, Lockman S, et al. HIV disease is associated with increased biomarkers of endothelial dysfunction despite viral suppression on long-term antiretroviral therapy in Botswana. Cardiovasc J Afr. 2018;29:155– 61.

44. Gleason RL, Caulk AW, Seifu D, Parker I, Vidakovic B, Getenet H, et al. Current Efavirenz (EFV) or ritonavir-boosted lopinavir (LPV/r) use correlates with elevate markers of atherosclerosis in HIV-infected subjects in Addis Ababa, Ethiopia. PLoS One. 2015;10:e0117125.

45. Volpe GE, Tang AM, Polak JF, Mangili A, Skinner SC, Wanke CA. Progression of carotid intima-media thickness and coronary artery calcium over 6 years in an HIV-infected cohort. J Acquir Immune Defic Syndr. 2013;64:51–7.

46. Fourie CMT, Schutte AE, Smith W, Kruger A, van Rooyen JM. Endothelial activation and cardiometabolic profiles of treated and never-treated HIV infected Africans. Atherosclerosis. 2015;240:154–60.

47. George JA, Venter WDF, Van Deventer HE, Crowther NJ. A longitudinal study of the changes in body fat and metabolic parameters in a South African population of HIV-positive patients receiving an antiretroviral therapeutic regimen containing stavudine. AIDS Res Hum Retroviruses. 2009;25:771–81.

48. Bandera A, Colella E, Rizzardini G, Gori A, Clerici M. Strategies to limit immune-activation in HIV patients. Expert Rev Anti Infect Ther. 2017;15:43–54

49. Siedner MJ, Kim J-H, Nakku RS, Bibangambah P, Hemphill L, Triant VA, et al. Persistent Immune Activation and Carotid Atherosclerosis in HIV-Infected Ugandans Receiving Antiretroviral Therapy. J Infect Dis. 2016;213:370–8.

50. Borges ÁH, Neuhaus J, Sharma S, Neaton JD, Henry K, Anagnostou O, et al. The Effect of Interrupted/Deferred Antiretroviral Therapy on Disease Risk: A SMART and START Combined Analysis. J Infect Dis. 2019;219:254–63.

CHAPTER

10

Lipid levels, insulin resistance and cardiovascular risk over 96 weeks of antiretroviral therapy: a randomised controlled trial comparing low-dose stavudine and tenofovir

A.G. Vos, M. Chersich, K.Klipstein-Grobusch, P. Zuithoff, M.A. Moorhouse, S.T. Lalla-Edward, A. Kambugu, N. Kumarasamy, D.E. Grobbee, R.E. Barth, W.D.Venter

Retrovirology. 2018 Dec 14;15(1):77

Abstract

Background HIV infection and antiretroviral treatment are associated with changes in lipid levels, insulin resistance and risk of cardiovascular disease (CVD). We investigated these changes in the first 96 weeks of treatment with low-dose stavudine or tenofovir regimens.

Methods This is a secondary analysis of a double blind, randomised controlled trial performed in South-Africa, Uganda and India comparing low-dose stavudine (20 mg twice daily) with tenofovir in combination with efavirenz and lamivudine in antiretroviral-naïve adults (n=1067) (Clinicaltrials.gov, NCT02670772). Over 96 weeks, data were collected on fasting lipids, glucose and insulin. Insulin resistance was assessed with the HOMA-IR index and 10-year CVD risk with the Framingham risk score (FRS). A generalized linear mixed model was used to estimate trends over time.

Results Participants were on average 35.3 years old, 57.6% female and 91.8% Black African. All lipid levels increased following treatment initiation, with the sharpest increase in the first 24 weeks of treatment. The increase in all lipid subcomponents over 96 weeks was higher among those in the stavudine than the tenofovir group. Insulin resistance increased steadily with no difference detected between study groups. Framingham risk score rose from 1.90% (1.84-1.98%) at baseline to 2.06 (1.98-2.15%) at week 96 for the total group, with no difference between treatment arms (p = 0.144). Lipid changes were more marked in Indian than African participants.

Conclusion Lipid levels increased in both groups, with low-dose stavudine resulting in a worse lipid profile compared to tenofovir. Insulin resistance increased, with no difference between regimens. CVD risk increased over time and tended to increase more in the group on stavudine. The low CVD risk across both arms argues against routine lipid and glucose monitoring in the absence of other CVD risk factors. In high risk patients, monitoring may only be appropriate at least a year after treatment initiation.

Introduction

Globally, cardiovascular disease (CVD) is the leading cause of mortality[1]. Low- and middle-income countries (LMICs) share this burden: the leading cause of death has changed from infectious diseases to ischaemic heart disease over the last two decades[1]. Timely recognition and treatment of cardiovascular risk factors are key for reducing the burden of CVD. Human immunodeficiency virus (HIV) infection and treatment with antiretroviral therapy (ART) affects risk factors for CVD[2-5] and some studies indicate that HIV infection increases the risk of myocardial infarction or stroke by up to 50%[6,7].

The contribution of ART to CVD risk is less clear, and risk profiles vary by antiretroviral drug[8]. Protease inhibitors and efavirenz, a non-nucleoside reverse transcriptase inhibitor, are well known for their adverse effects on lipid and glucose metabolism[9-11]. Stavudine, a nucleoside reverse transcriptase inhibitor (NRTI), has considerable metabolic side effects. It is associated with mitochondrial toxicity, resulting in lipodystrophy, disturbances in lipid levels and an increase in insulin resistance[12-15]. Stavudine was part of first-line ART for many years globally, as it was well tolerated in the first few months of treatment, cheap, widely available in twice-daily fixed-dose formulations and effective in suppressing viral load[16]. When the extent of mitochondrial toxicity became apparent, use of the drug declined rapidly. Between 2012 and 2016, a clinical trial was initiated, comparing a lower dose of stavudine to the current most commonly used first-line drug, tenofovir disoproxil fumarate ('tenofovir'). The overall conclusion was that low-dose stavudine was equally effective as tenofovir in reducing viral load after 48 weeks, but that lipoatrophy occurred more often in the low-dose stavudine group[17].

Extensive metabolic and toxicity monitoring allow us to conduct an in-depth analysis of the effects of ART initiation with low-dose stavudine or tenofovir on lipid levels, insulin resistance and CVD risk, an important analysis as the vast majority of people on ART are taking regimens containing tenofovir. We present these results in this paper.

Methods

A randomised 1:1 double blind placebo-controlled trial was conducted in Johannesburg, South Africa, Kampala, Uganda and Chennai, India to assess the efficacy and safety of treatment with either low dose stavudine (20 mg twice a day (BD)) or tenofovir (300 mg daily) tablets administered in combination with lamivudine (150 mg BD) and efavirenz (600 mg daily) over 96 weeks (Clinicaltrials.gov, NCT02670772). The methods including quality control and safety evaluation are described in detail elsewhere[17]. In brief, data were captured in an electronic data system. Computerised and manual

procedures as well as regular site visits were implemented to optimise data quality. Monitoring and support was undertaken by Pharmaceutical Product Development (PPD, Wilmington, USA). 1067 HIV-positive, ART-naive participants aged 18 years and over with unsuppressed HIV viral load were included from clinical trial sites. Recruitment in India was stopped early due to a regulatory change, and it was decided to raise the number of participants recruited in the remaining sites so as to reach the target number timeously. Exclusion criteria were age above 65 years for the Indian site, pregnancy, CD4+ > 350 cells/mL, hepatitis B antigen positivity, or an estimated glomerular filtration rate (eGFR) < 60 mL/min, calculated using the Cockcroft-Gault equation. Visits took place at baseline, one month, three months and every three months thereafter until 96 weeks. Data collected at baseline included demographic information, medical history including use of medication. In the South African site only, additional data on employment, marital status, having children, as well as data on current smoking, alcohol and drug use were collected.

At each visit, weight, heart rate and blood pressure were measured and blood samples taken. Blood pressure was measured once in a seated position after a 5-minute rest. Blood sampling was performed after an overnight fast and included lipids, glucose, insulin, HIV viral load and CD4+ count. Blood samples were analysed by contracted and accredited laboratory services (see Additional file 1 for more details). Insulin resistance was quantified with the HOMA-IR, using the following formula: fasting glucose (mmol/L) * fasting insulin (mU/L))/22.5[18]. Framingham risk score (FRS) was calculated using the 10-year CVD risk score equation, and only available for South Africa, as the risk score includes smoking history, which was only collected at the South African site[19].

The study was approved by the Human Ethics Research Committee of the University of the Witwatersrand, ethics clearance number 111112, the Research and Ethics Committee of the Joint Clinical Research Centre, Uganda, and the Uganda National Council for Science and Technology clearance number HS 1219, and the IRB & Ethics Research Committee of YRGCARE. All participants provided written informed consent prior to participation. All participants in the stavudine arm were switched to a tenofovir containing regimen at the end of follow as per the national HIV treatment guidelines.

Statistical analysis

Demographics were reported as means and standard deviations, or medians with interquartile ranges as appropriate. Non-normal outcomes were log transformed to obtain a normal distribution. In line with logistic regression models, we used a logit transformation for the predicted probabilities based on the Framingham risk model. Lipid levels, glucose, insulin, HOMA-IR and FRS measurements over the 96 week period were analysed with a linear mixed model (estimated with restricted maximum likelihood)

with a random intercept and a random effect for time. We included a quadratic term for time (i.e. time2), considering that outcomes are expected to level off at a certain point in time. Treatment arm, time, time^2 and the interaction between treatment arm and both time and time^2 were included as fixed effects together with a correction for age, sex, site of inclusion (South-Africa, Uganda or India), body mass index and viral load at baseline. P-values for the interactions between treatment and both time and time2 were estimated with likelihood ratio tests. Likelihood ratio tests were chosen, as it allows estimation of one p-value (per outcome) for both interactions. For these tests, models were refitted with maximum likelihood estimation[20]. The estimated mean values at week 0, 24 weeks, 48 weeks, 72 weeks and 96 weeks for all outcomes were displayed graphically (transformed back to the original scale when applicable). The trend over time (per month) per treatment arm was calculated for all outcomes. Results are presented as regression coefficients (β) with 95% confidence intervals. Statistical analysis was done with SPSS version 24 (IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.). Validity of the models was evaluated with residual analyses with SAS software version 9.4 (SAS Institute Inc., Cary, NC, USA).

Results

The mean age of participants was 35.3 years, the majority were female (57.6%), 24.2% was married, 76.0% was employed and most were of African descent (91.8%). The median nadir CD4+ cell count was 206 cells/μL (IQR 124-272). There were no differences in demographics, CVD risk factors or HIV-related factors between the study arms or study sites at baseline (Table 1). Participant retention was similar in each arm, with an overall median follow-up time of 95.4 weeks (Interquartile range (IQR) 95.0–95.7 weeks). Viral suppression (<50 copies/mL) was reached in 71.1% in the stavudine arm and 76.7% in the tenofovir arm at the end of follow up. In total, 12.2% had a follow-up time of less than 48 weeks. Alcohol use was the most frequently reported substance used, followed by smoking and illegal drug use.

Table 1. Demographics, HIV disease and cardiovascular disease risks of patients at baseline

	All (n=1067)	**Stavudine** (n=533)	**Tenofovir** (n=534)
Sex, female (n, %)	615 (57.6)	324 (60.8)	291 (54.5)
Age in years	35.3 (8.2)	35.5 (8.4)	35.0 (8.1)
Race (n, %) African	979 (91.8)	489 (91.7)	490 (91.8)
Indian	86 (8.1)	43 (8.1)	43 (8.1)
Other	2 (0.2)	1 (0.2)	1 (0.2)
Site (n, %) South Africa	600 (56.2)	300 (56.3)	300 (56.2)
Uganda	381 (35.7)	190 (35.6)	191 (35.8)
India	86 (8.1)	43 (8.1)	43 (8.1)
Married[1] (n, %)	139 (24.2)	69 (24.1)	70 (24.2)
Having children[1] (n, %)	492 (85.9)	243 (84.1)	249 (87.7)
Employed[1] (n, %)	434 (76.0)	220 (76.1)	214 (75.9)
CD4+ cell count (cells/μL) (median, IQR)	206 (124 – 272)	206 (128 – 274)	206 (123 – 270)
Log HIV viral load (copies/mL)	11.26 (1.61)	11.34 (1.64)	11.18 (1.58)
History of hypertension[2] (n, %)	59 (5.5)	28 (5.3)	31 (5.8)
History of diabetes mellitus[2] (n, %)	6 (0.6)	4 (0.8)	2 (0.4)
History of dyslipidemia[2] (n, %)	2 (0.2)	1 (0.2)	1 (0.2)
Current smoking[3] (n, %)	86 (15)	44 (15.3)	42 (14.7)
Current alcohol use[3] (n, %)	203 (35.4)	101 (35.1)	102 (35.8)
Current drug use[3] (n, %)	17 (3.0)	8 (2.8)	9 (3.2)
Systolic blood pressure (mmHg)	118.3 (13.2)	117.5 (13.5)	119.1 (12.8)
Diastolic blood pressure (mmHg)	72.9 (10.2)	72.5 (10.6)	73.4 (9.9)
Body mass index (kg/m^2)	23.5 (4.4)	23.6 (4.6)	23.5 (4.2)
Follow-up time, median months, IQR	95.4 (95.0-95.7)	95.4 (95.0–95.7)	95.4 (95.1–95.8)
Total-C (mmol/L)	4.30 (1.02)	4.39 (1.11)	4.22 (0.93)
HDL-C (mmol/L)	1.32 (0.49)	1.37 (0.54)	1.27 (0.44)
LDL-C (mmol/L)	2.47 (0.86)	2.50 (0.93)	2.44 (0.79)
TG (mmol/L) (median, IQR)	1.00 (0.74 – 1.37)	1.00 (0.76 – 1.40)	0.98 (0.72 – 1.33)
Fasting glucose (mmol/L)	4.77 (0.97)	4.72 (0.64)	4.81 (1.20)
Fasting insulin (pmol/L) (median, IQR)	38.20 (25.70 – 55.56)	40.28 (27.09 – 57.64)	36.81 (24.31 – 54.17)
HOMA-IR (median, IQR)	1.13 (0.74 – 1.75)	1.18 (0.78 – 1.78)	1.09 (0.71 – 1.70)
Framingham risk score (%) (median, IQR)	1.86 (1.02 – 3.45)	1.90 (1.05 – 3.44)	1.82 (0.98 – 3.46)

Figure 1 shows the trend in lipid levels following treatment initiation (Figure 1). Total-C, HDL-C and LDL-C increased over the course of 96 weeks with a sharper increase in the group receiving stavudine than in the group receiving tenofovir, $p < 0.001$ for Total-C and HDL-C, and $p = 0.036$ for LDL-C (Table 2, see Additional file 2). TG levels decreased from week 24 in the group on tenofovir and the estimated mean at week 96 was almost identical to the estimated mean at baseline (1.148 mmol/L (95% CI 1.102 – 1.197) at baseline versus 1.154 mmol/L (95% CI 1.097 – 1.212) at week 96).

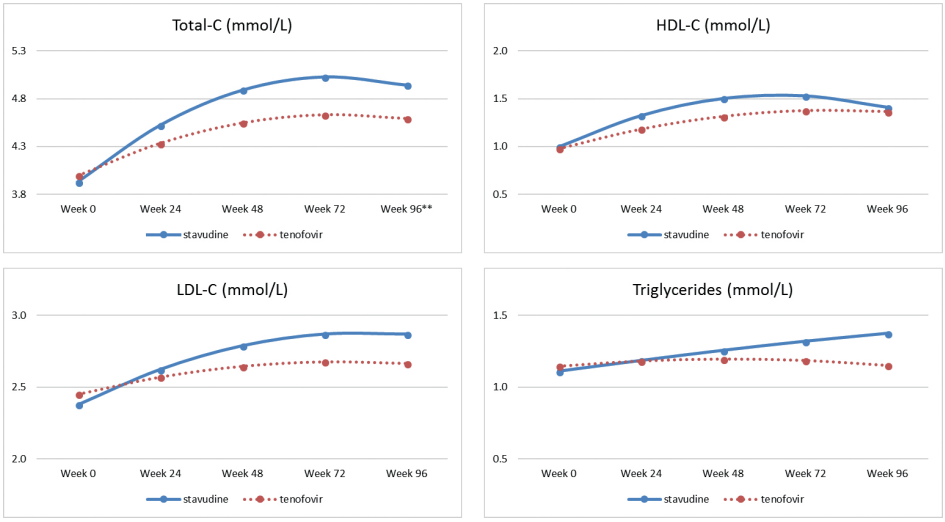


Figure 1. Trends in estimated marginal mean lipid levels in each study group. HDL-C, high density lipoprotein cholesterol; LDL-C, low densiy lipoprotein cholesterol; Total-C, total cholesterol.

Glucose values were similar in both groups, with a steep increase in the first 48 weeks (from 4.63 mmol/L (95% CI 4.56 – 4.69) at baseline to 5.13 mmol/L (95% CI 5.06 – 5.20) at week 48) and values tended to level off from week 48 to reach 5.12 mmol/L (95% CI 5.05 – 5.31) at week 96 (see Figure 2 for trend per treatment arm and Additional file 2). Insulin increased over 96 weeks, with no difference detected between arms ($p = 0.677$, Table 2). The increase in insulin resistance per month (with HOMA-IR) was not different for participants on stavudine versus tenofovir ($p = 0.698$; Table 2)

531207-L-bw-Vos
Processed on: 20-5-2019

Table 2. Estimated changes per month in lipid levels, insulin resistance and Framingham risk score.

Variable		**Increase per month** regression coefficient (95% CI)[#]	**Increase per month²** regression coefficient (95% CI)[#]	***p***
Total-C (mmol/L)				
	stavudine	0.1275 (0.1035 – 0.1514)	-0.0037 (-0.0047 – -0.0027)	
	tenofovir	0.0719 (0.0621 – 0.0818)	-0.0020 (-0.0025 - -0.0016)	<0.001
HDL-C (mmol/L)				
	stavudine	0.0721 (0.0572 – 0.0870)	-0.0024 (-0.0031 - -0.0018)	
	tenofovir	0.0433 (0.0372 – 0.0495)	-0.0012 (-0.0014 - -0.0009)	<0.001
LDL-C (mmol/L)				
	stavudine	0.0516 (0.0324 – 0.0709)	-0.0013 (-0.0021 - -0.0005)	
	tenofovir	0.0254 (0.0175 – 0.0334)	-0.0007 (-0.0010 - -0.0004)	0.036
Log TG (mmol/L)				
	stavudine	0.0125 (-0.0017 – 0.0268)	-0.0001 (-0.0007 – 0.0005)	
	tenofovir	0.0073 (0.0014 – 0.0132)	-0.0003 (-0.0006 - -0.0001)	0.058
Glucose (mmol/L)				
	stavudine	0.0689 (0.0393 – 0.0980)	-0.0021 (-0.0033 - -0.0009)	
	tenofovir	0.0679 (0.0558 – 0.0800)	-0.0020 (-0.0026 - -0.0015)	0.991
Insulin (pmol/L)				
	stavudine	0.3941 (-1.3102 – 2.0984)	0.0045 (-0.0659 – 0.0749)	
	tenofovir	0.0042 (-0.7001 – 0.7086)	0.0231 (-0.0059 – 0.0521)	0.677
Log HOMA-IR				
	stavudine	0.0242 (0.0006 – 0.0479)	-0.0004 (-0.0014 – 0.0006)	
	tenofovir	0.0158 (0.0060 – 0.0255)	-0.0002 (-0.0006 – 0.0003)	0.756
Logit Framingham risk score[*]				
	stavudine	0.0100 (-0.0050 – 0.0251)	-0.0001 (-0.0008 – 0.0005)	
	tenofovir	-0.0047 (-0.0109 – 0.0016)	0.0002 (0.0000 – 0.0005)	0.144

[*]Data for South African site only. [#] The model includes adjustment for age, gender, site, body mass index and viral load. Month², time was used as a quadratic term to take non-linearity into account. P-values were estimated with a likelihood ratio test for both interactions. See methods for details. Abbreviations: CI: confidence interval, HDL-C: high density lipoprotein cholesterol, LDL-C: low density lipoprotein cholesterol, OR: odds ratio, TG: triglycerides, Total-C: total cholesterol.

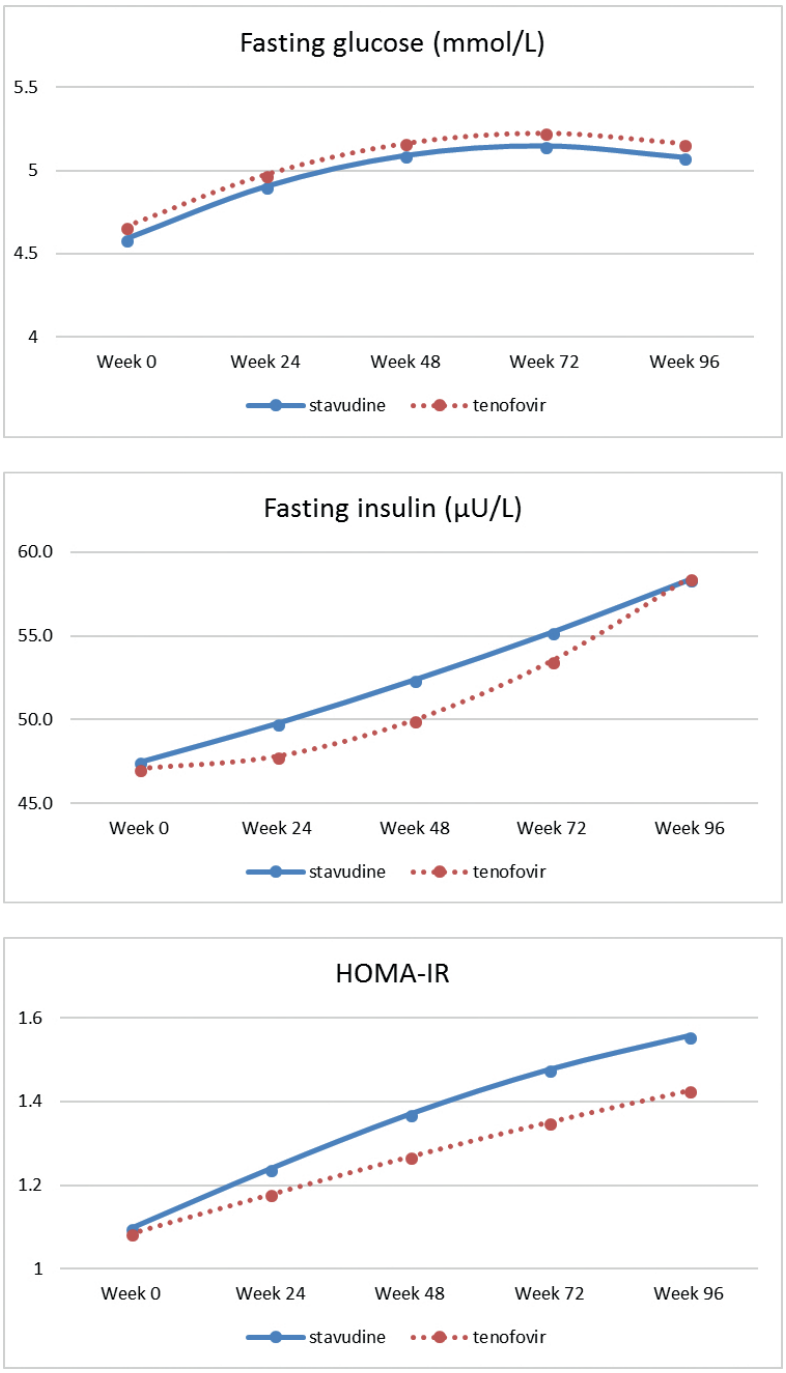


Figure 2. Trends in estimated marginal mean levels of indicators of insulin resistance.

Framingham risk score rose from 1.90% (1.84-1.98%) at baseline to 2.06 (1.98-2.15%) at week 96 for the total group. (see Figure 3 for trend per treatment arm and Additional file 2). FRS in the stavudine group was significantly lower at baseline than in the tenofovir group, 1.82% (95% CI 1.73 – 1.92) and 2.00% (95% CI 1.89 – 2.10) respectively, p = 0.013. The risk in the group on stavudine went up to 2.11% (95% CI 1.99 – 2.24) at week 96. The risk in the group on tenofovir decreased initially to 1.94% (95% CI 1.85 – 2.05) at week 48 to increase to 2.01% (95% CI 1.90 – 2.13) at week 96. The overall trend between the groups was not significantly different, p = 0.144.

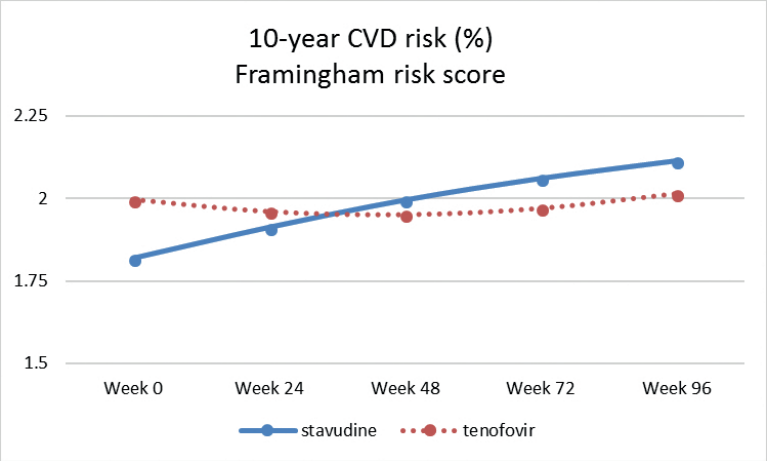


Figure 3. Trend in estimated marginal mean levels of 10-year CVD risk according to the Framingham risk score

Age, sex and BMI were associated with all lipid subcomponents, glucose, insulin, HOMA-IR and FRS (Additional file 3. Supplementary tables 3a-3h). The 10-year CVD risk according to the FRS was almost double for males compared to females at week 96, 2.88% (95% CI 2.68 – 3.00) for males versus 1.50% (95% CI 1.42 – 1.58) for females, p < 0.001. Log viral load at baseline was associated with all lipid subcomponents (with a higher log viral load linked to a lower Total-C, HDL-C and LDL-C) but not to glucose, insulin, HOMA-IR or FRS. Indian participants had a worse lipid profile and glucose homeostasis compared to their African counterparts. For example, at baseline Total-C was 4.38 mmol/L (95% CI 4.21 – 4.55) for the Indian site and 3.72 mmol/L (95% CI 3.66 – 3.79) for the South African site, and fasting glucose was 5.05 mmol/L (95% CI 4.90 – 5.22) for the Indian site versus 4.45 mmol/L (95% CI 4.38 – 4.51) for the South African site, p < 0.001 for both comparisons. In addition to these differences in baseline levels, Indian participants had a sharper increase in total-C, LDL-C and TG, and a larger decrease

in HDL-C with treatment (See Additional file 3. Supplementary Tables 3a-3d). The effect of stavudine and tenofovir on lipid components, as described above, was similar for all three populations (South Africa, Uganda and India).

Discussion

Initiation of low-dose stavudine and tenofovir with a backbone of lamivudine and efavirenz resulted in a rise in lipid levels with a worse lipid profile for stavudine compared to tenofovir. Insulin resistance went up but there was no difference in trend between both groups. CVD risk was low in general and with no statistically significant difference between the groups over time.

Untreated HIV infection is characterized by an increase in TG and a decrease in both LDL-C and HDL-C[8,21,22]. The disturbance in lipid levels is likely a result of chronic inflammation that accompanies HIV infection[23]. Chronic inflammation influences the way lipids are metabolized and activated. Oxidized LDL and HDL may directly induce monocyte and endothelial cell activation, and this is related to the development of CVD [24].

Studies on initiation of ART have attributed both improvement, as well as deterioration, in lipid levels to ART[8,22,24-26]. A meta-analysis of cardiovascular risk factors in relation to HIV and ART in sub-Saharan Africa found that ART was associated with an increase in HDL-C and a decrease in TG, but also with an increase in LDL-C[2]. Liu et al. investigated changes in lipid levels during three years following ART initiation in Tanzania. They found an increase in LDL and HDL, and a decrease in TGs in the first 6 months. Between six months and three years HDL levels topped off and TG levels continued to increase [25]. Our results are broadly in line with these findings. We did not see a decrease in TG between treatment initiation and week 96, but the TG level in the group receiving tenofovir at week 96 was approximately the same as the level before treatment initiation. Our results underline the importance of taking treatment duration into account.

There is no agreed threshold to define insulin resistance in an urban African population[27] but based on a study in an urban Ghanaian population, a HOMA-IR cut-off of 2.3 seems appropriate[28]. According to this definition, neither low-dose stavudine nor tenofovir resulted in significant insulin resistance. Stavudine's mitochondrial toxicity is likely dose dependent[29,30], and the low dose used in this study may account for the lack of impact on glucose metabolism. We can, however, not exclude the possibility that the drug's deleterious effects could still occur with more extended durations of treatment.

Lipid levels and insulin resistance are increased in both treatment arms. This may partly rely on the effects of efavirenz, as this drug is known to alter lipid levels and glucose metabolism [11,15]. Our findings underline the need for more 'lipid-friendly' ART, of which dolutegravir is a promising alternative[31].

CVD risk according to the 10-year FRS increased in the total group with 0.16% between baseline and week 96. This was statistically significant, but it's questionable whether this slight increase over almost two years is clinically relevant. There was a trend towards a sharper increase in CVD risk in the group receiving stavudine, most likely reflecting the steeper increase in LDL-C and TG in this group compared to the group on tenofovir. At the same time, it would be important to consider the relative contribution of ART to well-known modifiable cardiovascular risk factors such as obesity, smoking, diet, and physical inactivity.

Indian participants had substantially worse lipid profiles and glucose homeostasis compared to the African participants. This is a well-known phenomenon that is likely dependent on genetic factors and lifestyle factors such as being underweight during infancy, diet and physical activity[32-34].

Strengths of the study are the large, representative sample including participants from three countries, the randomized design, the standardized and regular assessment of lipids, glucose and insulin, and the follow up duration of almost two years. However, some limitations need to be recognized. Information on smoking was only collected for the South African site, relied on patient reporting as opposed to biochemical measures, and only at baseline. No information on family history of CVD was collected, so we could not quantify CVD risk with other CVD risk prediction scores such as the Data collection on Adverse events of Anti-HIV Drugs (D:A:D) score, which is considered to be a more accurate risk prediction tool for HIV-infected populations than the FRS[35]. Also, we could not examine effects of the metabolic syndrome as waist circumference had not been measured. In line with an intention-to-treat approach for clinical trials, we only corrected for viral load at baseline, even though viral load at follow-up was available in the data. Both adherence and the proportion of participants with suppressed viral load between treatment groups were approximately equal. We therefore assume that any bias is small. Finally, the study did not include an HIV-negative control group to compare our findings to age-related, HIV-unrelated changes in lipid levels and insulin resistance over time.

Conclusion

This study showed that low-dose stavudine has more deleterious effects on lipids than tenofovir. However, the impact of stavudine on this short term appears to be small, and it's questionable whether the increase in lipid levels is clinical relevant in this relatively young population with a low CVD risk. The need to monitor lipid and glucose levels has to be determined using CVD risk calculators that take other risk factors like age and hypertension into account. There should be extra awareness for CVD risk prevention in the Indian population. We recommend to measure lipids and glucose at the earliest a year after treatment initiation, as levels change substantially following treatment initiation.

References

1. GBD 2016 Causes of Death Collaborators. Global, regional, and national age-sex specific mortality for 264 causes of death, 1980-2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet. 2017;390: 1151-1210. doi: S0140-6736(17)32152-9 [pii].
2. Dillon DG, Gurdasani D, Riha J, Ekoru K, Asiki G, Mayanja BN, et al. Association of HIV and ART with cardiometabolic traits in sub-Saharan Africa: a systematic review and meta-analysis. Int J Epidemiol. 2013;42: 1754-1771. doi: 10.1093/ije/dyt198 [doi].
3. Kingery JR, Alfred Y, Smart LR, Nash E, Todd J, Naguib MR, et al. Short-term and long-term cardiovascular risk, metabolic syndrome and HIV in Tanzania. Heart. 2016;102: 1200-1205. doi: 10.1136/heartjnl-2015-309026 [doi].
4. Nduka CU, Stranges S, Sarki AM, Kimani PK, Uthman OA. Evidence of increased blood pressure and hypertension risk among people living with HIV on antiretroviral therapy: a systematic review with meta-analysis. J Hum Hypertens. 2016;30: 355-362. doi: 10.1038/jhh.2015.97 [doi].
5. Nou E, Lo J, Grinspoon SK. Inflammation, immune activation, and cardiovascular disease in HIV. AIDS. 2016;30: 1495-1509. doi: 10.1097/QAD.0000000000001109 [doi].
6. Freiberg MS, Chang CC, Kuller LH, Skanderson M, Lowy E, Kraemer KL, et al. HIV infection and the risk of acute myocardial infarction. JAMA Intern Med. 2013;173: 614-622. doi: 10.1001/jamainternmed.2013.3728 [doi].
7. Zanni MV, Schouten J, Grinspoon SK, Reiss P. Risk of coronary heart disease in patients with HIV infection. Nat Rev Cardiol. 2014;11: 728-741. doi: 10.1038/nrcardio.2014.167 [doi].
8. Dube MP, Cadden JJ. Lipid metabolism in treated HIV Infection. Best Pract Res Clin Endocrinol Metab. 2011;25: 429-442. doi: 10.1016/j.beem.2011.04.004 [doi].
9. Clotet B, Negredo E. HIV protease inhibitors and dyslipidemia. AIDS Rev. 2003;5: 19-24.
10. Stein JH. Dyslipidemia in the era of HIV protease inhibitors. Prog Cardiovasc Dis. 2003;45: 293-304. doi: 10.1053/pcad.2003.4 [doi].
11. Sinxadi PZ, McIlleron HM, Dave JA, Smith PJ, Levitt NS, Haas DW, et al. Plasma Efavirenz Concentrations Are Associated With Lipid and Glucose Concentrations. Medicine (Baltimore). 2016;95: e2385. doi: 10.1097/MD.0000000000002385 [doi].
12. Fleischman A, Johnsen S, Systrom DM, Hrovat M, Farrar CT, Frontera W, et al. Effects of a nucleoside reverse transcriptase inhibitor, stavudine, on glucose disposal and mitochondrial function in muscle of healthy adults. Am J Physiol Endocrinol Metab. 2007;292: E1666-73. doi: 00550.2006 [pii].
13. Gallant JE, Staszewski S, Pozniak AL, DeJesus E, Suleiman JM, Miller MD, et al. Efficacy and safety of tenofovir DF vs stavudine in combination therapy in antiretroviral-naive patients: a 3-year randomized trial. JAMA. 2004;292: 191-201. doi: 10.1001/jama.292.2.191 [doi].

14. George JA, Venter WD, Van Deventer HE, Crowther NJ. A longitudinal study of the changes in body fat and metabolic parameters in a South African population of HIV-positive patients receiving an antiretroviral therapeutic regimen containing stavudine. AIDS Res Hum Retroviruses. 2009;25: 771-781. doi: 10.1089/aid.2008.0308 [doi].
15. Mouton JP, Cohen K, Maartens G. Key toxicity issues with the WHO-recommended first-line antiretroviral therapy regimen. Expert Rev Clin Pharmacol. 2016;9: 1493-1503. doi: 10.1080/17512433.2016.1221760 [doi].
16. World HO. Antiretroviral therapy for HIV infection in adults and adolescents. Recommendations for a public health approach. 2010 revision. . 2010. doi: NBK138540 [bookaccession].
17. Venter WDF, Kambugu A, Chersich MF, Becker S, Hill A, Arulappan N et al. Efficacy and safety of tenofovir disoproxil fumarate versus low-dose stavudine over 96 weeks: a multi-country randomised, non-inferiority trial. J Acquir Immune Defic Syndr. 2018 Nov 12.
18. Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modeling. Diabetes Care. 2004;27: 1487-1495. doi: 27/6/1487 [pii].
19. Framingham Heart Study10-year CVD risk score. https ://www.framingham heart study .org/fhs-risk-functions/cardiovascular-disease-10-yearrisk/. Accessed 27 April 2018. doi: 10.1097/QAI.0000000000001908.
20. Fitzmaurice GM, Laird NM, Ware JH. Jason Roy, Applied Longitudinal Analysis. Journal of Biopharmaceutical Statistics. 2013;Second Edition: 491.
21. Grunfeld C, Pang M, Doerrler W, Shigenaga JK, Jensen P, Feingold KR. Lipids, lipoproteins, triglyceride clearance, and cytokines in human immunodeficiency virus infection and the acquired immunodeficiency syndrome. J Clin Endocrinol Metab. 1992;74: 1045-1052. doi: 10.1210/jcem.74.5.1373735 [doi].
22. Dave JA, Levitt NS, Ross IL, Lacerda M, Maartens G, Blom D. Anti-Retroviral Therapy Increases the Prevalence of Dyslipidemia in South African HIV-Infected Patients. PLoS One. 2016;11: e0151911. doi: 10.1371/journal.pone.0151911 [doi].
23. Miedema F, Hazenberg MD, Tesselaar K, van Baarle D, de Boer RJ, Borghans JA. Immune activation and collateral damage in AIDS pathogenesis. Front Immunol. 2013;4: 298. doi: 10.3389/fimmu.2013.00298 [doi].
24. Funderburg NT, Mehta NN. Lipid Abnormalities and Inflammation in HIV Inflection. Curr HIV/AIDS Rep. 2016;13: 218-225. doi: 10.1007/s11904-016-0321-0 [doi].
25. Liu E, Armstrong C, Spiegelman D, Chalamilla G, Njelekela M, Hawkins C, et al. First-line antiretroviral therapy and changes in lipid levels over 3 years among HIV-infected adults in Tanzania. Clin Infect Dis. 2013;56: 1820-1828. doi: 10.1093/cid/cit120 [doi].
26. Nduka C, Sarki A, Uthman O, Stranges S. Impact of antiretroviral therapy on serum lipoprotein levels and dyslipidemias: a systematic review and meta-analysis. Int J Cardiol. 2015;199: 307-318. doi: 10.1016/j.ijcard.2015.07.052 [doi].

27. Tang Q, Li X, Song P, Xu L. Optimal cut-off values for the homeostasis model assessment of insulin resistance (HOMA-IR) and pre-diabetes screening: Developments in research and prospects for the future. Drug Discov Ther. 2015;9: 380-385. doi: 10.5582/ddt.2015.01207 [doi].

28. Meeks KA, Stronks K, Adeyemo A, Addo J, Bahendeka S, Beune E, et al. Peripheral insulin resistance rather than beta cell dysfunction accounts for geographical differences in impaired fasting blood glucose among sub-Saharan African individuals: findings from the RODAM study. Diabetologia. 2017;60: 854-864. doi: 10.1007/s00125-017-4216-4 [doi].

29. Hill A, Ruxrungtham K, Hanvanich M, Katlama C, Wolf E, Soriano V, et al. Systematic review of clinical trials evaluating low doses of stavudine as part of antiretroviral treatment. Expert Opin Pharmacother. 2007;8: 679-688. doi: 10.1517/14656566.8.5.679 [doi].

30. McComsey GA, Lo Re V,3rd, O'Riordan M, Walker UA, Lebrecht D, Baron E, et al. Effect of reducing the dose of stavudine on body composition, bone density, and markers of mitochondrial toxicity in HIV-infected subjects: a randomized, controlled study. Clin Infect Dis. 2008;46: 1290-1296. doi: 10.1086/529384 [doi].

31. Quercia R, Roberts J, Martin-Carpenter L, Zala C. Comparative changes of lipid levels in treatment-naive, HIV-1-infected adults treated with dolutegravir vs. efavirenz, raltegravir, and ritonavir-boosted darunavir-based regimens over 48 weeks. Clin Drug Investig. 2015;35: 211-219. doi: 10.1007/s40261-014-0266-2 [doi].

32. Anand SS, Yusuf S, Vuksan V, Devanesen S, Teo KK, Montague PA, et al. Differences in risk factors, atherosclerosis and cardiovascular disease between ethnic groups in Canada: the study of health assessment and risk in ethnic groups (SHARE). Indian Heart J. 2000;52: S35-43.

33. Frank AT, Zhao B, Jose PO, Azar KM, Fortmann SP, Palaniappan LP. Racial/ethnic differences in dyslipidemia patterns. Circulation. 2014;129: 570-579. doi: 10.1161/CIRCULATIONAHA.113.005757 [doi].

34. Patel SA, Shivashankar R, Ali MK, Anjana RM, Deepa M, Kapoor D, et al. Is the "South Asian Phenotype" Unique to South Asians?: Comparing Cardiometabolic Risk Factors in the CARRS and NHANES Studies. Glob Heart. 2016;11: 89-96.e3. doi: 10.1016/j.gheart.2015.12.010 [doi].

35. Friis-Moller N, Ryom L, Smith C, Weber R, Reiss P, Dabis F, et al. An updated prediction model of the global risk of cardiovascular disease in HIV-positive persons: The Data-collection on Adverse Effects of Anti-HIV Drugs (D:A:D) study. Eur J Prev Cardiol. 2016;23: 214-223. doi: 10.1177/2047487315579291 [doi].

Additional material

Supplementary table 1. Title: Laboratory methods.

Laboratory methods	
Total cholesterol, HDL-C, TG, glucose	Cobas Integra 400 autoanalyzer (Roche Diagnostics Ltd., Indianapolis, Ind., USA)
LDL-C	Calculated using the Friedman formula: LDL-C = Total-C – HDL-C – (0.45 * TG)
Insulin	ABBOTT ARCHITECT
CD4 cell count	EPICS XL/MCL Analyzer Cytomics FC500 Flow Cytometer MPL/Cellmek (Beckman Coulter Inc. California, USA)
HIV viral load	Roche Cobas Amplicor/Cobas AmpliPrep/Cobas Taqman/Easymag/EasyQ Analyser

HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; TG, triglycerides.

Supplementary table 2. Title: Estimated means with 95% confidence intervals per outcome.

	Baseline	**Week 24**	**Week 48**	**Week 72**	**Week 96**
Total-C (mmol/L)					
stavudine	3.93 (3.85 – 4.02)	4.54 (4.44 – 4.61)	4.89 (4.80 – 4.98)	5.03 (4.94 – 5.12)	4.94 (4.84 – 5.05)
tenofovir	4.00 (3.92 – 4.08)	4.33 (4.25 – 4.42)	4.55 (4.46 – 4.63)	4.63 (4.54 – 4.72)	4.59 (4.49 – 4.69)
HDL-C (mmol/L)					
stavudine	1.00 0.96 – 1.04)	1.32 (1.29 – 1.36)	1.50 (1.46 – 1.54)	1.53 (1.49 – 1.57)	1.41 (1.36 – 1.46)
tenofovir	0.98 (0.94 – 1.02)	1.18 (1.15 – 1.22)	1.32 (1.28 – 1.36)	1.38 (1.33 – 1.42)	1.37 (1.32 – 1.41)
LDL-C (mmol/L)					
stavudine	2.38 (2.31 – 2.45)	2.62 (2.55 – 2.70)	2.79 (2.71 – 2.87)	2.87 (2.78 – 2.95)	2.87 (2.78 – 2.96)
tenofovir	2.45 (2.38 – 2.52)	2.57 (2.50 – 2.64)	2.65 (2.57 – 2.72)	2.68 (2.59 – 2.76)	2.67 (2.57 – 2.76)
TG (mmol/L)					
stavudine	1.11 (1.06 – 1.16)	1.18 (1.14 – 1.23)	1.26 (1.20 – 1.31)	1.32 (1.26 – 1.38)	1.37 (1.31 – 1.45)

Supplementary table 2. Continued

	Baseline	Week 24	Week 48	Week 72	Week 96
tenofovir	1.15 (1.10 – 1.20)	0.18 (1.14 – 1.23)	1.20 (1.15 – 1.25)	1.19 (1.14 – 1.24)	1.15 (1.10 – 1.21)
Glucose (mmol/L)					
stavudine	4.59 (4.50 – 4.67)	4.90 (4.82 – 4.98)	5.09 (5.00 – 5.12)	5.15 (5.06 – 5.23)	5.08 (4.98 – 5.18)
tenofovir	4.66 (4.58 – 4.75)	4.98 (4.90 – 5.05)	5.16 (5.08- 5.25)	5.23 (5.14 – 5.31)	5.16 (5.07 – 5.26)
Insulin (µmol/L)					
stavudine	47.48 (44.26 – 50.71)	49.80 (47.08 – 52.52)	52.40 (48.71 – 56.09)	55.24 (50.82 – 59.66)	58.37 (52.43 – 64.32)
tenofovir	47.07 (43.88 – 50.27)	47.80 (45.14 – 50.46)	49.96 (46.33 – 53.59)	53.49 (49.17 – 57.81)	58.45 (52.71 – 64.19)
HOMA-IR					
stavudine	1.10 (1.03 – 1.16)	1.24 (1.18 – 1.30)	1.37 (1.29 – 1.45)	1.48 (1.39 – 1.56)	1.56 (1.46 – 1.66)
tenofovir	1.09 (1.02 – 1.15)	1.18 (1.12 – 1.24)	1.27 (1.20 – 1.34)	1.35 (1.28 – 1.43)	1.43 (1.34 – 1.52)
FRS					
stavudine	1.82 (1.73 – 1.92)	1.91 (1.82 – 2.01)	1.99 (1.89 – 2.10)	2.06 (1.96 – 1.27)	2.11 (1.99 – 2.24)
tenofovir	2.00 (1.89 – 2.10)	1.96 (1.87 – 2.06)	1.95 (1.85 – 2.05)	1.97 (1.87 – 2.07)	2.01 (1.90 – 2.13)

This table presents estimates of the means for each treatment group at 4 follow-up measurements based on a linear mixed model with correction for age, sex, site of inclusion (South-Africa, Uganda or India), body mass index and viral load at baseline (see methods for details).

Legend: FRS: Framingham Risk Score, HDL-C: high density lipoprotein cholesterol, LDL-C: low density lipoprotein cholesterol, TG: triglycerides, Total-C: total cholesterol

Supplementary Table 3a: generalized linear mixed models for Total-C (mmol/L)

		Regression coefficient (95% CI)	*P*
Intercept		3.0826 (2.5934 – 3.5719)	< 0.001
Stavudine		-0.0670 (-0.1668 – 0.0328)	0.188
Tenofovir		REF	
Time per month		0.0719 (0.0621 – 0.0818)	< 0.001
Stavudine * time		0.0555 (0.0415 – 0.0696)	< 0.001
Tenofovir * time		REF	
Time per month^2		-0.0020 (-0.0025 - -0.0016)	< 0.001
Time per month^2 * stavudine		-0.0017 (-0.0022 - -0.0011)	< 0.001
Time per month^2 * tenofovir		REF	
Age (years)		0.0206 (0.0148 – 0.0264)	< 0.001
Sex	Male	-0.1403 (-0.2403 - -0.0402)	0.006
	Female	REF	
Site	South Africa	REF	
	Uganda	0.0749 (-0.0270 – 0.1769)	0.150
	India	0.6572 (0.4769 – 0.8377)	< 0.001
BMI (kg/m^2)		0.0333 (0.0216 – 0.0451)	< 0.001
Log viral load (copies/mL)		-0.1578 (-0.2267 - -0.0889)	< 0.001

Supplementary Table 3b: generalized linear mixed models for HDL-C (mmol/L)

		Regression coefficient (95% CI)	*P*
Intercept		1.5426 (1.3234 – 1.7618)	< 0.001
Stavudine		0.0208 (-0.0296 – 0.0712)	0.419
Tenofovir		REF	
Time per month		0.0433 (0.0372 – 0.0495)	< 0.001
Stavudine * time		0.0287 (0.0200 – 0.0375)	< 0.001
Tenofovir * time		REF	
Time per month^2		-0.0012 (-0.0014 - -0.0009)	< 0.001
Time per month^2 * stavudine		-0.0013 (-0.0016 - -0.0009)	< 0.001
Time per month^2 * tenofovir		REF	
Age (years)		0.0040 (0.0014 – 0.0065)	0.003
Sex	Male	-0.1266 (-0.1712 - -0.0819)	< 0.001
	Female	REF	
Site	South Africa	REF	
	Uganda	-0.0994 (-0.1449 – 0.0540)	< 0.001
	India	-0.2550 (0.3357 – 0.1742)	< 0.001
BMI (kg/m^2)		-0.0064 (-0.0116 – -0.0013)	0.017
Log viral load (copies/mL)		-0.0762 (-0.1070 - -0.0454)	< 0.001

Supplementary Table 3c: generalized linear mixed models for LDL-C (mmol/L)

		Regression coefficient (95% CI)	*P*
Intercept		1.6638 (1.2303 – 2.0973)	< 0.001
Stavudine		-0.0714 (-0.1584 – 0.0157)	0.108
Tenofovir		REF	
Time per month		0.0254 (0.0175 – 0.0334)	< 0.001
Stavudine * time		0.0262 (0.0149 – 0.0375)	< 0.001
Tenofovir * time		REF	
Time per month^2		-0.0007 (-0.0010 - -0.0004)	< 0.001
Time per month^2 * stavudine		-0.0006 (-0.0011 - -0.0001)	0.010
Time per month^2 * tenofovir		REF	
Age (years)		0.0136 (0.0085 – 0.0188)	< 0.001
Sex	Male	-0.0990 (-0.188 - -0.0103)	0.029
	Female	REF	
Site	South Africa	REF	
	Uganda	0.1012 (0.0108 – 0.1916)	0.028
	India	0.6367 (0.4768 – 0.7965)	< 0.001
BMI (kg/m^2)		-0.0278 (0.0174 – 0.0382)	< 0.001
Log viral load (copies/mL)		-0.1117 (-0.1728 - -0.0507)	< 0.001

Supplementary Table 3d: generalized linear mixed models for log TG (mmol/L)

		Regression coefficient (95% CI)	*P*
Intercept		-1.3038 (-1.5338 – -1.0738)	< 0.001
Stavudine		-0.0331 (-0.0838 – 0.0175)	0.199
Tenofovir		REF	
Time per month		0.0254 (0.0175 – 0.0334)	< 0.001
Stavudine * time		0.0262 (0.0149 – 0.0375)	< 0.001
Tenofovir * time		REF	
Time per month^2		0.0073 (0.0014 - 0.0131)	0.016
Time per month^2 * stavudine		0.0053 (-0.0031 - -0.0136)	0.217
Time per month^2 * tenofovir		REF	
Age (years)		0.0055 (0.0028 – 0.0082)	< 0.001
Sex	Male	0.1530 (0.1061 - 0.1999)	0.029
	Female	REF	
Site	South Africa	REF	
	Uganda	0.1358 (0.0880 – 0.1836)	< 0.001
	India	0.5651 (0.4803 – 0.6499)	< 0.001
BMI (kg/m^2)		0.0215 (0.0160 – 0.0270)	< 0.001
Log viral load (copies/mL)		0.0883 (0.0560 – 0.1206)	< 0.001

Supplementary Table 3e: generalized linear mixed models for glucose (mmol/L)

		Regression coefficient (95% CI)	*P*
Intercept		3.4479 (2.9766 – 3.9190)	< 0.001
Stavudine		-0.0758 (-0.1823 – 0.0308)	0.163
Tenofovir		REF	
Time per month		0.0679 (0.0558 – 0.0800)	< 0.001
Stavudine * time		0.0007 (-0.0165 – 0.0179)	0.933
Tenofovir * time		REF	
Time per month^2		-0.0021 (-0.0026 - -0.0015)	< 0.001
Time per month^2 * stavudine		-0.0001 (-0.0078 - 0.0007)	0.891
Time per month^2 * tenofovir		REF	
Age (years)		0.0093 (0.0038 – 0.0149)	0.001
Sex	Male	0.1530 (0.1061 - 0.1999)	0.029
	Female	REF	
Site	South Africa	REF	
	Uganda	-0.0756 (-0.1733 – 0.0221)	0.129
	India	0.6150 (0.4413 – 0.7887)	< 0.001
BMI (kg/m^2)		0.0245 (0.0132 – 0.0357)	< 0.001
Log viral load (copies/mL)		0.0016 (-0.0645 – 0.0678)	0.961

Supplementary Table 3f: generalized linear mixed models for insulin (pmol/L)

		Regression coefficient (95% CI)	*P*
Intercept		10.4996 (-4.5763 – 25.5756)	0.172
Stavudine		0.4126 (-3.6727 – 4.4979)	0.843
Tenofovir		REF	
Time per month		0.0042 (-0.7001 – 0.7086)	0.991
Stavudine * time		0.3898 (-0.6101 – 1.3898)	0.445
Tenofovir * time		REF	
Time per month^2		0.0231 (-0.0059 – 0.0521)	0.118
Time per month^2 * stavudine		-0.0187 (-0.0601 – 0.0223)	0.891
Time per month^2 * tenofovir		REF	
Age (years)		-0.5081 (-0.6839 – -0.3325)	< 0.001
	Sex Male	-5.4980 (-8.5468 – -2.4493)	< 0.001
	Female	REF	
Site	South Africa	REF	
	Uganda	-4.736 (-7.8346 – -1.6378)	0.003
	India	19.166 (13.6368 – 24.6955)	< 0.001
BMI (kg/m^2)		2.2421 (1.8834 – 2.6010)	< 0.001
Log viral load (copies/mL)		-0.0724 (-2.1758 – 2.0310)	0.946

Supplementary Table 3g: generalized linear mixed models for log HOMA-IR

Log HOMA-IR		Regression coefficient (95% CI)	*P*
Intercept		-0.8821 (-1.1714 – -0.5928)	< 0.001
Stavudine		0.0098 (-0.0635 – 0.0832)	0.793
Tenofovir		REF	
Time per month		0.0158 (0.0060 – 0.0255)	0.002
Stavudine * time		0.0085 (-0.0054 – 0.0223)	0.231
Tenofovir * time		REF	
Time per month^2		-0.0002 (-0.0006 – 0.0003)	0.465
Time per month^2 * stavudine		-0.0002 (-0.0008 – 0.0004)	0.455
Time per month^2 * tenofovir		REF	
Age (years)		-0.0097 (-0.0131 – -0.0063)	< 0.001
Sex	Male	-0.0945 (-0.1531 – -0.0359)	0.002
	Female	REF	
Site	South Africa	REF	
	Uganda	-0.1839 (-0.2435 – -0.1243)	< 0.001
	India	0.4052 (0.2986 – 0.5117)	< 0.001
BMI (kg/m^2)		0.0519 (0.0450 – 0.0588)	< 0.001
Log viral load (copies/mL)		0.0119(-0.0286 – 0.0523)	0.565

Supplementary Table 3h: generalized linear mixed models for logit FRS

		Regression coefficient (95% CI)	*P*
Intercept		-7.7377 (-8.0744 – -7.4009)	< 0.001
Stavudine		-0.0949 (-0.1697 – -0.0201)	0.013
Tenofovir		REF	
Time per month		-0.0047 (-0.0109 – 0.0016)	0.141
Stavudine * time		0.0147 (0.0059 – 0.0236)	0.001
Tenofovir * time		REF	
Time per month^2		0.0002 (-0.0000 – 0.0005)	0.083
Time per month^2 * stavudine		-0.0004 (-0.0007 – -0.0000)	0.049
Time per month^2 * tenofovir		REF	
Age (years)		0.0810 (0.0765 – 0.0855)	< 0.001
Sex	Male	0.6516 (0.5791 – 0.7241)	< 0.001
	Female	REF	
BMI (kg/m^2)		0.0278 (0.0194 – 0.0362)	< 0.001
Log viral load (copies/mL)		0.0049 (-0.0419 – 0.0516)	0.839

FRS: Framingham Risk Score

CHAPTER

11

General discussion and conclusion

Sub-Saharan African (SSA) is heavenly burdened with HIV[1]. Since the roll-out of antiretroviral therapy (ART) the face of the epidemic has changed from a deadly disease to a chronic condition[2,3]. People living with HIV (PLHIV) are aging and, as a result, non-communicable diseases (NCDs) are expected to increase in this population[4]. Currently, in PLHIV aged 50 and over about two-thirds have two or more comorbid conditions[5].

Due to globalization and urbanization, the socio-economic situation in SSA is changing, resulting in more sedentary lifestyles and unhealthy food patterns[6]. These factors are closely linked to a high burden of risk factors for cardiovascular disease (CVD), notably hypertension and obesity.

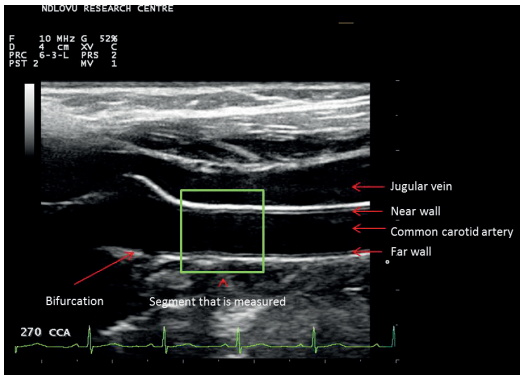
Research from high income-countries (HICs) showed that the risk of CVD is increased in PLHIV, with a twofold risk of developing CVD for PLHIV[7]. The underlying mechanism was postulated to consist of the triad of immune activation by the virus, metabolic side effects of ART and traditional CVD risk factors[8]. In this thesis, we aimed to gain insight into the relation between HIV, immune activation and CVD risk, and we started with summarizing the evidence on the value of immune markers in estimating CVD risk in PLHIV. Besides, we aimed to gain insight into the burden of cardiovascular risk factors and the estimated CVD risk in PLHIV over HIV-negative people, whilst considering ART. Subsequently, lessons learned and the way forward will be discussed.

Lessons learned

Search for immune markers to identify (subclinical) atherosclerosis

A substantial number of studies aimed to understand the pathophysiology of CVD in HIV or aimed to improve CVD risk prediction in PLHIV. In these studies, one or more immune markers were measured, and associated one by one to surrogate markers for CVD. We consolidated the evidence on this topic in two systematic reviews (**chapter 2 and chapter 3**). In total, 31 immune markers were studied in relation to carotid intima-media thickness (CIMT) and 34 immune markers were related to nine other surrogate CVD outcomes. CRP, IL-6 and d-dimer were studied most frequently in relation to CIMT, but no consistent relation with CIMT was observed.

Carotid intima-media thickness (CIMT) is an ultrasound based measurement of the thickness of the intima-media layer of the carotid artery. The carotid artery include the common carotid artery (CCA) and the bifurcation (bulb), where the CCA splits in the internal and external carotid artery. A participant lies in a supine position when the measurements are taken with an ultrasound machine using a linear probe. The thickness of the near wall and the far wall of the CCA is measured over a length of one centimeter proximal to the bifurcation, and at the bifurcation. The measurements at the CCA are taken at three standardized angles at both sides using a Meijer's Arc. The thickness of the intima-media layer is measured offline with dedicated software what results in a mean and maximum thickness of the segment. CIMT is normally presented as the average (including near wall and far wall measurements of all angles) of the mean thickness and/or maximum thickness of the intima-media layer in the CCA and/or in the bulb.



The correlation of a single marker of immunity to a surrogate marker of CVD comes with limitations. A single immune marker is most likely unable to represent the complex process of immune activation and its influence on the arterial vessel wall. Second, a wide variety of immune markers was studied in a variety of populations; this prevents fully understanding the value of a specific marker across different stages of HIV infection and across different populations. Third, the HIV-positive population is still relatively young which makes it harder to discover end organ damage. Another limitation refers to the use of surrogate CVD markers as outcome rather than clinical CVD. Surrogate outcomes for CVD are associated with an increased risk of CVD, but there is no one-to-one relationship with CVD. This might obscure a possible relation between an immune marker and overt CVD. Finally, most of the studies investigating the value of an immune marker had a cross-sectional design, and, of the few longitudinal studies, the longest follow-up duration was rather short with three years. Based on these results, we recommend to first assess the value of well-known markers in longitudinal studies with a sufficient follow-up duration before "embarking on a fishing expedition to find any immune-marker 'associated' with CIMT".

Despite these objections, the above mentioned studies did shed light on the level of immune activation and the pathophysiology of HIV on the arterial vessel wall. In line with this the utility of a novel marker, Lp-PLA_2 (**chapter 4**) was investigated. Lp-PLA_2 is a phospholipase which complexes with low-density lipoprotein and catalyzes the release of pro-inflammatory lipid mediators. It has been linked to CVD risk (plaque formation and instability) specifically in patients with underlying inflammatory diseases[9]. We observed that Lp-PLA_2 strongly correlated to lipids as well as to viral load and CD4+ cell count in virally unsuppressed HIV-positive patients. This knowledge helps to understand

the pathophysiology of atherosclerosis in HIV infection as it suggests that inflammation linked to the virus may be driving increased levels of Lp-PLA_2 and hence CVD risk.

Lower prevalence of cardiovascular risk factors for PLHIV

The burden of CVD risk factors was consistently observed to be lower in PLHIV than in the HIV-negative population. We observed this trend in both the rural and the urban South African populations (**chapter 6 and chapter 7**). This finding differs from results in high-income countries, where an excess burden of hypertension, obesity and smoking in PLHIV[10-12] has been reported. This contrasting finding can most likely be explained by the differences in the socio-demographics between the HIV epidemic in high-income settings versus SSA. The majority of PLHIV in HIC are white men belonging to risk groups such as men having sex with men, clients of sex workers or intravenous drug users. In contrast, most PLHIV in SSA do not belong to a risk population, and the majority is female[1].

Subclinical atherosclerosis and CVD risk in HIV infection

HIV infection is associated with lower levels of conventional CVD risk factors on the one hand and, on the other hand, with ongoing immune activation reflected by a higher CRP level in PLHIV compared to the HIV-negative population. The main question is how the interplay between these factors would translate into long-term CVD risk.

Rural population

The results of the Ndlovu Cohort Study showed that CIMT was increased in PLHIV on antiretroviral therapy (ART) from the age of 30 years compared to the HIV-negative population and that the influence of HIV on CIMT increased with age (**chapter 6**).

Duration of HIV at enrolment was associated with a decrease in CIMT in participants up to 49 years. In the age group 30-49 years, a low CD4+ cell count was associated with in an increase in CIMT. No effect of viral load on CIMT was observed in any of the age groups. From a pathophysiological point it is difficult to understand the seemingly contradictory findings on HIV duration and CD4+ cell count on CIMT, as well as the lack of association between viral load and CIMT. In this regard, our results are accompanied by multiple studies on the relation between HIV and HIV-related characteristics and CIMT, where some studies report positive associations, some no association, and some negative associations for HIV, CD4+ cell count and viral load on CIMT[13-16].

The explanation likely needs to be sought in the complex interaction between HIV and the immune system[17,18], differences in the burden of co-infections like hepatitis C, Cytomegaly virus and tuberculosis between study populations[19-21], immune dysregulation at the moment of ART initiation[13] and the level of virological control during treatment[22].

No influence of HIV-related characteristics was seen in participants aged 50 years and over, regardless of ART status. This data suggests that HIV characteristics do not influence CIMT anymore in the older participants or that the influence of HIV is overruled by conventional CVD risk factors.

Urban population

Analysis of subclinical atherosclerosis in the urban group, however, did not show any influence of HIV or ART on CIMT. There was no indication for an age dependent influence of HIV on CIMT nor were any of the HIV characteristics like CD4+ cell count and viral load associated with CIMT (**Chapter 8**).

Comparison between outcomes in the rural and the urban population

Our results indicate that the association between HIV, ART and CIMT differs between a rural and an urban population. The methods between both studies were comparable, so it is unlikely that this explains the difference. However, a direct comparison between the outcomes in the rural and urban group should be interpreted with caution as the number of participants and the recruitment process varied between the urban and the rural site. The rural group included 1927 participants whereas the urban group consisted of 548 participants. In the rural population, participants were recruited from the general population, whereas in the urban population participants were recruited from randomized clinical trials (RCTs). However, it is unlikely that recruitment from RCTs has a significant impact on generalizability to an urban HIV-positive population. The RCTs recruited participants from public HIV testing and treatment facilities in the inner city of Johannesburg and exclusion criteria were mainly related to hepatitis B co-infection, renal impairment and abnormal liver function, all conditions that are rare in a relatively young, HIV-positive population.

In a subsequent analysis, determinants of CIMT in both the rural and the urban setting were analyzed (**chapter 9**)**.** The median age was comparable between the rural and the urban site (38.0 years versus 37.5 years respectively), but the rural study included a significant number of PLHIV aged 50 and over (n=204, 23%), whereas in the urban population only 50 participants (13%) were 50 years or older. We found a significant positive interaction between age and HIV, as well as between age and several CVD risk factors. These results indicate that the effect of HIV and CVD risk factors on CIMT increases with age. This may, in turn, explain in part why we did not find a relation between HIV and CIMT in the urban population whereas CIMT was increased in PLHIV in the rural population from the age of 30 years. It seems that the urban participants may not yet have been old enough to develop subclinical CVD related to HIV and ART. The findings on the positive association of HIV and ART with CIMT were confirmed in

a subsequent analysis using the functional surrogate outcome heart rate variability (HRV) (**chapter 8**), including a subset of the NCS participants. PLHIV had a lower HRV, indicating a higher CVD risk compared to HIV-negative participants.

Summarizing, our data suggests that despite the favorable CVD risk profile in PLHIV, the aging HIV-positive population is at increased risk for CVD. No difference in CVD risk was observed in the group of participants younger than 30 years in both the urban and the rural site. Therefore, future studies should focus on older participants to further elucidate the relation between HIV, ART and CVD.

The influence of ART on CVD risk

ART regimens have changed substantially over the last decade towards drugs with fewer side effects. Therefore, toxicity related to lipodystrophy, insulin resistance and disturbances in lipid levels are seen less often[23]. Still, initiation of ART has a substantial influence on lipids and glucose homeostasis. CVD risk, however, hardly seems to change in the first two years following ART initiation (**chapter 10**). Results from the Ndlovu Cohort Study showed that ART was related to increased CIMT, as was the duration of ART. Results in the urban population did not show a difference in subclinical CVD in patients using ART, either first- or second-line, and those not using ART. There are some considerations to be taken into account when interpreting these results. First, the rural participants were on first-line ART for longer periods (median duration of treatment of 57 months) than participants in the urban group (median duration of treatment of 39 months), with a larger spread (the 75% percentile was 97 months for the rural group versus 48 months for the urban group). This might indicate that a number of the NCS participants have been exposed to older antiretroviral drugs that were known for their adverse metabolic side effects like stavudine[24].

Second, urban participants had better virological control than the rural participants. Of all urban participants, 91% had undetectable viremia on first-line ART and 95% on second-line ART. This was only 82% for the rural participants on first-line, and 59% for participants on second-line ART.

The adequate virological control could partly explain why there was no effect of ART on subclinical CVD in the urban group[25]. Third, as mentioned above, the smaller proportion of participants aged 50 years and over in the urban group could explain why no effect of ART was seen on subclinical CVD, while there could have been an effect if the population would have been older.

In summary, ART does seem to increase subclinical CVD risk in an aging population with insufficient virological control. ART, including first- and second-line, might have limited

influence on the development of CVD in a population with good virological control, but this hypothesis has to be confirmed in an elderly population.

Practical lessons

Every research project comes with its own challenges, but this is likely even more the case in resource-limited settings. The lessons learned are not new and have been addressed before in platforms like the WHO bulletin 'Lessons from the Field'[26], but we would like to highlight the most important lessons that we learned during the past years.

1). Questionnaires should be as concise as possible, and every question should be evaluated on its value to answer the research questions before inclusion in the questionnaire. This applies to resource rich settings, but even more to resource limited settings. The questionnaires on socio-demographics and lifestyle factors are often taken by lay people or counselors. With a concise questionnaire it is easier to train the research staff on how to interpret a question and how to prevent contradictory answers to questions. For example, in the NCS baseline questionnaire participants are asked three times about their HIV and ART status. We observed a substantial number of inconsistent answers. Besides, questions should be evaluated carefully on the information that they collect, and if all required information is included. For example: in one of the projects smoking was assessed with 11 questions, but we were still unable to calculate pack years in previous smokers as there was no question assessing the average number of cigars/cigarettes per day for people who had quit smoking, and we were not able to retrieve stopping dates as this information was only registered in 'weeks, months or years'. This question could have been shortened while maintaining the same information that is currently used for the analysis. A questionnaire should be piloted thoroughly prior to implementation; to adapt to its cultural differences; to test feasibility and to evaluate if the critical information is incorporated.

2) Study protocols should be as straightforward as possible, and the amount of information collected needs to be a tradeoff between what is needed to answer a research question and what is feasible from a time and resources perspective. For example: the CIMT protocol in one of the studies required 30 different images per participant (15 of the right side, 15 of the left side). Research personnel with no previous experience in performing ultrasounds were trained over a weekend only. At a performance review a couple of months later, it turned out that only a few images per participants were collected and that the quality was insufficient. When the protocol was changed to eight images per participants and additional training was performed, completeness of data increased to over 95% and the quality improved significantly.

3) Following the implementation of any protocol, regular performance reviews should be scheduled. This is true for any setting, but even more so for settings where it is not common to ask for feedback or to discuss insecurities regarding specific tasks. For the CIMT scans there was no performance review implemented, so the issue with the quality was only picked up after a couple of months. Another example is the measurement of blood pressure. At one of the research sites the automatic blood pressure devices using an arm cuff were replaced after some time with devices using a wrist cuff. As no performance reviews with physical visits in the research rooms were done, this was only picked up two years later, when the data showed an unlikely increase in blood pressure between baseline and follow-up measurements.

4) Perceptions of research in the study population should be carefully investigated before and after implementation of a project to adequately address barriers for participation and fear/mistrust during participation. A common barrier to participate in HIV research relates to stigma and non-disclosure of the HIV status. An example of mistrust during participation relates to the amount of blood that was taken, and what the blood samples were used for. A frequently heard complaint in one of the studies was that too much blood was taken. Participants were discouraged by the number of tubes, but did not realize that only about 50 mL's of blood was taken, equivalent to approximately three table spoons. It is likely not enough to include this information in the information sheet before enrolment, but active search for barriers and areas of mistrust during a survey will help to address these issues to improve participant satisfaction and retention in follow-up.

The way forward

Considering the lessons we learned through the research that is addressed in this thesis, the following recommendations can be made.

1. There is an urgent need for more data regarding the burden of CVD in PLHIV as this is needed to inform policy makers how to organize the health care system. Our research suggests that CVD risk is increased in an aging HIV-positive population on ART, but this has to be confirmed with CVD endpoints. This can be reached in three ways, in order of desirability;

a. A country wide registry system that captures information on NCD's in the HIV-positive population together with HIV-related information. An example of such a system is TIER.net. TIER.net is an online electronic database that monitors patients on HIV and TB treatment[27] and it has been implemented in a number of SSA countries like South-Africa, Malawi and Mozambique [28]. Although the platform is still working

to increase coverage of public health facilities, it could be used to incorporate information on NCDs, and more specifically, CVD risk factors and the occurrence of CVD endpoints like stroke and myocardial infarction. This would allow getting insight into the epidemiology of CVD in the HIV-infected population, and it could be used to integrate and monitor care for HIV and NCDs, like what is currently implemented for HIV and TB.

b. Longitudinal studies addressing CVD outcomes in PLHIV. The NCS is an example of a longitudinal study aiming to assess CVD endpoints. Data quality in research studies would likely be higher than in a national registration system, but longitudinal studies are resource intense and restrained by loss to follow-up.

c. Cross-sectional studies that assess the burden of CVD with surrogate outcomes by comparing PLHIV to HIV-negative participants. These studies should focus on older populations as the effect of HIV on the CVD system likely increases with age. We do not have a set age threshold but based on our data we would advise to include people from the age of 40 years or even 50 years.

2. To gain insight in the pathophysiology of atherosclerosis in HIV infection a panel of immune markers rather than a single immune marker should be evaluated considering the complexity of the interaction between HIV, ART and the immune system. Measurement of a panel of immune markers allows for cluster analysis and identification of immune activation patterns. In a recent article, two clusters of inflammatory markers were identified that were predictive for the presence respectively absence of coronary artery plaque in the general population[29]. Participants in different phases of HIV infection (newly diagnosed, on treatment and virally suppressed, on treatment and not virally suppressed, as well as participants on different ART regiments) should be included to evaluate immune activation patterns at different stages. Outcomes can be linked to surrogate outcomes of CVD, but preferably to real CVD events. A first analysis comparing immune activation in HIV-positive participants on ART to HIV-negative participants is underway.

3. It is time to prioritize incorporation of NCD care in routine HIV care now that we know there is a signal that CVD risk is increased in PLHIV in SSA, and considering the high burden of CVD risk factors. South Africa was the first African country to launch a guideline to promote integration of HIV, TB and NCD care in 2016[30], but this is not yet widely implemented. A systematic review that assessed integration of HIV and NCD care showed that over the past years several integrated chronic care initiatives were undertaken in SSA, most of them describing small-scale interventions. Integration of

HIV and NCD care seems feasible when using the existing platform of HIV care[31]. Although NCDs comprise more diseases than just CVD, we argue for prioritizing CVD care as ischemic heart disease and stroke are among the leading causes of years of life lost[32]. The focus therefore should be on management of conventional CVD risk factors such as blood pressure, glucose and lipid levels, obesity and lack of physical activity. Steps to improve care need to be related to prevention, detection, linkage to care, retention in care and adequate management while in care. Lessons learned from the HIV field should be considered and built upon. Recently, a research agenda was set by members of the HIV/NCS Integration project. This is a project initiated by the National Institute of Health (NIH) that focusses on the integration of HIV and NCD care in SSA. They concluded that the cascade of care needs to be addressed at three levels; clinical, health systems and community. Among the proposed areas of research priority were service delivery models, health workforce education, medication supply chain management, development of new financing and sustainability models and data generation and integration through research and informatics platforms to advance research-informed policy [33].

Conclusion

There is a high burden of conventional CVD risk factors such as hypertension, obesity and DM type 2 in the African population. PLHIV have fewer conventional CVD risk factors than the HIV-negative population. Despite this, the aging HIV-positive population on ART is likely to have an increased risk of CVD compared to the HIV-negative population. Markers of immune activation such as CRP and Lp-PLA2 help to understand the pathophysiology of CVD in HIV infection. However, it is unlikely that a single marker of immune activation will be identified that is able to distinguish between people with a low and a high CVD risk as long as only surrogate outcomes for CVD are available to estimate the burden of CVD. The next step in research needs to be directed towards integration of NCD services, including CVD services, in the HIV care cascade. The general population should not be forgotten and lessons from the HIV field should be used to extend NCD care to the whole population.

References

1. UNAIDS. UNAIDS 2018 Estimates. 2018. Available: http://www.unaids.org/sites/default/files/media_asset/unaids-data-2018_en.pdf. Date last visited: 28 February 2019
2. Legarth RA, Ahlstrom MG, Kronborg G, Larsen CS, Pedersen C, Pedersen G, et al. Long-Term Mortality in HIV-Infected Individuals 50 Years or Older: A Nationwide, Population-Based Cohort Study. J Acquir Immune Defic Syndr. 2016;71: 213-218. doi: 10.1097/QAI.0000000000000825 [doi].
3. Pillay-van Wyk V, Msemburi W, Laubscher R, Dorrington RE, Groenewald P, Glass T, et al. Mortality trends and differentials in South Africa from 1997 to 2012: second National Burden of Disease Study. The Lancet Global Health. 2016;4: e642-e653. doi: https://doi.org/10.1016/S2214-109X(16)30113-9.
4. d'Arminio Monforte A, Diaz-Cuervo H, De Luca A, Maggiolo F, Cingolani A, Bonora S, et al. Evolution of major non-HIV-related comorbidities in HIV-infected patients in the Italian Cohort of Individuals, Naive for Antiretrovirals (ICONA) Foundation Study cohort in the period 2004-2014. HIV Med. 2019;20: 99-109. doi: 10.1111/hiv.12683 [doi].
5. Maciel RA, Kluck HM, Durand M, Sprinz E. Comorbidity is more common and occurs earlier in persons living with HIV than in HIV-uninfected matched controls, aged 50 years and older: A cross-sectional study. Int J Infect Dis. 2018;70: 30-35. doi: S1201-9712(18)30045-6 [pii].
6. Scott A, Ejikeme CS, Clottey EN, Thomas JG. Obesity in sub-Saharan Africa: development of an ecological theoretical framework. Health Promot Int. 2013;28: 4-16. doi: 10.1093/heapro/das038 [doi].
7. Shah ASV, Stelzle D, Lee KK, Beck EJ, Alam S, Clifford S, et al. Global Burden of Atherosclerotic Cardiovascular Disease in People Living With HIV. Circulation. 2018;138: 1100-1112. doi: 10.1161/CIRCULATIONAHA.117.033369 [doi].
8. Zanni MV, Schouten J, Grinspoon SK, Reiss P. Risk of coronary heart disease in patients with HIV infection. Nat Rev Cardiol. 2014;11: 728-741. doi: 10.1038/nrcardio.2014.167 [doi].
9. Goncalves I, Edsfeldt A, Ko NY, Grufman H, Berg K, Bjorkbacka H, et al. Evidence supporting a key role of Lp-PLA2-generated lysophosphatidylcholine in human atherosclerotic plaque inflammation. Arterioscler Thromb Vasc Biol. 2012;32: 1505-1512. doi: 10.1161/ATVBAHA.112.249854 [doi].
10. 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;92: 2506-2512. doi: jc.2006-2190 [pii].
11. Vidrine DJ. Cigarette smoking and HIV/AIDS: health implications, smoker characteristics and cessation strategies. AIDS Educ Prev. 2009;21: 3-13. doi: 10.1521/aeap.2009.21.3_supp.3 [doi].

12. Mdodo R, Frazier EL, Dube SR, Mattson CL, Sutton MY, Brooks JT, et al. Cigarette smoking prevalence among adults with HIV compared with the general adult population in the United States: cross-sectional surveys. Ann Intern Med. 2015;162: 335-344. doi: 10.7326/M14-0954 [doi].
13. Kaplan RC, Kingsley LA, Gange SJ, Benning L, Jacobson LP, Lazar J, et al. Low CD4+ T-cell count as a major atherosclerosis risk factor in HIV-infected women and men. AIDS. 2008;22: 1615-1624. doi: 10.1097/QAD.0b013e328300581d [doi].
14. Stein JH, Brown TT, Ribaudo HJ, Chen Y, Yan M, Lauer-Brodell E, et al. Ultrasonographic measures of cardiovascular disease risk in antiretroviral treatment-naïve individuals with HIV infection. AIDS. 2013;27: 929-937. doi: 10.1097/QAD.0b013e32835ce27e [doi].
15. Delaney JA, Scherzer R, Biggs ML, Shliplak MG, Polak JF, Currier JS, et al. Associations of antiretroviral drug use and HIV-specific risk factors with carotid intima-media thickness. AIDS. 2010;24: 2201-2209. doi: 10.1097/QAD.0b013e32833d2132 [doi].
16. Schoffelen AF, de Groot E, Tempelman HA, Visseren FL, Hoepelman AI, Barth RE. Carotid intima media thickness in mainly female HIV-infected subjects in rural South Africa: Association with cardiovascular but not HIV-related factors. Clin Infect Dis. 2015;61: 1606-1614. doi: 10.1093/cid/civ586 [doi].
17. Miedema F, Hazenberg MD, Tesselaar K, van Baarle D, de Boer RJ, Borghans JA. Immune activation and collateral damage in AIDS pathogenesis. Front Immunol. 2013;4: 298. doi: 10.3389/fimmu.2013.00298 [doi].
18. Nou E, Lo J, Grinspoon SK. Inflammation, immune activation, and cardiovascular disease in HIV. AIDS. 2016;30: 1495-1509. doi: 10.1097/QAD.0000000000001109 [doi].
19. Maniar A, Ellis C, Asmuth D, Pollard R, Rutledge J. HIV infection and atherosclerosis: evaluating the drivers of inflammation. Eur J Prev Cardiol. 2013;20: 720-728. doi: 10.1177/2047487312447843 [doi].
20. Lichtner M, Cicconi P, Vita S, Cozzi-Lepri A, Galli M, Lo Caputo S, et al. Cytomegalovirus coinfection is associated with an increased risk of severe non-AIDS-defining events in a large cohort of HIV-infected patients. J Infect Dis. 2015;211: 178-186. doi: 10.1093/infdis/jiu417 [doi].
21. Boulougoura A, Sereti I. HIV infection and immune activation: the role of coinfections. Curr Opin HIV AIDS. 2016;11: 191-200. doi: 10.1097/COH.0000000000000241 [doi].
22. Data Collection on Adverse Events of Anti-HIV drugs (D:A:D) Study Group, Smith C, Sabin CA, Lundgren JD, Thiebaut R, Weber R, et al. Factors associated with specific causes of death amongst HIV-positive individuals in the D:A:D Study. AIDS. 2010;24: 1537-1548. doi: 10.1097/QAD.0b013e32833a0918 [doi].
23. Domingo P, Mateo MG, Gutierrez MDM, Vidal F. Tolerability of current antiretroviral single-tablet regimens. AIDS Rev. 2018;20: 141-149. doi: 10.24875/AIDSRev.M18000025 [doi].

24. George JA, Venter WD, Van Deventer HE, Crowther NJ. A longitudinal study of the changes in body fat and metabolic parameters in a South African population of HIV-positive patients receiving an antiretroviral therapeutic regimen containing stavudine. AIDS Res Hum Retroviruses. 2009;25: 771-781. doi: 10.1089/aid.2008.0308 [doi].

25. Borges AH, Neuhaus J, Sharma S, Neaton JD, Henry K, Anagnostou O, et al. The effect of interrupted/deferred antiretroviral therapy on disease risk: A SMART and START combined analysis. J Infect Dis. 2019;219: 254-263. doi: 10.1093/infdis/jiy442 [doi].

26. World Health Organisation. Bulletin of the World Health Organization - Lessons from the field. https://www.who.int/bulletin/volumes/83/1/editorial10105/en/2005.

27. Osler M, Hilderbrand K, Hennessey C, Arendse J, Goemaere E, Ford N, et al. A three-tier framework for monitoring antiretroviral therapy in high HIV burden settings. J Int AIDS Soc. 2014;17: 18908. doi: 10.7448/IAS.17.1.18908 [doi].

28. Chowles T. Tier.Net Gets Global Interest TIER.Net. https://ehealthnews.co.za/tier-net-global-2/ Last updated October 2014. Date last visited: 22 February 2019.

29. Bom MJ, Levin E, Driessen RS, Danad I, Van Kuijk CC, van Rossum AC, et al. Predictive value of targeted proteomics for coronary plaque morphology in patients with suspected coronary artery disease. EBioMedicine. 2019;39: 109-117. doi: S2352-3964(18)30610-8 [pii].

30. South African Department of Health. Adherence guidelines for HIV, TB and NCDs. Policy and service delivery guidelines for linkage to care, adherence to treatment and retention in care. South Africa, 2016, February.

31. Haldane V, Legido-Quigley H, Chuah FLH, Sigfrid L, Murphy G, Ong SE, et al. Integrating cardiovascular diseases, hypertension, and diabetes with HIV services: a systematic review. AIDS Care. 2018;30: 103-115. doi: 10.1080/09540121.2017.1344350 [doi].

32. GBD 2016 Causes of Death Collaborators. Global, regional, and national age-sex specific mortality for 264 causes of death, 1980-2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet. 2017;390: 1151-1210. doi: S0140-6736(17)32152-9 [pii].

33. Vorkoper S, Kupfer LE, Anand N, Patel P, Beecroft B, Tierney WM, et al. Building on the HIV chronic care platform to address noncommunicable diseases in sub-Saharan Africa: a research agenda. AIDS. 2018;32 Suppl 1: S107-S113. doi: 10.1097/QAD.0000000000001898 [doi].

Appendix

A

Nederlandse samenvatting
Dankwoord – Acknowlegdements
List of publications
Curriculum vitae

Samenvatting in het Nederlands *(Summary in Dutch)*

Sinds de ontdekking van het humaan immuundeficiëntie virus (HIV) in de jaren '80 is infectie met HIV een belangrijk gezondheidsprobleem in de hele wereld. Circa 70% van alle mensen met een HIV infectie leeft in sub-Sahara Afrika (SSA). Door behandeling met een combinatie van antiretrovirale middelen (cART) is HIV een chronische ziekte geworden en is de levensverwachting van mensen met HIV zo goed als genormaliseerd. Inmiddels is duidelijk geworden dat een HIV infectie geassocieerd is met een tweemaal verhoogd risico op hart- en vaatziekten (HVZ) vergeleken met het risico op HVZ in de algemene bevolking. De pathofysiologie is multifactorieel en berust waarschijnlijk op de volgende drie factoren: 1) activatie van het immuunsysteem door het HIV virus, 2) bijwerkingen van cART en 3) klassieke risicofactoren voor HVZ.

Ondanks het feit dat de meeste mensen met een HIV infectie leven in SSA, is er maar weinig onderzoek verricht naar HIV en HVZ in deze regio. Onderzoeksgegevens uit het westen kunnen waarschijnlijk niet direct toegepast worden op de SSA HIV-positieve populatie omdat er verschillen zijn in socio-demografische karakteristieken tussen de HIV epidemie in de westerse wereld en in SSA. Daarom is het belangrijk om inzicht te krijgen in de relatie tussen HIV, cART en HVZ in de SSA populatie. Hiernaast is het belangrijk om inzicht te krijgen in hoe HIV en cART het immuunsysteem beïnvloeden en in hoe dit vervolgens samenhangt met het risico op het ontwikkelen van HVZ. Dit proefschrift richt zich in het **eerste gedeelte** op de relatie tussen HIV, immuun activatie en HVZ. In het **tweede gedeelte** word gekeken naar de epidemiologie van HVZ bij mensen met een HIV infectie, en naar de invloed van HIV en cART op het risico om HVZ te ontwikkelen. Harde klinische uitkomsten zoals een myocardinfarct of een herseninfarct zijn zeldzaam in de relatief jonge HIV-positieve populatie, daarom is gebruik gemaakt van zogenaamde surrogaat markers om de aanwezigheid van HVZ in te schatten. Een voorbeeld van een dergelijke surrogaat marker is een carotis intima-media dikte (CIMT) meting. Hiermee kan de aanwezigheid van subklinische atherosclerose in kaart gebracht worden.

Deel een

Hoofdstuk twee en **hoofdstuk drie** zijn beide systematische reviews van de literatuur waarin de relatie tussen markers van immuun activatie en HVZ of een surrogaat marker voor HVZ beschreven wordt. In **hoofdstuk twee** worden HVZ en de CIMT meting besproken. Het optreden van HVZ was geassocieerd met de immuun markers C-reactief proteine, interleukine-6 en d-dimeer, maar er werd geen consistente relatie gevonden tussen immuun markers en CIMT. In **hoofdstuk drie** worden de andere

surrogaat markers voor HVZ besproken zoals *pulse wave velocity*, *flow mediated dilation* en beeldvorming, zoals een CT scan van de coronair arteriën waarmee de mate van calcificatie geschat kan worden. Ook nu werd geen eenduidige relatie gevonden tussen een immuun marker en een surrogaat marker van HVZ. Het ontbreken van een associatie tussen een immuun marker en een surrogaat marker voor HVZ kan deels berusten op de heterogeniteit tussen de studies waarin dit onderzocht werd, alsook door het gebruik van surrogaat markers voor HVZ in plaats van het klinische eindpunt HVZ. **Hoofdstuk vier** zoomt in op een specifieke immuun marker, namelijk Lipoproteine geassocieerd Phosfolipase A2 (Lp-PLA2). Dit is een fosfolipase dat een verbinding aangaat met low density lipoproteine, en het vrijkomen van pro-inflammatoire lipiden katalyseert. Lp-PLA2 is eerder beschreven als een risicofactor voor HVZ. In **hoofdstuk vier** wordt beschreven dat Lp-PLA2 sterk correleert met HIV virale lading en het aantal CD4+ cellen in patiënten waarin HIV niet onderdrukt is met cART.

Deel twee

Hoofdstuk vijf beschrijft de rationale en het studiedesign van de Ndlovu Cohort Studie (NCS). Dit is een longitudinale studie in Limpopo, Zuid-Afrika, waarin 1040 HIV-negatieve participanten en 887 HIV-positieve participanten geïncludeerd werden. Het doel van de studie is inzicht te krijgen in de rol van HIV en cART bij het ontwikkelen van HVZ. **Hoofdstuk zes** beschrijft de resultaten van de NCS zoals verzameld tijdens inclusie in de studie. Mensen met HIV hebben minder traditionele risicofactoren voor het ontwikkelen van HVZ dan mensen zonder HIV infectie, maar het risico op HVZ, volgens de CIMT meting, is toch verhoogd voor mensen met HIV en cART vanaf de leeftijd van 30 jaar in vergelijking tot mensen zonder HIV. **Hoofdstuk zeven** beschrijft een studie waarin gekeken word naar het risico op HVZ bij mensen met HIV, maar nu wordt HVZ geëvalueerd met *heart rate variability*, een andere surrogaat uitkomst voor HVZ. In deze studie werd een gedeelte van de NCS participanten geïncludeerd. Mensen met HIV blijken een lagere variabiliteit in de hartslag te hebben; dit suggereert een verhoogd risico op HVZ, in vergelijking tot mensen zonder HIV infectie. In **hoofdstuk acht** word een stedelijke populatie beschreven. Hierin wordt gekeken naar het risico op HVZ volgens CIMT meting en *carotid distensibility*, een functionele surrogaat uitkomst voor HVZ waarbij de mate waarin de arterie kan uitzetten de mate van atherosclerose weerspiegelt. Het effect van HIV en cART werd onderzocht door de volgende vier groepen met elkaar te vergelijken: mensen met HIV die nog geen cART kregen, mensen met HIV op eerstelijns cART behandeling, op tweedelijns cART behandeling en HIV-negatieve controles. In tegenstelling tot de resultaten van de NCS werd nu geen relatie tussen HIV, cART en een van de surrogaat uitkomsten voor HVZ geobserveerd. In

hoofdstuk negen ligt de focus op de determinanten van CIMT in zowel de landelijke groep (de NCS) als in de stedelijke groep. Leeftijd en klassieke risicofactoren voor HVZ zijn de belangrijkste determinanten van carotis intima-media dikte in beide groepen, en de invloed van een aantal klassieke risicofactoren op CIMT neemt toe bij het ouder worden. In de NCS zijn zowel HIV en ART gerelateerd aan CIMT. HIV is geassocieerd met een lagere CIMT, maar dit effect neemt af bij het ouder worden, en cART is geassocieerd met een hogere CIMT. In de NCS werden meer participanten van 50 jaar en ouder geïncludeerd dan in de stedelijke groep; dit zou kunnen verklaren waarom we zagen dat HIV en ART CIMT beïnvloeden in de landelijke groep, maar (nog niet) in de stedelijke groep. Hiernaast was er minder virologische controle in de NCS dan in de stedelijke groep. Dit zou ook een verklaring kunnen zijn waarom mensen met HIV een hogere CIMT hadden in de NCS dan de mensen zonder HIV, terwijl we dit effect niet zagen in de stedelijke groep. **Hoofdstuk tien** beschrijft een gedetailleerde analyse van het effect van het starten van behandeling met cART op cholesterol waarden, glucose en insuline metabolisme en HVZ risico volgens de Framingham risico score. Cholesterolwaarden en insulineresistentie nemen toe na het starten van cART, maar ondanks dat het risico op HVZ ook toeneemt is het risico laag na een follow-up duur van 96 weken.

Dankwoord - Acknowledgements

Terugblikkend op de achterliggende jaren heb ik reden om dankbaar te zijn voor iedereen die betrokken was bij mijn promotie traject. Dit proefschrift zou niet tot stand zijn gekomen zonder de medewerking en het vertrouwen van de studie participanten, de begeleiding door mijn promotie team en de steun vanuit de cirkel van collega's, vrienden en familie.

Als eerste wil ik alle participanten bedanken, zowel de participanten in de Ndlovu Cohort Studie als de participanten in mijn studie in Johannesburg, voor hun tijd en het vertrouwen dat ze me als onderzoeker gaven. Ik mocht even delen in hun leven, en hun verhalen horen, mooie, en verdrietige verhalen, die me altijd bij zullen blijven.

Geachte professor Grobbee, beste Rick. Hartelijk dank voor het vertrouwen dat je in mij stelde door mij dit promotie traject te laten doen. Dank voor wat ik van je leerde de afgelopen jaren. Dank dat ik de afgelopen jaren langere tijd in Zuid-Afrika kon doorbrengen voor het onderzoek. Dank voor het stabiele platform dat je bood: je was er als het nodig was en met je feedback kon ik weer verder.

Geachte Dr. Klipstein-Grobusch, beste Kerstin. Je bent de meest betrokken begeleider die ik ooit ontmoet heb. Je was er altijd de afgelopen jaren, ongeacht of het wel of geen vakantie was, en ongeacht het tijdstip van de dag. Ik kon met al mijn vragen bij je terecht, of het nu om logistieke zaken ging, het uitvoeren van het onderzoek of feedback tijdens het schrijven van artikelen. Ik heb veel aan je te danken, hartelijk dank voor alles!

Geachte Dr. Barth, beste Roos. Het was heerlijk om jou als co-promotor te hebben. Met je klinische kennis kon je goed sturen op wat relevant was voor de praktijk. Met je nuchtere blik en helikopter view hielp je me om de dingen in het juiste perspectief te blijven zien. Na een overleg met jou leek alles weer gemakkelijk op te lossen. Hartelijk dank voor de afgelopen jaren!

Dear professor Venter, dear Francois. It was an unexpected blessing to have you as my supervisor from Wits Reproductive Health and HIV Institute, University of the Witwatersrand, Johannesburg. You believed in my capacities and gave me room to fly, even when I was still hovering around. Your name opened doors when I needed to arrange practicalities to make the research happen. Thanks for introducing me to rock climbing, and the outdoor climbing weekends, where all the research troubles dissolved while facing the rock. Thank you for everything!

Geachte professor Bots, beste Michiel. Dank je voor je begeleiding de afgelopen jaren. Ik kon altijd bij je aankloppen als ik even vastzat en niet wist hoe met de CIMT data om te gaan.

Geachte professor Devillé, beste Walter. Je bent voor mij onlosmakelijk verbonden aan de Ndlovu Cohort Study, waar je in alle dagelijkse beslommeringen die het onderzoek mee bracht een rots in de branding was. Het was altijd gezellig met je bij te praten en ik herinner gezellige braais en een 11 km run onder de hete Afrikaanse zon waar je jezelf aansloot bij ons als master- en PhD studenten. Hartelijk dank voor alles!

Geachte dokter Tempelman, beste Hugo. Een speciaal woord van dank aan jou. Ik zie het als een voorrecht dat ik mee mocht meekijken en als PhD student kon participeren in de onderzoeksarm van de Ndlovu Care Group. Ik ben onder de indruk van wat je in Elandsdoorn opgebouwd hebt, je visie (dank voor de inspirerende avond waarin je je visie op de organisatie hebt uitgelegd m.b.v. de pyramide van Maslow!) en je passie waarmee je streeft naar verbetering van de leefsituatie van de mensen rondom de Ndlovu Care Group.

Hartelijk dank aan alle leden van het Ndlovu Research Consortium, voorzover nog niet hierboven genoemd. In het bijzonder hartelijk dank aan professor Coutinho, professor Hoepelman en Dr. Wensing voor jullie vertrouwen in mij en de mogelijkheid als PhD student mee te werken aan de Ndlovu Cohort Studie.

Dear prof. Dr. Crowther, dear Nigel, thank you for your advice and pratical support with the laboratory space in the past years. Dear Dr. S. Louw and Dr. E. Mayne, thank you for the collaboration that resulted in a nice article.

Dear Dr. Scheuermeier, dear Karine. It was a pleasure to meet you halfway through my PhD project and a nice surprise when you took the post for scientific coordinator at the Ndlovu Research Consortium. Your enthusiasm and energy inspire me to keep going and strive for the best. Thanks for your friendship.

I would also like to thank the Ndlovu Cohort Study team: Miss Mophoko Molepo, professional nurse, Miss Bathabile Mahlangu, research nurse, Miss Stephina Rakgotho, receptionist and Sir Bhuti Nyath, community liaison officer. You have worked hard to attend to all of the participants over the past years. Without you there would not have been any participants in the study. Thank you for the collaboration over the past years.

A special thanks to Miss Ellen Taylor, research nurse, who worked with me on the Caput_Cappuchino project in Johannesburg. Your laughter, work-ethic and interaction with participants made me smile at the research site every day.

Thanks to my South-African colleagues, Samanta Lalla-Edward, Michelle Moorhouse, Godspower Akpomiemie, Raees Mahomed, as well as the DRV and Advance study teams. Thanks for your practical support and help.

Lieve pa en ma, woorden zijn niet genoeg om te zeggen dat ik jullie dankbaar ben. Dank voor jullie liefde en de warmte van het gezin. Dank voor de vrijheid die jullie me hebben gegeven bij het kiezen van mijn studie. Dank voor de opvoeding waarin jullie me hebben geleerd hebben wie God is, geleerd hebben verantwoordelijkheid te dragen, niet op te geven en dat elke moeilijkheid ook een mogelijkheid met zich meebrengt of iets positiefs in zich draagt.

Lieve Marian en Jilles, Willemien en Anton, Jan en Heidi, Niels en Sanne, Gerdien en Wijnand, Marleen en Gert, alle neefjes en nichtjes. Dank jullie wel voor jullie belangstelling naar mijn onderzoek en voor de keren dat jullie mij hebben bezocht in Zuid-Afrika. Ik vind het altijd heerlijk om met jullie af te spreken en de band te voelen die er is, die dieper is dan vriendschap.

Vrienden, jullie verrijken mijn leven; zonder jullie was ik niet de persoon geweest die ik nu ben. Een aantal van jullie wil ik in het bijzonder noemen. Erika Pors, onze vriendschap is begonnen op weg naar de kerk, toen ik je herkende aan je rode jas. Dank je wel dat je er altijd was, dat ik je kon vertellen over de mooie dingen, verdrietige dingen, mijn planning die weer eens te optimistisch was, onderzoek frustraties en nog veel meer. Dank je wel voor je luisterend oor, je praktische hulp, ook nu als paranimf. Je bent een zegen in mijn leven! Jeanet en Niels van Ewijk, jullie zijn me dierbaar. Dank dat ik altijd bij jullie terecht kan en lief en leed met jullie kan delen. Willemien van de Berg, zolang ik me herinner zijn we al bevriend. Dat maakt me blij en dankbaar. Dank voor alles wat ik met jou mocht delen, voor je luisterend oor. Jij, samen met Willem en de kinderen, zijn een zegen in mijn leven! Esther Westland, Annie Liefaard, Petra en Jack de Vos, Gerdien en Martijn van Genderen, Willemien Spelt, Bertine Vermeer, Evelien Koetsier, Herwin Looman, Elma Zweistra, Aline Hanskamp, Netty Post, Elze de Vries en alle andere vrienden, dank jullie wel voor jullie vriendschap, support en gezelligheid. Janneke Everse – de Kloe en Mariëlle Fieret, het was een fijne tijd met jullie als huisgenootjes. Dank voor de gezelligheid en de praktische hulp. Hanna de Vries, Eefje de Gelder en Regina Hofland, met jullie heb ik de PhD ups en downs kunnen delen. Het was altijd weer verfrissend te zien dat de thema's hetzelfde zijn, hoewel we een PhD deden binnen verschillende vakgebieden. Dank voor jullie support en de geruststelling dat ondanks de vele downs, waar we allemaal doorheen gingen, ik bij jullie zag dat de eindstreep gehaald werd of in het zicht is.

Collega's binnen het Julius Centrum en het UMC Utrecht, Joyce Brown, Daniel Boateng, Hannah Brown en alle andere global health PhD fellows, Ismay van Loon, Nienke Plantinga, Charlotte Hakkers, het was fijn om met jullie op te trekken, samen te lunchen en de onderzoeksperikelen te kunnen delen.

Beste Annelieke Hulzebosch, Meri Varkila, Stan Antonides, Justine van Kieftenburg, Joyce Peper, Klariska Hoeve, Maren Kummerow, Geert Roozen, Vita Jongen, Noor Godijk en Melvin Draaijer. Met plezier heb ik jullie begeleid tijdens jullie master stage. Dank voor jullie bijdrage aan het onderzoek.

Dear Theresa Benson-Onaji, dear Tess. It was a blessing to meet you in Johannesburg when we became flatmates and close friends. Thanks for your friendship, for all the moments that we shared when we both started to find our way in Johannesburg as foreigners. We walked a major part of the PhD journey together, although our research field was completely different. My time in South-Africa was filled with sunlight and warmth, and you were a main part of the joy. Thanks for everything!

To my South African family and friends. The Aitken family, dear Mike, Blanche and Sue. You became my South-African family and words cannot tell how grateful I am for what you did for me. Dear bootcamp friends, Rob Purves, Matthew Cartwright, Dewald and Birte Veldsman, Laura Unterslak, Elizabeth Wallis, the Hugo brothers, Marie-Louise, Themba, Jason, Chris, Natalie, Ephraim, Johan and others. It was amazing to train with you up to four times a week. Dear Rob, you did a great job and you are an excellent motivator. The training cleared my head after a long day at the research site or behind the laptop and helped a lot to keep the right balance in my life. And to the amazing friendships formed at church, at rock climing or just randomly, Chileshe, Pfungwa, Fiona, Funni and all other guys from Connect. Dear Clem, the coffee breaks in the canteen at medical school were moments to look forward to. Thank you all for your friendship.

Maartje en Maarten, het voelde altijd als thuiskomen bij jullie. Ik vond het heerlijk om jullie kant op te komen, samen te praten en samen te braaien. Ik reken jullie bij mijn familie in Zuid-Afrika.

A special thank you to Edwin Seda, the guy who stole my heart just before I came back to The Netherlands. Many thanks for the beautiful design of the cover *Kipenzi changu*.

Tot slot, ere wie ere toekomt. Ik wil God, mijn hemelse Vader, erkennen en bedanken voor het leven, gezondheid en passie voor het onderzoek en mijn klinische werk. Hierdoor heb ik de achterliggende periode het onderzoek kunnen uitvoeren en deze thesis kunnen schrijven. Soli Deo Gloria!

List of publications

M.E. Hamaker, **A.G.Vos**, C.H. Smorenburg, S.E.J.A. de Jong, B.C. van Munster. The value of a Comprehensive Geriatric Assessment in predicting treatment tolerance and prognosis in older cancer patients. Review. The Oncologist, 2012 Aug 31.

M.E. Hamaker, J.M. Jonker, S.E. de Rooij, **A.G.Vos**, C.H. Smorenburg, B.C. van Munster Frailty screening tools for predicting outcome of a comprehensive geriatric assessment in older cancer patients: a systematic review. The Lancet Oncology Vol 13 October 2012.

A.G. Vos, M.A. van de Ree. Hypertension and use of an intrauterine levonorgestrel-releasing device. Letter to the editor. The Netherlands Journal of Medicine, November 2012, vol. 70, no 9

Alinda G. Vos, Michèle H.J. Goossens, Steven F.T. Thijsen, Sanjay U.C. Sankatsing. Mycoplasma Pneumonia associated Mucositis. Ned Tijdschr Geneeskd. 2013;157:A6375

A.G.Vos, M.W.M. Wassenberg, J. de Hoog, J.J. Oosterheert. Diagnosis and treatment of tuberculous uveitis in a low endemic setting. A retrospective case study. International Journal of Infectious Diseases, 2013 may 22, S1201-9712(13)00154-9

A.G. Vos, N. Salamia, R.E. Barth, K.Klipstein, D.E.Grobbee. Pro-inflammatory markers in relation to cardiovascular disease in HIV infection. A systematic review. PLoS One. 2016 Jan 25;11(1)

A.G. Vos, A. Hulzebosch, D.E. Grobbee, R.E. Barth, K.Klipstein-Grobusch. Association Between Immune Markers and Surrogate Markers of Cardiovascular Disease in HIV Positive Patients. A Systematic Review. PLoS One. 2017 Jan 13;12(1).

Alinda Vos, Hugo Tempelman, Walter Devillé, Roos Barth, Annemarie Wensing, Mirjam Kretzschmar, Kerstin Klipstein-Grobusch, Andy Hoepelman, Kiki Tesselaar, Sue Aitken, Mashudu Madzivhandila, Cuno Uiterwaal, Francois Venter, Roel Coutinho, Diederick E. Grobbee. HIV and Risk of Cardiovascular Disease in Sub Saharan Africa. Rationale and design of the Ndlovu Cohort Study. European Journal of Preventive Cardiology 0(00) 1–8. April 2017

Vos AG, Chersich MF, Klipstein-Grobusch K, Zuithoff P, Moorhouse MA, Lalla-Edward ST, Kambugu A, Kumarasamy N, Grobbee DE, Barth RE, Venter WD. Lipid levels, insulin resistance and cardiovascular risk over 96 weeks of antiretroviral therapy: a randomised controlled trial comparing low-dose tavudine and tenofovir. Retrovirology. 2018 Dec 14;15(1):77.

V.W. Jongen, S.T. Lalla-Edward, **A.G. Vos**, N.G. Godijk, H.A. Tempelman, D.E. Grobbee, W.L.J. Devillé, K.Klipstein-Grobusch. Hypertension in a rural community in South Africa: What they know, what they think they know and what they recommend. BMC Public Health. 2019 Mar 25;19(1):341.

Venter WDF, Kambugu A, Chersich MF, Becker S, Hill A, Arulappan N, Moorhouse M, Majam M, Akpomiemie G, Sokhela S, Poongulali S, Feldman C, Duncombe C, Ripin DHB, **Vos A**, Kumarasamy N. Efficacy and Safety of Tenofovir Disoproxil Fumarate Versus Low-Dose Stavudine Over 96 Weeks: A Multicountry Randomized, Noninferiority Trial. J Acquir Immune Defic Syndr. 2019 Feb 1;80(2):224-233.

M.R.J. Varkila#, **A.G. Vos#,** R.E. Barth, H.A. Tempelman, W. Devillé, R.A. Coutinho, D. E. Grobbee, K. Klipstein-Grobusch. The influence of HIV infection on pulmonary function in a rural African population. # shared first author. PLoS One. 2019 Jan 15;14(1

Curriculum Vitae

Alinda Vos was born on the 19th of December 1985 in Nederhemert (Gelderland), The Netherlands. In 2004 she completed secondary school at Gomarus College, Gorinchem. She studied Medicine at Utrecht University from 2004 to 2010. During her medical training she spent 10 weeks at Nkhoma Hospital in Malawi and eight weeks at Plateau Mission Hospital in Kenya for an elective clinical internship. In her final year of Medicine she performed a scientific internship at the Department of Infectious Diseases, University Medical Center Utrecht, The Netherlands.

Upon graduation in September 2010, she started working as a medical doctor at the Internal Medicine department of the Diakonessenhuis in Utrecht, The Netherlands, under supervision of Dr. A.F. Muller. In December 2011 she started her residency in Internal Medicine, while she continued to work at the Diakonessenhuis until May 2014. During her years at the Diakonessenhuis she participated in research that resulted in a desire to pursue a PhD degree. She started her PhD in May 2014 at the Julius Center, UMC Utrecht, under the supervision of prof. Dr. D.E. Grobbee, Dr. K. Klipstein-Grobusch and Dr. R.E. Barth, and this led to the current thesis. Alongside the PhD project, she was enrolled in the master program Epidemiology Postgraduate, offered by the Julius Center. From 2015 to 2017 she spent most of her time in South-Africa where she was supervised by prof. Dr. F. Venter. She obtained a position as an honorary researcher at the University of the Witwatersrand from January 2015. In May 2017 she resumed her clinical training in Internal Medicine at the University Medical Center Utrecht, under the supervision of prof. Dr. H.A.H. Kaasjager. She interrupted her training two more times for a couple of months to complete her research project in Johannesburg. In April 2019 she started her haematology training under the supervision of Dr. A. van Rhenen.