HIV-related arterial stiffness in Malawian adults is associated with proportion of PD-1 expressing CD8 T-cells and reverses with anti-retroviral therapy

Christine KELLY PhD [1,2,3], Henry C MWANDUMBA PhD [2,4,5], Robert S HEYDERMAN PhD [2,6], Kondwani JAMBO PhD [2,4], Raphael KAMNG'ONA BSc [2], Mishek CHAMMUDZI BSc [2], Irene SHEHA BSc [2], Ingrid PETERSON PhD [2], Alicja RAPALA [7], Jane MALLEWA PhD [5], A. Sarah WALKER PhD [8], Nigel KLEIN PhD [9], Saye KHOO PhD[1]

[1]Insitute of Translational Medicine University of Liverpool, Liverpool, UK.

[2]Malawi-Liverpool-Wellcome Trust Clinical Research Programme, College of Medicine, Blantyre, Malawi.

[3]HIV Molecular Research Group, University College Dublin, Ireland.

[4] Department of Clinical Sciences, Liverpool School of Tropical Medicine, Liverpool, UK

[5]Department of Medicine, Queen Elizabeth Central Hospital, Blantyre, Malawi.

[6]Division of Infection and Immunity UCL, London, UK.

[7]Institute for Cardiovascular Sciences, UCL, London, UK.

[8]MRC Clinical Trials Unit UCL, London, UK.

[9]UCL Great Ormond Street Institute of Child Health, London, UK.

Foot notes

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Corresponding author

Dr. Christine Kelly, Nelson Street, Dublin 7, Ireland

T +353 1 7164552, christine.kelly@ucd.ie

Alternate Corresponding author

Professor Saye Khoo, Aston Street, Liverpool, UK

T +44 (0)151 794 5560, S.H.Khoo@liverpool.ac.uk

Keywords

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Article Summary: Arterial stiffness is increased in Malawian adults with low CD4 during the first 3 months of antiretroviral therapy, compared to adults without HIV. Hypertension is an important traditional risk factor and immune activation, including CD8 exhaustion, also contributes to arterial stiffness.

Abstract (199 words)

Background

The contribution of immune activation to arterial stiffness and its reversibility in HIV-infected adults in sub-Saharan Africa is unknown.

Methods

HIV-uninfected and HIV-infected Malawian adults initiating antiretroviral therapy (ART) with CD4 <100 cells/μl were enrolled (2-weeks post-ART for HIV-infected) and followed for 44-weeks. We evaluated the relationship between carotid femoral pulse wave velocity (cfPWV) and T-cell activation (HLADR+CD38+), exhaustion (PD1+) and senescence (CD57+), and monocyte subsets using normal regression.

Results

In 279 HIV-infected and 110 HIV-uninfected adults, 142(37%) had hypertension. HIV was independently associated with 12% higher cfPWV (p=0.02) at baseline, and week-10 (14% higher, p=0.02) but resolved by week-22. CD4 and CD8 T-cell exhaustion(PD1+) were independently associated with higher cfPWV at baseline (p=0.02). At 44-weeks, arterial stiffness improved more in those with greater decreases in %CD8 and %CD8PD1+ T-cells (p=0.01, p=0.03 respectively). When considering HIV-infected participants alone, adjusted arterial stiffness at week-44 tended to be lower in those with higher baseline %CD8PD1+ T-cells (p=0.054).

Conclusions

PD-1+ expressing CD8 T-cells are associated with HIV-related arterial stiffness, which remains elevated during the first three months on ART. Resources to prevent cardiovascular disease in sub-Saharan Africa should focus on blood pressure reduction, and those with low CD4 during early ART.

Word count: 3500

Introduction

Sub-Saharan Africa (SSA) has the highest burden of HIV worldwide, with 25.6 million people living with the disease.[1] The region also faces an accelerated epidemic of non-communicable diseases including cardiovascular disease (CVD).[2] Mortality from CVD is high in SSA, causing an estimated one million deaths per year.[3] This rate is predicted to increase, with non-communicable disease-related mortality estimated to surpass infection-related deaths by 2030.[3] Studies from high-income settings suggest the risk of CVD is approximately doubled in people living with HIV, even after adjusting for confounders such as socio-economic status, traditional cardiovascular risk factors and viral hepatitis co-infection.[4, 5] Several processes may contribute, including high HIV viral load, side-effects of antiretroviral therapy (ART) - particularly protease-inhibitors, and the effects of chronic immune activation despite effective ART.[6] Immune activation may be driven by persistent low level HIV viraemia, microbial translocation, and subclinical infections.[7, 8] However, the risk of CVD in people living with HIV in SSA has not been well characterised. It is likely that immune activation differs in low-income settings due to the effects of more advanced HIV disease at presentation, more frequent acute coinfections, and malnutrition with disruption to the gut barrier.[9, 10] Traditional cardiovascular risk factors also vary, with hypertension being more prevalent than diabetes, dyslipidaemia or obesity - although with epidemiological transition and increasing urbanisation in the region, this may change in the near future.[11, 12]

Large cohorts documenting cardiovascular events have not been established in SSA, limiting the assessment of cardiovascular risk. One physiological marker of cardiovascular risk is carotid femoral pulse wave velocity (cfPWV), a gold standard measurement of arterial stiffness.[13-15] Although adjusted for concurrent blood pressure, the reading can be affected by blood viscosity and ambient

temperature.[13] Nevertheless it has been shown to be reliable and reproducible, and has been validated against clinical outcomes in high-income settings;[16-18] a cfPWV in the top versus bottom tertile is associated with a greater than 2-fold increased risk of myocardial infarction/stroke.[18] The 2007 European Society of Cardiology consensus guidelines proposed a 12m/s threshold as high risk for CVD events.[14] A few small studies have assessed arterial stiffness in people living with HIV in low-income SSA,[19-23] but none have evaluated the impact of chronic immune activation over time.

This study therefore aimed to characterise the contribution of immune activation to arterial stiffness in HIV-infected Malawian adults initiating ART with advanced immune suppression, in comparison to HIV-uninfected adults, and to determine how this changed over time on ART.

Methods

Study design

Adults over 18 years old presenting for HIV testing at the voluntary testing clinic, the HIV outpatient clinic and the medical inpatient wards at the Queen Elizabeth Central Hospital, Blantyre, Malawi were recruited into a prospective cohort from January 2014 until June 2015. Adults with a new HIV diagnosis were approached consecutively and were eligible if they were ART-naïve, had CD4 <100 cells/uL and provided informed written consent. Adults who were confirmed HIV-uninfected, after self-presenting for an asymptomatic HIV test at the same voluntary testing clinic, were eligible if they had no current illness and no history of infection in the previous month by clinician assessment and medical notes review, and provided informed written consent. Exclusion criteria were living outside the Blantyre area, inability to attend follow-up visits, pregnancy, or being too unwell to participate as judged by the study clinicians. Because HIV-uninfected participants were younger, an exclusion

criterion of <35 years was applied for this group from March 2014. From January 2014 until January 2015, HIV-infected participants were co-recruited with the REALITY trial (NCT01825031). REALITY assessed interventions to reduce early mortality following ART initiation in those with CD4<100 cells/μL. Participants were simultaneously randomised to three study interventions in addition to standard triple-drug ART and cotrimoxazole; 12 weeks' adjunctive raltegravir, a package of opportunistic infection prophylaxis, and/or Ready to Use Supplementary Food.[24-26]

At enrolment and 44 weeks later, participants underwent a detailed clinical assessment including traditional cardiovascular risk factors and infection history, and fasting blood draw including sodium citrate-containing samples for immunophenotyping. To reduce the burden of study participation, and to ensure starting ART was prioritised in the severely immunosuppressed HIV-infected population, the baseline study visit was conducted at week 2 following ART initiation. cfPWV was assessed on all participants at enrolment, and 10, 22 and 44 weeks later. All participants provided informed written consent and ethical approval was granted by the College of Medicine Research and Ethics Committee (COMREC), University of Malawi (P.09/13/1464) and the University of Liverpool Research and Ethics Committee (UoL000996).

Outcome measurement

cfPWV was measured using a Vicorder device (Skidmore Medical, London, UK). The distance was the length from the sternal notch to the umbilicus and then the top mid-point of the femoral cuff, multiplied by 0.8 as per consensus guidelines. Wave forms were saved and a random sample was reviewed by an experienced independent assessor, blinded to HIV status, at three time points during the study to ensure consistent quality. The intra-operator concordance correlation coefficient for 10 participants was 0.99 (95%CI 0.96–1.00).

Immunophenotyping of peripheral blood mononuclear cells

For flow cytometry, whole blood was processed within 4 hours of collection to isolate peripheral blood mononuclear cells (PBMCs) using Lymphoprep (Axis-Shields-Diagnostics) as previously described.[27] Cells were analysed using a CyAn ADP 9 colour flow cytometer (Beckman Coulter). The T-cell panel included CD3 BV510, CD4 V450, CD38 PE Cy7, HLA-DR AF700, PD1 APC, and CD57 FITC (all from BD Biosciences) and CD8 PE (Biolegend). The monocyte panel included HLA-DR AF700, CD14 PE Cy7 and CD16 PE (all from BD Biosciences). Anti-mouse Igk isotype control and negative control particles (BD biosciences) were used for compensation. A standardised gating strategy was followed for both panels (T-cells Supplementary Figure 1A, monocytes Supplementary Figure 1B). Monocyte subsets were identified as previously.[28]

Statistical analysis

As data validating a clinically relevant cfPWV threshold in SSA were not available, the 12m/s threshold in European consensus guidance was used to guide sample size calculations.[14] Recruiting 300 HIV-infected and 100 HIV-uninfected participants provided 80% power to detect an odds ratio (OR) of 1.5 associated with HIV, assuming 25% of HIV-uninfected participants had cfPWV >12 m/s.[14] After enrolment, it was clear that this threshold was rarely reached, and therefore the planned analysis considered the primary outcome, cfPWV, as a continuous variable.

Factors affecting HIV and arterial stiffness were identified *a priori* using a causal diagram (Supplementary Figure 2) as either potential mediators (on the mechanistic pathway between them) or confounders (associated with HIV and arterial stiffness but not on the mechanistic pathway). Categorical and continuous variables were compared between HIV-infected and uninfected adults using chi-squared and rank-sum tests, respectively. Correlations between continuous variables were compared using Spearman's rho. To avoid undue influence from outliers, continuous variables were truncated at their 97.5% and 2.5% percentiles for regression models. Normal linear regression models

were constructed, with log10 cfPWV (approximately normal) as the outcome, and HIV infection as the primary exposure. Confounders and mediators with univariate p value <0.2 for association with cfPWV were considered and backwards elimination (exit p=0.2) used to identify a final model. Where two variables were strongly co-linear, the variable with the strongest univariate association with cfPWV was considered for inclusion in the multivariate model. Independent effects of immunophenotyping parameters on this model were then also assessed. Overall changes in $\log_{10}$ cfPWV over the first 44 weeks from enrolment were estimated using random effects models, considering the impact of HIV on cfPWV both at baseline (intercept) and over time (interaction with time). Regression models for cfPWV 44 weeks after enrolment in HIV-infected participants adjusted for baseline cfPWV and factors identified as confounders or mediators at baseline, and additionally considered the impact of immune parameters. Adjusting for baseline means these models identify predictors of change from baseline.

All analysis was undertaken using Stata v13.1 (Statacorp, USA).

Results

2107 adults with a new HIV diagnosis were screened, of whom 279 (13%) were recruited. 170 (61%) were co-recruited with REALITY and 109 (39%) were not (Figure 1). Most exclusions [1477 (70%)] were due to CD4 >100 cells/mm^3 (complete list in Supplementary Table 1). 110 HIV-uninfected adults were also recruited. Although the HIV-infected and uninfected groups were of similar age (median 36.6 versus 34.8 years, Table 1), HIV-infected participants were more likely to be male and have a lower level of education. Three (3%) HIV-uninfected versus five (2%) HIV-infected adults had a diagnosis of hypertension at enrolment; a further 46 (42%) and 88 (32%) respectively were discovered to have hypertension during the study. HIV-infected participants had advanced immune

suppression (median CD4 count 41 cells/μL, median HIV viral load 5.06 log10 copies/ml), although only 54 (19%) were WHO HIV disease stage 3 or 4.

All but one HIV-infected participant initiated standard first-line therapy with tenofovir+lamivudine+efavirenz (one zidovudine+lamivudine+nevirapine). In total, 28 (7%) participants withdrew or were lost to follow-up and 24 (6%) participants died. Of those lost to follow-up or death, 13(29%) occurred within the first 2 weeks, 16(36%) between 2 and 10 weeks and 16(36%) after 10 weeks (Figure 1). One HIV-uninfected participant died from a hypertension-related intra-cranial bleed. The 23 deaths in HIV-infected participants were due to pulmonary/disseminated TB (6), cryptococcal meningitis (3), Kaposi's Sarcoma (3), gastroenteritis (1), and TB meningitis (1). The cause of death was unknown for 9 participants.

Arterial stiffness at enrolment

At enrolment, the median(IQR) cfPWV was 7.3 (IQR 6.5-8.2) m/s in HIV-infected versus 7.2 (6.2-8.0) m/s in uninfected participants (p=0.07). cfPWV was greater than 12 m/s for 5 patients, 4 of whom had HIV infection.

Immune activation at enrolment

As expected, compared with HIV-uninfected participants, HIV-infected adults had higher activated (CD38+HLADR+) CD4 and CD8 T-cells (both p<0.0001). HIV-infected adults also had a higher proportion of exhausted (PD1+) CD4 and CD8 T-cells (both p<0.0001). In contrast, there were no differences between HIV-infected and uninfected participants in classical ($CD14^{++}CD16^{-}$), intermediate ($CD14^{++}CD16^{+}$) and non-classical ($CD14^{+}CD16^{+}$) monocytes (p=0.79, 0.10, 0.59 respectively; Table 1).

T-cell PD1 expression is independently associated with HIV-related arterial stiffness 2 weeks post ART initiation

Factors univariately associated with baseline cfPWV with p<0.2 are shown in Table 2A; neither WHO stage nor a diagnosis of acute co-infection were associated with baseline cfPWV (p=0.25 and 0.23 respectively). Independently, every 10-year increase in age was associated with a 18% (95% CI 14-23%) higher cfPWV (p<0.0001) (univariable associations in Supplementary Figure 3), with no evidence of effect modification between age and HIV (interaction p=0.73). Every 10mmHg higher diastolic blood pressure was also associated with a 9% (4-13%) higher cfPWV (p<0.0001), and women had 9% (2-16%) lower cfPWV (p=0.001).

Adjusting for these confounders (Table 2A), there was weak evidence that HIV-infected participants had a 7% higher cfPWV (95% CI -1% to +16%, p=0.08). Adjusting for confounders and mediators, HIV was significantly associated with cfPWV, with a 12% higher cfPWV in HIV-infected participants (95% CI 2-23% p=0.02) (Table 2A). The effect of gender weakened with the addition of potential mediators (haemoglobin, weight, cholesterol, recent infection) to the model, but effects of age and diastolic blood pressure remained. cfPWV was 2% higher with every 1 g/dL higher haemoglobin (p=0.07), which may be a marker of plasma viscosity. Concurrent infection at HIV diagnosis was not associated with cfPWV [adjusted fold change +9% (95% CI -2% to +21%, p=0.13]. When immune variables were considered in addition to this model (Table 2B), exhausted CD4 and CD8 T-cells were each independently associated with cfPWV (p=0.02), and the independent effect of HIV was lost. HIV remained significantly associated with cfPWV excluding those with WHO stage 3 and 4 (fold change 12% (95% CI 3 to 24%, p=0.01).

HIV-related arterial stiffness improves on ART

At week-44, 228 (82%) HIV-infected and 103 (94%) uninfected participants remained on-study. All HIV-uninfected participants were retested for HIV at the week 44 visit and none had acquired a new infection.

HIV-infected participants still had significantly higher cfPWV at week 10 (adjusted p=0.02), but not at week 22 or 44 (p=0.46 and 0.59, Figure 2). Overall, from enrolment through 44 weeks, cfPWV declined by 9% (95% CI 4 to 15%, p=0.002) in HIV-infected participants but did not change significantly in HIV-uninfected participants [change +2% (95% CI -7% to +12%, p=0.69; heterogeneity p=0.04]. In a sensitivity analysis, a similar effect of HIV on cfPWV was found at each timepoint when only including participants who completed the study: enrolment fold change +10% (CI interval 0% to 21%) , week 10 fold change +13% (CI interval +2% to +27%, p= 0.022), week 24 fold change -4% (CI interval -13% to +6%, p=0.45) , week 44 fold change 3% (CI interval -7% to 14%, p=0.60).

For HIV-infected participants, median (IQR) CD4 count had increased to 144 (99-218) cells/uL. %CD8 T-cells decreased significantly (82% (84–96) to 60% (54–67), p<0.0001). Only %activated CD4 T-cells and %exhausted CD4 and CD8 T-cells decreased significantly by week-44 (74% (62–86) to 61% (50–69) p<0.0001; 54% (31-67) to 32% (22–48) p<0.0001; 38% (29–50) to 32% (21–47%) p=0.007 respectively, Supplementary Table 2).

Resolution of higher CD8 T-cell PD1 expression is associated with lower arterial stiffness at 44 weeks post ART initiation

Next, we examined the impact of baseline factors on cfPWV 44 weeks post ART initiation, adjusting for baseline cfPWV (equivalent to predictors for change in cfPWV) and age, sex, baseline

haemoglobin and diastolic blood pressure (Table 3). There was a trend towards higher %CD8PD1+ T-cells at baseline being independently associated with lower arterial stiffness 44 weeks post ART initiation (4% lower week-44 cfPWV for every 10% higher %CD8PD1+ at enrolment, p=0.054). When REALITY intervention arms were added one by one to the same model (adjusted for age, sex, and baseline haemoglobin, diastolic blood pressure, CD8PD1+ T cells and baseline cfPWV), there was no evidence of association between week 44 cfPWV and randomisation to enhanced infection prophylaxis [fold change vs standard-prophylaxis 2% (95% CI -9% to +11%, p=0.76)]; or enhanced nutritional support [fold change vs standard support +8% (95% CI -3% to +20%, p=0.15)]. However, there was a trend towards lower adjusted cfPWV at week 44 in the 82 (29%) randomised to adjunctive raltegravir for 12 weeks at ART initiation [fold change vs standard ART alone -11% (95% CI -21 to -1%, p=0.04)]. This effect of raltegravir on week 44 cfPWV was not lost by adding HIV viral load at baseline[-12% (95% CI -22 to 0%, p=0.05)], week 12 [-14% (95% CI -25% to 0%, p=0.04] or week 24 [-12% (95% CI -24% to +1%, p=0.08].

Examining the relationship between change in immune markers and change in cfPWV from baseline to week 44, we found greater decreases in %CD8 T-cells, %CD8PD1+ T-cells and proportion of intermediate monocytes were associated with greater improvements in arterial stiffness univariably (p=0.01, p=0.03, p=0.054 respectively, Table 4). Adjusting for baseline factors as well as change in %CD8 and %CD8PD1+ T-cells, we found that the baseline effect of %CD8PD1+ cells on week 44 cfPWV was attenuated and that there was instead a trend towards a lower cfPWV at week 44 in those with a greater decrease in %CD8PD1+ cells (p=0.079; Table 3). Overall, these data suggest that resolution of an initially high proportion of CD8 PD1+ T-cells is associated with an improvement in arterial stiffness over 44 weeks of ART.

Discussion

We have demonstrated that arterial stiffness is increased in Malawian adults with advanced HIV during the first 3 months of ART and that T-cells expressing PD1 are associated with this effect. Further, we have shown that this effect is reversible, in a cohort initiating ART with severe immunosuppression and with only modest CD4 count gains on ART. Those with the highest proportion of PD1 expressing CD8 T-cells seemed to benefit the most from ART, demonstrating lower arterial stiffness 44 weeks post ART initiation. This is superimposed on a high background prevalence of hypertension.

Hypertension was the most important traditional risk factor for cardiovascular disease.[29] Our findings are consistent with studies from the region showing 30-50% prevalence in the general population, with as few as 7% aware of their diagnosis.[30] In regions further along the epidemiological transition,[11] lifestyle-related CVD risk factors such as obesity and diabetes are becoming more important and are compounded by HIV infection.[31] Intervention to prevent CVD in low-income countries is urgently needed, before traditional risk factors intersect with the increased risk associated with HIV.

HIV-infected participants had a 12% higher adjusted arterial stiffness at ART initiation compared to our HIV-uninfected population. Persisting higher arterial stiffness in the first 3 months of ART is consistent with work from Benjamin et al showing vasculitis as the pathological phenotype of vascular injury during this period, potentially reflecting an IRIS phenomenon.[32] Excess mortality during the first 3 months of ART is well-recognised in patients initiating ART with very low CD4 counts.[10, 33] The REALITY trial assessed interventions to reduce this, but even with enhanced infection prophylaxis, 24-week mortality was still 8.9%.[34] Vascular inflammation, driven by high immune activation, may contribute to some of the adverse events seen during the first three months in those initiating ART with low CD4 counts.

In particular, CD8PD1+ T-cells have previously been associated with endothelial dysfunction in patients with HIV infection.[35-38] In a cross-sectional study of 358 participants of the SCOPE cohort (of whom 75% were virologically suppressed on ART), PD1+ T-cells were raised in untreated and treated HIV infection and CD8PD1+ expression was particularly associated with markers of HIV antigenaemia including CD8 T-cell activation and HIV viral load.[39] Given the association with reductions in arterial stiffness demonstrated here, the CD8PD1+ expression pathway warrants further investigation. However, in an environment where concurrent acute and latent infections are common, it may be that improvements in CD8PD1+ expression and arterial stiffness were not due to ART and control of HIV alone.[40, 41] Rather, there may have been a protective effect from cotrimoxazole co-administration, preventing co-infections such as malaria or bacterial infections. An 'ART care effect' may have contributed to improvements over the study period, whereby patients who are engaged in care in a low-income setting experience benefits such as frequent monitoring or better access to care.

Study strengths include prospective follow-up with robust assessment of clinical and cardiovascular measures, and a comprehensive longitudinal characterisation of both monocyte and T-cell surface activation markers from fresh PBMCs in a large cohort of HIV-infected and uninfected participants. As HIV is a generalised epidemic in Malawi, the HIV-infected population is likely to have a broadly similar traditional cardiovascular risk profile to HIV-uninfected adults enrolled from the same facility. Further, all but one of our participants received the same standard first-line ART regimen, meaning that choices relating to the specific ART regime cannot be confounders of the associations identified.

Limitations include the fact that cfPWV has not been validated to predict cardiovascular events in low-income SSA, however, cfPWV has been validated robustly elsewhere.[42, 43] The relatively small differences identified in continuous cfPWV may have uncertain clinical relevance and our

hypothesised poor outcome (cfPWV>12 m/s) was rare; likely because our power calculations were based on studies from high-income settings, and much older populations than the HIV-infected population in SSA.[44-46] Overall, our study is still the largest to address this issue in the region, and demonstrates similar magnitudes of effect of HIV as traditional risk factors such as age (18% per 10 years older) and blood pressure (9% per 10mmHg higher).

Our HIV-uninfected population was selected to reflect generally healthy adults with similar socio-demographics. Comparisons with our severely immunosuppressed HIV population therefore reflect the extremes, and this also limits the generalisability of our study to unselected HIV populations. However, WHO stage, CD4 count and the presence of co-infections and their clinical markers, were not independently associated with arterial stiffness, suggesting HIV effects may exist across the disease spectrum. Acute co-infections at the time of diagnosis were rare, limiting our power to assess their effects, but may have contributed to an IRIS type phenomenon. To avoid overburdening potentially clinically unwell patients who urgently needed to start ART, enrolment assessments were performed 2 weeks after ART initiation. As early mortality is high and some limited normalisation may have occurred, our findings are likely a best-case scenario.[24]

Lastly, the size of the effect of CD8PD1+ T-cells on arterial stiffness was modest and is likely to represent one component of several concurrent complex mechanisms involved in the pathogenesis of endothelial damage in people with HIV in this setting. Nevertheless, exhaustion was the strongest immune predictor of cfPWV on ART, and other immunophenotyping parameters did not have independent effects after adjusting for PD1 expression.

This is the first time that the dynamics of arterial stiffness in the first few months of ART have been documented longitudinally and related to cellular markers of immune activation in HIV-infected

adults. We have confirmed hypertension as the primary CVD risk factor in the region and demonstrated that consequences of immune activation can be reversed irrespective of CD4 recovery with early benefits for vasculature. Further studies into infection-driven immune activation, as a risk factor for CVD are warranted, but guidelines for prevention of CVD in HIV are urgently needed and should focus on hypertension reduction and close monitoring for those starting ART at lower CD4 counts.

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The REALITY trial group consists of:

Participating Centres: Joint Clinical Research Centre (JCRC), Kampala, Uganda (coordinating centre for Uganda): P Mugyenyi, C Kityo, V Musiime, P Wavamunno, E Nambi, P Ocitti, M Ndigendawani. JCRC, Fort Portal, Uganda: S Kabahenda, M Kemigisa, J Acen, D Olebo, G Mpamize, A Amone, D Okweny, A Mbonye, F Nambaziira, A Rweyora, M Kangah and V Kabaswahili. JCRC, Gulu, Uganda: J Abach, G Abongomera, J Omongin, I Aciro, A Philliam, B Arach, E Ocung, G Amone, P Miles, C Adong, C Tumsuiime, P Kidega, B Otto, F Apio. JCRC, Mbale, Uganda: K Baleeta, A Mukuye, M Abwola, F Ssennono, D Baliruno, S Tuhirwe, R Namisi, F Kigongo, D Kikyonkyo, F Mushahara, D Okweny, J Tusiime, A Musiime, A Nankya, D Atwongyeire, S Sirikye, S Mula, N Noowe. JCRC, Mbarara, Uganda: A Lugemwa, M Kasozi, S Mwebe, L Atwine, T Senkindu, T Natuhurira, C Katemba, E Ninsiima, M Acaku J Kyomuhangi, R Ankunda, D Tukwasibwe, L

Ayesiga. University of Zimbabwe Clinical Research Centre, Harare, Zimbabwe: J Hakim, K Nathoo, M Bwakura-Dangarembizi, A Reid, E Chidziva, T Mhute, GC Tinago, J Bhiri, S Mudzingwa, M Phiri, J Steamer, R Nhema, C Warambwa, G Musoro, S Mutsai, B Nemasango, C Moyo, S Chitongo, K Rashirai, S Vhembo, B Mlambo, S Nkomani, B Ndemera, M Willard, C Berejena, Y Musodza, P Matiza, B Mudenge, V Guti. KEMRI Wellcome Trust Research Programme, Kilifi, Kenya: A Etyang, C Agutu, J Berkley, K Maitland, P Njuguna, S Mwaringa, T Etyang, K Awuondo, S Wale, J Shangala, J Kithunga, S Mwarumba, S Said Maitha, R Mutai, M Lozi Lewa, G Mwambingu, A Mwanzu, C Kalama, H Latham, J Shikuku, A Fondo, A Njogu, C Khadenge, B Mwakisha. Moi University Clinical Research Centre, Eldoret, Kenya: A Siika, K Wools-Kaloustian, W Nyandiko, P Cheruiyot, A Sudoi, S Wachira, B Meli, M Karoney, A Nzioka, M Tanui, M Mokaya, W Ekiru, C Mboya, D Mwimali, C Mengich, J Choge, W Injera, K Njenga, S Cherutich, M Anyango Orido, G Omondi Lwande, P Rutto, A Mudogo, I Kutto, A Shali, L Jaika, H Jerotich, M Pierre. Department of Medicine and Malawi-Liverpool Wellcome Trust Clinical Research Programme, College of Medicine, Blantyre, Malawi: J Mallewa, S Kaunda, J Van Oosterhout, B O'Hare, R Heyderman, C Gonzalez, N Dzabala, C Kelly, B Denis, G Selemani, L Nyondo Mipando, E Chirwa, P Banda, L Mvula, H Msuku, M Ziwoya, Y Manda, S Nicholas, C Masesa, T Mwalukomo, L Makhaza, I Sheha, J Bwanali, M Limbuni.

Trial Coordination and Oversight: MRC Clinical Trials Unit at UCL, London, UK: D Gibb, M Thomason, AS Walker, S Pett, A Szubert, A Griffiths, H Wilkes, C Rajapakse, M Spyer, A Prendergast, N Klein. Data Management Systems: M Rauchenberger, N Van Looy, E Little, K Fairbrother.

Social Science Group: F Cowan, J Seeley, S Bernays, R Kawuma, Z Mupambireyi.

Independent REALITY Trial Monitors: F Kyomuhendo, S Nakalanzi, J Peshu, S Ndaa, J Chabuka, N Mkandawire, L Matandika, C Kapuya.

Trial Steering Committee: I Weller (Chair), E Malianga, C Mwansambo, F Miiro, P Elyanu, E Bukusi, E Katabira, O Mugurungi, D Gibb, J Hakim, A Etyang, P Mugyenyi, J Mallewa.

Data Monitoring Committee: T Peto (Chair), P Musoke, J Matenga, S Phiri.

Endpoint Review Committee (independent members): H Lyall (Co-Chair), V Johnston (Co-Chair), F Fitzgerald, F Post, F Ssali, A Prendergast, A Arenas-Pinto, A Turkova, A Bamford.

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Table 1. Baseline characteristics according to HIV status

		Complete cases	HIV-uninfected n=110 Median (IQR) or n (%)	HIV-infected n=279 Median (IQR) or n (%)	p value
Demographics					
	Age	388	34.8 (30.8 – 41.2)	36.6 (31.1 – 43.3)	0.41
	Female	389	66 (60%)	122 (44%)	0.004
	Primary school education or less	352	38 (40%)	136 (53%)	0.024
Traditional CV risk factors					
	Weight (kg)	375	60 (53 - 68)	53 (48 - 59)	<0.0001
	Waist: height ratio[b]	349	0.47 (0.44 – 0.53)	0.45 (0.43 – 0.49)	0.0003
	BMI (kg/m^2)	373	21.7 (20.2 – 25.2)	19.8 (18.3 – 21.9)	<0.0001
	Systolic BP (mmHg)	361	128 (114 - 134)	120 (108 - 128)	0.0001
	Diastolic BP (mmHg)	358	75 (68 - 82)	73 (68 - 80)	0.27
	History of smoking[c]	389	16 (15%)	56 (20%)	0.21
	History of alcohol use[c]	389	28 (25%)	119 (43%)	0.002
	Pre-existing CV diagnosis	370	1 (1%)	1 (0.4%)	0.47
	Prescribed CV medications	370	5 (5%)	4 (1.5%)	0.08
	Pre-existing diabetes	367	1 (1.0%)	1 (0.4%)	0.65
	Pre-existing hypertension	366	3 (3.0%)	5 (2.0%)	0.40
	New diagnosis of hypertension	358	46 (42%)	88 (32%)	0.055
	Fasting cholesterol (mmol/L)	377	4.0 (3.3 – 4.5)	3.6 (3.0 – 4.4)	0.049
	Fasting glucose (mmol/L)	327	4.6 (4.2 – 5.2)	4.9 (4.4 – 5.6)	0.015
	Creatinine (µmol/L)	381	62 (54 - 71)	65 (54 - 78)	0.13
Infection related factors					
	Heart rate (bpm)	360	72 (68 - 80)	82 (72 - 98)	<0.0001
	Haemoglobin (g/dL)	375	13.8 (12.7 – 14.7)	11.4 (10.0 – 13.0)	<0.0001
	Current infection at enrolment	377	3 (3%)[a]	57 (21%)	<0.0001
	TB		0 (0%)	2 (1%)	-
	Cryptococcal meningitis		0 (0%)	0 (0%)	-
	Pneumonia		0 (0%)	10 (4%)	-
	Gastroenteritis		1 (1%)	17 (6%)	-
	Malaria		2 (2%)	3 (1%)	-

		Complete cases	HIV-uninfected n=110 Median (IQR) or n (%)	HIV-infected n=279 Median (IQR) or n (%)	p value
Immune related factors					
	Lymphocytes (x10^9/L)	370	2.1 (1.6 – 2.6)	1.2 (0.8 – 1.7)	<0.0001
	Monocytes (x10^9/L)	323	0.30 (0.25 – 0.50)	0.40 (0.22 – 0.60)	0.053
	Absolute CD4 count (cells/µL)		-	41 (18 - 62)	-
	HIV viral load (log10 copies/mL)		-	5.06 (4.62 – 5.47)	-
	%CD4 Activated (CD38+HLADR+)	193	5% (3–9)	22% (11–34)	<0.0001
	%CD8 Activated (CD38+HLADR+)	290	11% (6–19)	34% (21–49)	<0.0001
	%CD4 Senescent (PD1+)	194	7% (4–9)	15% (9–24)	0.0001
	%CD8 Senescent (PD1+)	295	40% (27–53)	54% (44–64)	<0.0001
	Classical Monocytes ($CD14^{++}CD16^{-}$)	263	75% (65–81)	76% (66–83)	0.79
	Intermediate Monocytes ($CD14^{++}CD16^{+}$)	263	9% (7–14)	10% (6–13)	0.10
	Non-classical Monocytes ($CD14^{+}CD16^{+}$)	263	13% (10–22)	14% (9–21)	0.59

[a]Based on tests returned after enrolment at which point the participants reported, and physician confirmed, absence of infection.

[b]Waist:height ratio measures central obesity, a risk factor for metabolic syndrome. [47]

[c]Defined as either a past or current history of regular alcohol or smoking.

Table 2A. Predictors of carotid femoral Pulse Wave Velocity adult Malawians at enrolment

		Univariate analysis					Multivariate analysis including confounders N= 353 complete cases				Multivariate analysis including mediators and confounders n=335 complete cases			
		N evaluable	Fold change in cfPWV[b]	P value	95% CI		Fold change in cfPWV[b]	P value	95% CI		Fold change in cfPWV[b]	P value	95% CI	
	HIV	366	1.09	0.06	0.44	2.68	1.07	0.08	0.99	1.16	1.12	0.02	1.02	1.23
Potential confounders[a]														
	Age (per 10-year older)	366	1.23	<0.0001	1.18	1.27	1.18	<0.0001	1.14	1.23	1.18	<0.0001	1.13	1.23
	Female (vs male)	366	0.87	0.001	0.80	0.94	0.91	0.01	0.84	0.98	0.94	0.15	0.86	1.02
	Diastolic BP (per 10 mmHg higher)	353	1.13	<0.0001	1.09	1.18	1.09	<0.0001	1.04	1.13	1.07	0.001	1.03	1.13
Potential mediators[a]														
	Haemoglobin (per g/dL higher)	355	1.02	0.07	1.00	1.03	-	-	-	-	1.02	0.07	1.00	1.04
	Weight (per 10kg higher)	363	1.05	0.02	1.01	1.09	-	-	-	-	1.01	0.58	0.97	1.05
	Cholesterol (per mmol/L higher)	357	1.04	0.09	1.00	1.08	-	-	-	-	0.99	0.62	0.95	1.03
	Recent acute infection	364	1.11	0.08	0.99	1.25	-	-	-	-	1.09	0.11	0.98	1.22

Table 2B. Effect of the addition of T-cell exhaustion markers on the relationship between HIV and cfPWV

	Traditional risk factors model with HIV status added n=335				Traditional risk factors model with CD4 Exhaustion n=181				Traditional risk factors model with CD8 Exhaustion n=270			
	Fold change in cfPWV[b]	P value	95% CI		Fold change in cfPWV[b]	P value	95% CI		Fold change in cfPWV[b]	P value	95% CI	
Age (per 10-year older)	1.18	<0.0001	1.13	1.23	1.15	<0.0001	1.10	1.21	1.15	<0.0001	1.10	1.20
Female (vs male)	0.92	0.02	0.85	0.99	0.83	<0.0001	0.74	0.92	0.88	0.006	0.81	0.96
Diastolic BP (per 10mmHg higher)	1.07	0.001	1.03	1.13	1.10	0.01	1.04	1.17	1.10	<0.0001	1.05	1.15
Haemoglobin (per g/dL higher)	1.02	0.07	1.00	1.04	1.01	0.36	0.99	1.04	1.01	0.21	0.99	1.03
HIV infection	1.12	0.02	1.02	1.23	1.00	0.96	0.86	1.15	1.05	0.40	0.94	1.17
CD4 T-cell exhaustion (per 10% higher)	-	-	-	-	1.03	0.02	1.00	1.05	-	-	-	-
CD8 T-cell exhaustion (per 10% higher)	-	-	-	-	-	-	-	-	1.03	0.02	1.00	1.05

[a] see Supplementary Material Figure 2 for Directed Acyclic Graph and identification of confounders vs mediators. [b] log10 cfPWV was the outcome in linear regression models, providing model coefficients that correspond to fold (relative) changes when back transformed. Table 2A includes all relevant variables with p<0.2 for association with cfPWV in univariable analyses. Table 2B uses backwards elimination to select a final model from confounders and mediators in Table 2A and then considers additional effects of CD4 or CD8 exhaustion (similar effects in a model including both, n=178). There was no association between CD4/CD8 T-cell activation or CD4/CD8 senescence on cfPWV (p=0.77, 0.37, 0.98, 0.15 respectively)

Table 3. Factors associated with arterial stiffness at 44 weeks post ART initiation in HIV-infected adults

	Baseline factors only n=237				Baseline factors and change from baseline to week 44* n=174			
	Fold change	P value	95% Confidence Intervals		Fold change	P value	95% Confidence Intervals	
Baseline cfPWV (m/s) (per m/s higher)	2.70	<0.0001	1.83	4.00	1.15	<0.0001	1.09	1.22
Age (per 10 years older)	1.20	<0.0001	1.11	1.30	1.21	<0.0001	1.11	1.32
Baseline haemoglobin (per g/dL higher)	1.03	0.037	1.00	1.06	1.04	0.027	1.00	1.07
Baseline %CD8PD1+ (per 10% higher)	0.96	0.054	0.91	1.00	0.99	0.71	0.95	1.03
Change in %CD8PD1+ (per 10% higher) over 44 weeks	-	-	-	-	1.02	0.079	1.00	1.05
Change in %CD8 (per 10% higher) over 44 weeks	-	-	-	-	1.04	0.13	0.99	1.09

* including factors with univariable p<0.05 from Table 4

Table 4. Change in clinical and immune parameters and association with change in arterial stiffness 44 weeks post enrolment in HIV-infected adults

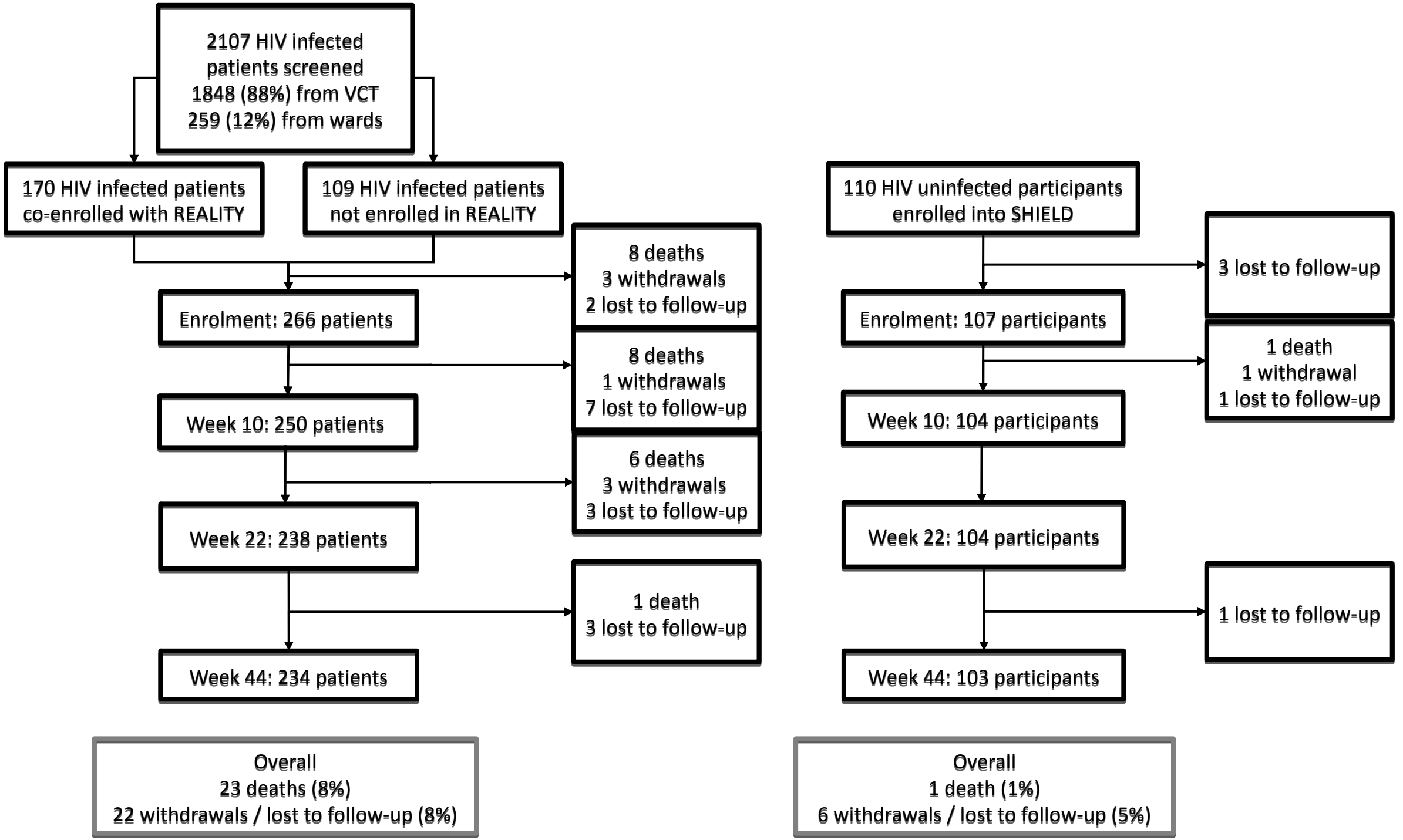
	Rho	P value
Δ Systolic BP (mmHg)	0.03	0.72
Δ Diastolic BP (mmHg)	0.07	0.29
Δ Weight (Kg)	-0.09	0.21
Δ Creatinine (µmol/L)	0.01	0.88
Δ Haemoglobin (g/dL)	0.03	0.72
Δ HIV viral load (copies/µL)	-0.08	0.28
Δ CD4 count (cells/µL)	-0.04	0.64
Δ %CD8	0.21	0.01
Δ CD4/CD8 ratio	-0.13	0.12
Δ %CD4 Activation	0.03	0.79
Δ %CD4 Exhaustion	0.14	0.22
Δ %CD4 Senescence	0.13	0.24
Δ %CD8 Activation	-0.03	0.72
Δ %CD8 Exhaustion	0.19	0.03
Δ %CD8 Senescence	0.04	0.67
Δ %Classical Monocytes	-0.16	0.07
Δ %Intermediate Monocytes	0.18	0.054
Δ %Non-classical Monocytes	0.01	0.91

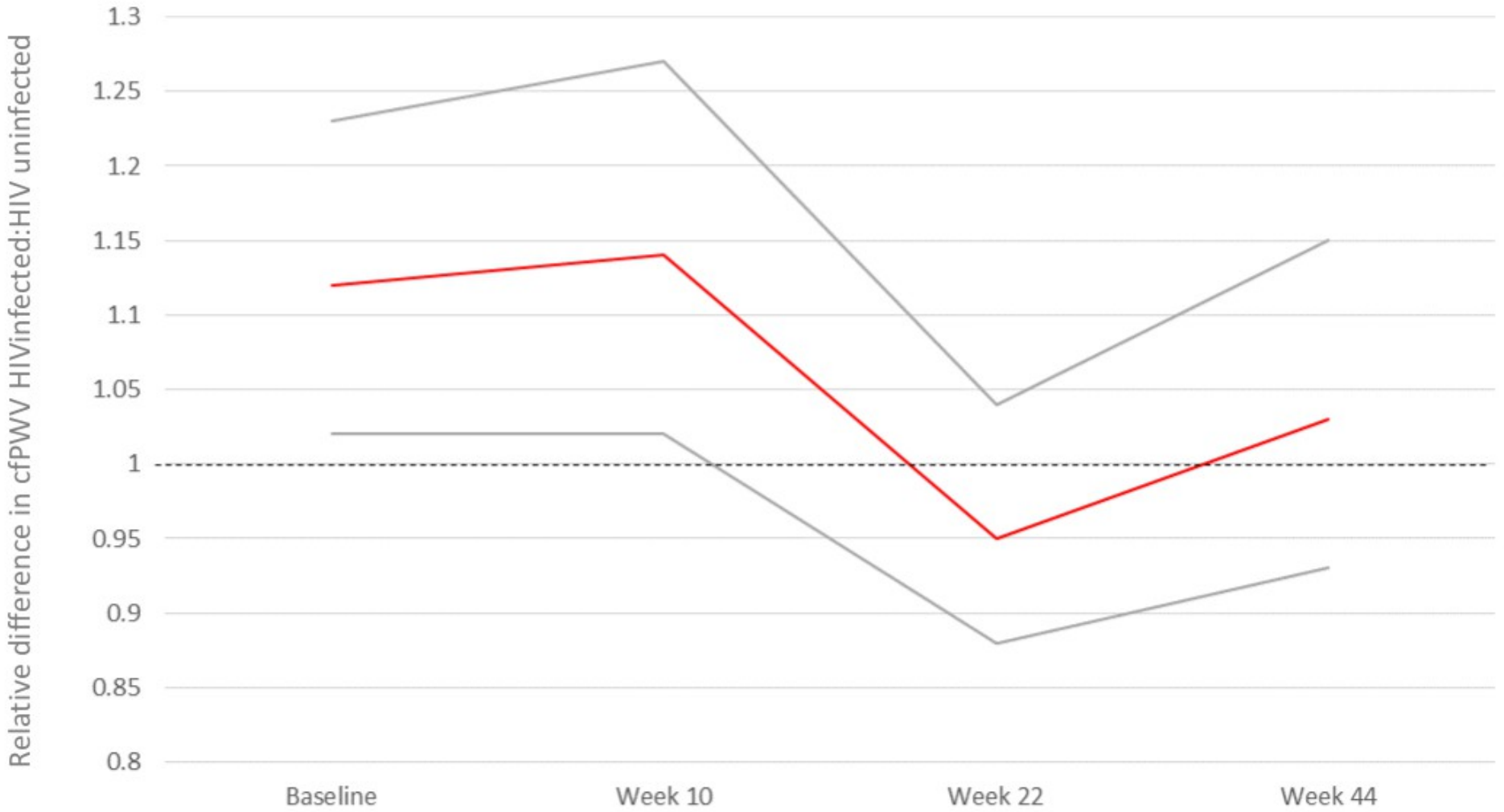
Figure 1. Recruitment Flow

Enrolment was carried out 2 weeks after screening

Figure 2. Adjusted effect of HIV on cfPWV over 44 weeks

Legend: The graph shows the effect of HIV on fold change in cfPWV adjusted for age, sex, diastolic blood pressure and haemoglobin. The same model is calculated for each individual timepoint. [a]The table below the graph shows the mean cfPWV and 95% confidence intervals according to HIV status at each timepoint.





	Baseline	Week 10	Week 22	Week 44
HIV infecteda	7.54 (7.36 – 7.72)	7.41 (7.21 – 7.61)	7.20 (7.02 – 7.39)	7.24 (7.04 – 7.45)
HIV uninfecteda	7.19 (6.92 – 7.45)	7.15 (6.89 – 7.41)	7.32 (7.05 – 7.58)	7.21 (6.93 – 7.50)