

Baseline Age is Independently Associated With the Rate of Arterial Stiffening Over 7 Years in a Zambian HIV Cohort

Longa Kaluba

`longa.banda@unza.zm`

University of Zambia

Theresa Chikopela

University of Zambia

Sepiso K Masenga

Mulungushi University

Benson M Hamooya

Mulungushi University

Fastone Goma

Center for Primary Care Research

Wilbroad Mutale

University of Zambia

Katongo H Mutengo

University of Zambia

Research Article

Keywords: HIV, arterial stiffness, pulse wave velocity, aging, Zambia, mixed-effects model, longitudinal

Posted Date: July 3rd, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-9939595/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Background

People living with HIV (PLWH) on antiretroviral therapy experience premature vascular aging. It remains unclear whether a baseline age of 50 years or older marks a linear progression of vascular aging or identifies a cohort experiencing steeper arterial stiffening.

Methods

This 7-year longitudinal study utilized data from 106 PLWH in Zambia. Annual measurements of age, CD4 + count, blood pressure (BP), and body mass index (BMI) were retrieved. Follow-up was restricted to years 1–7 to ensure adequate CD4 completeness ($> 85\%$). Estimated pulse wave velocity (ePWV) was derived using the Mattace-Raso equation. Linear mixed-effects models compared ePWV trajectories between baseline age groups (< 50 years, $n = 65$; ≥ 50 years, $n = 41$), adjusting for sex, BMI, and CD4 count.

Results

Baseline demographics, such as weight, CD4 count, creatinine, duration on ART, pulse pressure, mean arterial pressure, and BMI, were similar between groups. The ≥ 50 group commenced follow-up with a significantly higher median ePWV than the younger group (9.57 vs. 7.21 m/s, $p < 0.001$). After covariate adjustment, the ≥ 50 group stiffened 0.107 m/s faster per year than the younger cohort (95% CI: 0.033–0.181, $p = 0.005$), widening the ePWV gap to 3.01 m/s by year 6. BMI and male sex were independently significant predictors of steeper trajectories, while the female interaction was non-significant.

Conclusions

In Zambian PLWH, ≥ 50 years is independently associated with steeper ePWV trajectories. Cardiovascular screening and management of both BP and BMI should be integrated into routine HIV care well before patients reach this age.

INTRODUCTION

The rapid scale-up of antiretroviral therapy (ART) has fundamentally transformed HIV from a terminal illness into a manageable chronic condition. This success is particularly evident in East and Southern Africa, a region that bears over 68% of the global HIV burden [1] where expanding treatment access has led to a burgeoning population of older people living with HIV (PLWH). Within this regional context, Zambia presents a complex landscape; HIV prevalence remains significant but highly heterogeneous,

with district-level rates ranging from 4.2% to 23.5% [1]. However, this demographic shift has introduced a new clinical frontier, the management of non-communicable diseases (NCDs) in a population already predisposed to premature physiological decline.

Recent regional data, notably from South Africa, indicates that a vast majority of older PLWH are now managing at least one comorbid condition [2]. Crucially, the burden of disease has shifted away from traditional AIDS-related opportunistic infections toward "lifestyle" diseases typically associated with chronological aging, with hypertension and diabetes emerging as the most prevalent [3–5]. This trend is not confined to the elderly; younger PLWH are increasingly presenting with similar NCD patterns, necessitating lifelong dual-treatment regimens [2, 6]. This transition to multi-morbidity threatens to compromise long-term medication adherence, diminish quality of life, and substantially increase the per-capita cost of public health delivery [2].

Central to this multi-morbidity profile is accelerated vascular aging. Arterial stiffness, often quantified by Pulse Wave Velocity (PWV), serves as a robust proxy for cumulative cardiovascular risk and end-organ damage [7, 8]. An increase of 1 m/s in aortic PWV causes a 14%, 15%, and 15% increase in total CV events, CV mortality, and all-cause mortality, respectively [7, 9]. Carotid-femoral pulse wave velocity (cfPWV) is the gold standard for assessing arterial stiffness, but its reliance on specialized tonometry equipment limits its use in resource-constrained settings [7, 8]. Conversely, estimated PWV (ePWV) offers a validated, highly scalable surrogate. Mathematically derived from routine clinical metrics - age and mean blood pressure (MBP) - ePWV provides a population-level statistical projection of an individual's vascular aging trajectory rather than a direct anatomical measurement. Crucially, longitudinal validation studies confirm that ePWV correlates robustly with cfPWV and exhibits comparable prognostic power for predicting cardiovascular mortality at the cohort level [10]. Utilizing ePWV within large longitudinal cohorts thus provides a powerful, practical framework for characterizing shifting vascular phenotypes across distinct clinical strata over time.

While it is established that both HIV and aging independently contribute to vascular remodeling, the longitudinal trajectory of this "vascular aging-gap" - particularly in resource-limited settings like Zambia - remains poorly defined. It is unclear whether the crossing of the 50-year age threshold, a midlife age classification [11], represents a linear progression or a point of accelerated vascular aging. This study utilizes a 10-year longitudinal dataset from a Zambian cohort to characterize the trajectories of estimated PWV (ePWV) across two age strata (under 50 and over 50 years). By adjusting for risk factors such as BMI and CD4 count, we aim to determine the independent contribution of age status to the rate of arterial stiffening. Understanding these trajectories is vital for developing integrated care models that can address the mounting NCD burden within the aging HIV population.

METHODS

Study design

This was a retrospective longitudinal study conducted at a first-level hospital in Monze, Zambia. Participants were PLWH enrolled in the HIV clinic and maintained on ART, with serial clinical records spanning up to 24 annual visits.

Ethics Statement

This study received ethical clearance from the Mulungushi University School of Medicine and Health Sciences Research Ethics Committee (REC Ref: SMHS-MU4-2025-24; IORG: 0010344; IRB: 00012281; FWA: 0002888) and was officially authorized by the Zambia National Health Research Authority (NHRA) (Authorization No: NHRA-3084/26/12/2025). All protocols involving human subjects strictly adhered to institutional guidelines, national statutory regulations, and the ethical tenets of the Declaration of Helsinki.

Consent to participate

The Mulungushi University School of Medicine and Health Sciences Research Ethics Committee waived the requirement for informed consent to participate.

Consent for publication

Not applicable, as no identifiable data or images are present within the manuscript.

Study Population

A total of 106 participants with complete baseline data were included in the primary analysis with 65 in the baseline age <50 group (ranging from 35-49 years) and 41 in the baseline age ≥50 group (ranging from 50-84 years). The analysis was restricted to study years 1–7, the period during which CD4 count data exceeded 85% completeness across all participants. Beyond year 7 CD4 availability fell to 64%, introducing substantial missingness in a key covariate. Inclusion required at least one valid blood pressure measurement per year on record with participants with no valid BP recordings being excluded.

Data Analysis

Since direct carotid-femoral PWV (cfPWV) measurement was not available in this resource-limited setting, ePWV was derived using the validated Mattace-Raso reference equation:

$$ePWV = 9.587 - 0.402(age) + 0.004560(age^2) - 0.00002621(age^2 \times MAP) + 0.003176(MAP \times age) - 0.01832(MAP)$$

where MAP (mean arterial pressure) = $DBP + 0.4 \times (SBP - DBP)$, and age was updated annually across visits.

Pre-specified covariates in the primary adjusted model were BMI (kg/m^2 , time-varying), sex (male/female, time-invariant), and CD4 count ($\text{cells}/\mu\text{L}$, time-varying). Duration on ART was included as an additional covariate in sensitivity analysis SA-5.

Baseline characteristics were compared between groups using the Mann-Whitney U test for continuous variables (all non-normally distributed on Shapiro-Wilk testing) and Pearson's chi-squared or Fisher's exact test for categorical variables. Data were structured in long format and declared as a panel using xtset in Stata 15. Longitudinal trajectories were analysed using linear mixed-effects models (REML), which account for repeated measurements within the same participant and allow each individual to have their own baseline ePWV level and rate of change over time. The key result was the interaction between age group and time, which measured whether the baseline age ≥ 50 group stiffened at a faster annual rate than the baseline age < 50 group, independent of other covariates. Unstructured random-effects covariance was specified and validated in sensitivity analysis SA-6.

Power Calculation

A post-hoc power calculation was performed to evaluate the study's capacity to detect the observed divergence in annual arterial stiffening rates between the baseline age cohorts ($n = 65$ for < 50 years; $n = 41$ for baseline age ≥ 50 group). Based on the true random-slope standard deviation derived from the mixed-effects model ($SD = 0.109$) and a two-sided alpha of 0.05, the sample size of 106 participants provided 99.8% power to detect the adjusted difference in longitudinal slopes of 0.107 m/s per year.

Sensitivity Analysis

Nine pre-specified sensitivity analyses were conducted to test the robustness of the primary findings. These examined whether results held in participants with at least 71% within the 7-year window with ≥ 5 valid ePWV (SA-1) and after accounting for missing values through multiple imputation (SA-2) with 20 imputations. SA-3 restricted the sample to participants with at least three follow-up visits, and SA-4 used actual calendar time rather than visit number as the time variable. SA-5 compared alternative covariate specifications using model fit criteria, and SA-6 tested whether the random-effects structure of the primary model was appropriate. SA-7 tested whether the rate of arterial stiffening accelerated non-linearly over time. SA-8 tested the rate of arterial stiffening with categorized CD4 counts and SA-9 consisted of an age-restricted cohort validation designed to eliminate mid-study crossovers.

Exploratory analyses included sex-stratified trajectory models and a model incorporating visit-level hypertension as a time-varying covariate ($SBP \geq 140$ mmHg or $DBP \geq 90$ mmHg). Statistical significance was set at $p < 0.05$ (two-tailed). All coefficients are reported as unstandardised β with 95% confidence intervals derived from delta-method standard errors.

RESULTS

The study included 106 participants (65 baseline age < 50 , 41 baseline age ≥ 50). The two groups were well-matched at baseline with no statistically significant differences in weight, CD4 count, creatinine, duration on ART, SBP, diastolic BP, pulse pressure, MAP, or BMI. Sex distribution was similar (female: 55% vs 59%, $p = 0.75$). At baseline, the two age groups demonstrated highly comparable clinical profiles: NNRTI-based ART adherence was nearly universal (97% in those < 50 years vs. 95% in those ≥ 50 years),

and both groups maintained an identical mean follow-up duration of approximately 13.9 years ($p=0.64$). Baseline ePWV was significantly higher in the baseline age ≥ 50 group (median 9.57 vs 7.21 m/s, $p<0.001$) (Table 1).

Table 1. Clinical and Demographic Participant Characteristics – median (IQR)

Variable	Baseline Age <50 yrs (n=65)	Baseline age ≥ 50 yrs (n=41)	p-value
Weight, kg	62.5 (53, 75)	59.5 (51, 65)	0.12
Height, cm	164 (165, 170)	166 (160, 170)	1.00
Viral Load	20 (0, 20)	20 (17, 20)	0.29
Plasma Creatinine, $\mu\text{mol/L}$	106.0 (94.1, 122.2)	108.2 (86.4, 133.4)	0.85
CD4 count	200 (126, 307)	226 (109, 326)	0.59
Duration on ART, years	13.9 (12.9, 15.8)	13.8 (12.6, 16.4)	0.69
SBP (baseline), mmHg	120 (104, 132)	120 (110, 137)	0.26
DBP (baseline), mmHg	71 (65, 84)	77 (68, 85)	0.76
Pulse pressure (baseline), mmHg	43 (38, 56)	50 (40, 58)	0.44
BMI, kg/m^2	23.3 (19.5, 26.6)	21.2 (19.5, 23.7)	0.15
ePWV (baseline), m/s	7.21 (6.55, 8.01)	9.57 (8.12, 11.13)	<0.001
Baseline ART regimen: NNRTI base combination therapy*, n (%)	63 (97%)	39 (95%)	0.64

*All continuous variables non-normally distributed (Shapiro-Wilk $p<0.05$ except DBP and height). Values presented as median (IQR). Categorical variables compared using Pearson chi-squared or Fisher's exact test. *All participants were on NNRTI-based triple therapy (TLE or TDF-NVP)*

ePWV Trajectories; Unadjusted

Both groups show a rising trajectory with the baseline age ≥ 50 cohort demonstrating a visually steeper progression. Figure 1a displays the unadjusted longitudinal trajectories of estimated PWV over the 7-year follow-up period, stratified by age group at ART enrolment. While both groups show an age-related increase in arterial stiffness, the ≥ 50 years cohort demonstrates a visually steeper progression compared to the <50 years group.

Regression analysis of factors with ePWV Trajectory

In the unadjusted random-effects model, the baseline age ≥ 50 group had a significantly higher baseline ePWV (+2.34 m/s, 95% CI 1.73–2.96, $p<0.001$) and a significantly steeper annual rate of increase

(+0.092 m/s/year interaction term, 95% CI 0.02–0.17, p=0.02). The marginal annual rate of ePWV change was 0.14 m/s/year in the baseline age <50 group and 0.23 m/s/year in the baseline age ≥50 group.

After adjustment for BMI, sex, and CD4 count in a linear mixed-effects model, the divergent trajectory between age groups remained statistically significant (p=0.01). The baseline age ≥50 group commenced follow-up with an ePWV 2.37 m/s higher than the baseline age <50 group (95% CI 1.82–2.93, p<0.001) (Table 2). The interaction term, representing the differential annual rate of ePWV change, was +0.107 m/s per year (95% CI 0.03–0.18, p=0.01). By year 7, the predicted ePWV was 8.05 m/s in the baseline age <50 group and 11.06 m/s in the baseline age ≥50 group, a gap of 3.01 m/s compared to the baseline difference of 2.37 m/s.

BMI was a significant independent predictor of ePWV (+0.04 m/s per kg/m², p=0.02). Male sex was independently associated with higher ePWV (+0.66 m/s, p=0.02). CD4 count was significant (β=+0.00039 per cell/μL, p=0.03).

Table 2. Primary Adjusted ePWV Trajectory Model; Linear Mixed-Effects (REML, Unstructured Covariance)

Predictor Variable	β Coefficient	95% CI	p-value
Fixed Effects			
Baseline age ≥ 50 group; baseline difference	+2.37	1.82, 2.93	<0.001
Study year; baseline age <50 group slope (per year)	+0.10	0.05, 0.15	<0.001
Baseline age $\geq 50 \times$ Study year interaction (acceleration factor)	+0.11	0.03, 0.18	0.01
BMI (per kg/m ²)	+0.04	0.01, 0.07	0.02
Sex; Male (ref: Female)	+0.66	0.12, 1.21	0.02
CD4 count (per cell/ μ L)	+0.00039	0.000031, 0.00075	0.03
Intercept	+6.10	5.21, 6.99	<0.001
Random Effects			
Var (random slope; study year)	0.01	0.01, 0.03	-
Var (random intercept)	1.71	1.24, 2.36	-
Covariance (slope, intercept)	0.02	-0.04, 0.08	-
Var (residual)	0.51	0.45, 0.58	-
Model Fit			
Observations / Groups	653 / 102	-	-
Log restricted-likelihood	-904.63	-	-
Wald $\chi^2(6)$	169.77	-	<0.001

β = unstandardised REML coefficient. 95% CI from delta-method SEs. Reference: baseline age <50, Female sex. Blue shading = primary interaction term. N=653 obs with complete covariate data. SBP excluded – embedded in ePWV via MAP in the Mattace-Raso equation.

The baseline age ≥ 50 group's predicted ePWV exceeded 10 m/s from year 1 of follow-up onwards.

Blood Pressure Trajectories

Pulse pressure and SBP trajectories were modelled separately to characterise secondary vascular outcomes. Table 3 presents the pulse pressure model and Table 4 the SBP model. Pulse pressure showed a non-significant baseline group difference (baseline age ≥ 50 group 3.69 mmHg higher, $p=0.16$) but a significantly steeper annual increase in the baseline age ≥ 50 group (+1.21 mmHg/year, $p=0.04$). By

year 7, the baseline age <50 group's predicted pulse pressure was approximately 47.9 mmHg and the baseline age ≥50 group approximately 58.9 mmHg.

Table 3. Pulse Pressure Trajectory Model (Unadjusted); Linear Mixed-Effects

Predictor Variable	β Coefficient	95% CI	p-value
Fixed Effects			
Baseline age ≥50; baseline difference	+3.69	−1.40, 8.78	0.16
Study year; baseline age <50 group slope (per year)	+0.20	−0.52, 0.91	0.59
Baseline age ≥50 × Study year interaction	+1.21	0.06, 2.36	0.04
Intercept	+46.76	43.59, 49.93	<0.001
Model Fit			
Observations / Groups	742 / 106	-	-
Wald $\chi^2(3)$	17.14	-	0.001

β = unstandardised REML coefficient. Reference: baseline age <50. Predicted PP at year 6: baseline age <50 ~47.9 mmHg; baseline age ≥50 ~58.9 mmHg.

SBP at baseline did not significantly differ between groups (p=0.25), and the group × time interaction did not reach significance (+0.99 mmHg/year, p=0.16). By year 7, the baseline age <50 group's predicted SBP was approximately 127.6 mmHg and the baseline age ≥50 group approximately 138.1 mmHg (Table 4).

Table 4. Systolic Blood Pressure Trajectory Model (Unadjusted); Linear Mixed-Effects

Predictor Variable	β Coefficient	95% CI	p-value
Fixed Effects			
Baseline age ≥50 group; baseline difference	+4.54	−3.22, 12.30	0.25
Study year; baseline age <50 group slope (per year)	+0.79	−0.07, 1.66	0.07
Baseline age ≥50 × Study year interaction	+0.99	−0.40, 2.39	0.16
Intercept	+122.88	118.05, 127.70	<0.001
Model Fit			
Observations / Groups	742 / 106	-	-
Wald $\chi^2(3)$	16.24	-	0.001

β = unstandardised REML coefficient. Reference: baseline age <50. Baseline group difference non-significant ($p=0.25$); slope divergence non-significant ($p=0.16$) over the 7-year window.

Spearman's correlation between ePWV and pulse pressure was moderate in both groups (baseline age <50: $\rho=0.47$, $p<0.001$; baseline age ≥ 50 : $\rho=0.50$, $p<0.001$).

Effect of Hypertension on the Vascular Baseline Age <50-Gap

To explore the synergistic effect of hypertension and baseline age <50, Figure 1d presents predicted ePWV trajectories at fixed SBP levels of 110 mmHg (controlled) and 150 mmHg (uncontrolled). The vascular baseline age <50-gap between the two age groups was approximately 2.43 m/s by year 7 regardless of SBP level.

When visit-level hypertension was incorporated as a time-varying covariate ($SBP\geq 140$ or $DBP\geq 90$), it independently added a clinically substantial increment to ePWV per hypertensive visit ($p<0.001$). Crucially, the group interaction term remained significant at $p<0.001$ after this adjustment, confirming that the steeper stiffening in the baseline age ≥ 50 group operates independently of whether hypertension is present at any given visit.

SENSITIVITY ANALYSES

Nine pre-specified sensitivity analyses were conducted. Table 5 summarises the key interaction term across SA-1 and SA-2.

Table 5. Sensitivity Analyses; SA-1 Complete-Case and SA-2 Multiple Imputation

Predictor Variable	β Coefficient	95% CI	p-value
Fixed Effects (SA-1: ≥ 5 valid ePWV observations, $n=742$, 106 groups)			
Baseline age ≥ 50 group (≥ 50 yrs); baseline difference	+2.37	1.82, 2.93	<0.001
Study year slope (baseline age <50 group)	+0.10	0.05, 0.15	<0.001
Baseline age $\geq 50 \times$ Study year interaction	+0.11	0.03, 0.18	0.01
BMI	+0.04	0.01, 0.07	0.02
Sex; Male	+0.66	0.12, 1.21	0.02
CD4 count	+0.00039	0.000031, 0.00075	0.03
Fixed Effects (SA-2: Multiple Imputation, 20 imputations, $n=742$)			
Baseline age ≥ 50 group (≥ 50 yrs); baseline difference	+2.42	1.85, 2.98	<0.001
Study year slope (baseline age <50 group)	+0.12	0.08, 0.17	<0.001
Baseline age $\geq 50 \times$ Study year interaction	+0.10	0.03, 0.17	0.01
BMI	+0.04	0.01, 0.08	0.02
Sex; Male	+0.62	0.08, 1.16	0.03
CD4 count	+0.000265	-0.000099, 0.000630	0.15

SA-1: all 106 participants have 7 valid ePWV observations ($\text{max_epwv_obs}=7$) within the restricted 7-year window – SA-1 is therefore identical to the primary model for this dataset. SA-2: 20 chained-equations imputations for missing BMI and CD4. Both confirm the primary interaction finding. SA-2 interaction: +0.10 ($p=0.01$).

SA-6 confirmed that the unstructured random-effects covariance structure provided significantly better model fit than the exchangeable alternative ($p<0.001$) and produced a lower AIC. SA-7 showed that AIC marginally favoured a quadratic time trend over the linear model; the quadratic group interaction term was negative and statistically significant. The linear model was retained as the primary model.

SA-8 evaluated non-linear threshold effects of immune status on baseline ePWV by categorizing CD4 counts into clinical strata, utilizing the >500 cells/ μL group as the reference baseline. Compared to this reference, individuals within the moderate immunosuppression stratum (200–500 cells/ μL) exhibited significantly lower baseline ePWV ($\beta = -0.24$ m/s, $p = 0.01$), an effect even more pronounced in the severe immunosuppression stratum (<200 cells/ μL ; $\beta = -0.54$ m/s, $p < 0.01$). This step-wise gradient confirms that the positive linear association seen in our primary model (+0.00039 m/s per cell/ μL) is driven by the vascular transition *out* of severe immunosuppression, supporting the hypothesis that

quantitative immune recovery tracks with a greater burden of chronic vascular remodeling (Supplementary Table).

SA-9 To address the potential confounding effect of participants crossing the age-50 threshold during the 7-year follow-up, a sensitivity analysis was conducted by restricting the younger comparison cohort to individuals aged ≤ 42 years at baseline. This resulted in a restricted sub-cohort of 91 unique participants (50 in the ≤ 42 cohort and 41 in the ≥ 50 cohort), generating 182 total longitudinal observations. This restriction guaranteed that all individuals within the younger comparison group remained strictly under 50 years of age for the entirety of the 7-year follow-up. Within this 'pure' younger cohort, the longitudinal trajectory of arterial stiffness was statistically stable ($\beta = 0.07$ m/s per year, SE = 0.05, $p = 0.11$). Crucially, the interaction between age group (≥ 50 vs. ≤ 42) and time remained robust and statistically significant ($\beta = 0.13$ m/s per year, SE = 0.06, $p = 0.02$). This demonstrates that the older HIV-positive cohort exhibited a significantly accelerated trajectory of vascular aging ($\beta = 0.20$ m/s per year) compared to the younger cohort, confirming that the primary findings are robust, entirely data-driven, and not an artifact of age-threshold cross-overs (Supplementary Table).

EXPLORATORY ANALYSES

Sex-stratified models revealed that the trajectory divergence between age groups was significant in males but non-significant in females ($p=0.07$). Notably, the baseline ePWV gap was substantially larger in females (+3.74 m/s) than in males (+1.61 m/s). These sex-specific findings are presented in Table 6.

Table 6. Exploratory Analysis; Sex-Stratified ePWV Trajectory Models

Predictor Variable	β Coefficient	95% CI	p-value
<i>Fixed Effects; Males</i>			
Baseline age ≥ 50 group; baseline difference	+1.61	0.95, 2.27	<0.001
Study year slope	+0.13	0.12, 0.15	<0.001
Baseline age $\geq 50 \times$ Study year interaction	+0.05	0.03, 0.08	<0.001
BMI	+0.004	0.001, 0.006	<0.01
CD4 count	-0.000140	-0.000182, -0.000099	<0.001
<i>Fixed Effects; Females</i>			
Baseline age ≥ 50 group; baseline difference	+3.74	2.62, 4.86	<0.001
Study year slope	+0.13	0.10, 0.16	<0.001
Baseline age $\geq 50 \times$ Study year interaction	-0.05	-0.09, 0.004	0.07
BMI	+0.012	0.009, 0.015	<0.001
CD4 count	-0.000279	-0.000331, -0.000226	<0.001

Amber = non-significant interaction in females ($p=0.07$). Male interaction +0.05 m/s/year ($p<0.001$); female interaction -0.05 m/s/year ($p=0.07$). The vascular baseline age <50-gap trajectory is male-driven. Sex excluded as covariate in stratified models.

Duration on ART was not a significant predictor of ePWV in sensitivity analysis SA-5 ($\beta=-0.03$ m/s per year of ART, 95% CI -0.13 to +0.08, $p=0.63$).

DISCUSSION

The results of this 7-year longitudinal study show that individuals over the age of 50 at ART enrolment experience a 0.11 m/s faster annual increase in arterial stiffness than their younger counterparts, after adjustment for BMI, sex, and CD4 count. We further note that the rate of longitudinal vascular aging is not uniform across the lifespan, but exhibits a significant acceleration among individuals enrolling in care after the fifth decade of life.

This steeper trajectory of stiffening must be interpreted within the shifting clinical landscape of SSA. As ART continues to extend the lifespan of PLWH, the primary threat to long-term health has pivoted from opportunistic infections to non-communicable diseases (NCDs). The fact that the baseline age ≥ 50 group in our study began with an ePWV 2.37 m/s higher than the younger cohort underscores a

substantial loss of vascular reserve that occurs even before long-term follow-up begins. By year 7, this gap had widened to 3.01 m/s, confirming progressive divergence.

A critical insight from our analysis is that this aging-gap is not driven by blood pressure alone. When predicting ePWV at fixed SBP levels of 110 and 150 mmHg, the group divergence remained identical in magnitude, hypertension shifts both trajectories upward in parallel rather than selectively accelerating the older group. This suggests that hypertension may not merely add to the burden of HIV-related vascular damage but acts as an amplifier of a pre-existing gap. Given that a mere 2.0 mmHg increase in SBP can raise lifetime stroke mortality by 10% [12], the steeper trajectory of stiffening observed in our over-50 group suggests a critical window for intervention. Especially since a 5.0 mmHg reduction has been shown to decrease overall CVD events and all-cause mortality [12].

An important new finding in this analysis is the independent significance of BMI as a predictor of ePWV (+0.04 m/s per kg/m², p=0.02). In earlier analyses that included SBP as a covariate, BMI was non-significant, a consequence of SBP's strong association with both MAP (used in the ePWV equation) and BMI. With SBP removed, BMI's independent contribution to vascular stiffening is unmasked. This is consistent with evidence from SSA cohorts showing that adiposity contributes independently to arterial stiffening even in lean populations [13]. CD4 count was also independently significant in this model (p=0.03), suggesting that immunological recovery is a meaningful, if modest, vascular predictor when BP-related collinearity is removed.

Our exploratory sex-stratified analyses reveal that the steeper trajectory of stiffening observed in the older cohort is more pronounced among male participants. In females, while the baseline age ≥50 group commenced follow-up with a substantially higher ePWV (+3.74 m/s vs +1.61 m/s in males), the trajectories did not significantly diverge further over time (p=0.07). This pattern is consistent with broader evidence that the determinants of PWV differ by sex in African PLWH, a Tanzanian study found that HIV status and ART duration predicted PWV in men but not women, where metabolic and menopausal factors were more dominant [14]. The reversal of the sex advantage in arterial compliance with advancing age is well-established in the general population [14], and our findings suggest this dynamic may be modified by HIV in ways that warrant dedicated investigation. Sex-specific cardiovascular screening protocols within HIV care programmes may therefore be warranted [15].

Our sensitivity analysis identified a non-significant association between duration on ART and ePWV within the 7-year window (p=0.63), which contrasts with the protective signal observed in the earlier extended analysis. This discrepancy may reflect the restricted follow-up window, within which ART duration variation is insufficient to detect a modest protective effect. The protective signal from longer ART duration remains biologically plausible, supported by prospective evidence that ART initiation reduces arterial stiffness alongside inflammatory cytokines [16]. While regional data demonstrate that PLWH on long-term ART exhibit elevated oxidative stress, endothelial activation [17], and a higher augmentation index than HIV-negative controls [13], a longer duration of therapy may act to partially mitigate this underlying endothelial dysfunction over time. However, some studies have found a positive

association between longer ART duration and arterial stiffness, likely reflecting cumulative metabolic effects of older ART regimens [18]. The net vascular effect of ART duration likely depends on regimen type, metabolic profile, and degree of immune reconstitution. For Zambia's ART scale-up programme, the near-universal NNRTI distribution in our cohort provides a relatively homogeneous treatment context, limiting the ability to detect regimen-specific effects.

Intriguingly, our multivariable model demonstrated a small but statistically significant positive association between CD4 count and accelerated ePWV trajectories ($+0.00039$ m/s per cell/ μ L, $p=0.03$). Although higher CD4 counts are generally considered immunologically protective, similar paradoxical findings have been reported in treated longitudinal HIV cohorts and may reflect the vascular consequences of immune reconstitution. Persistent immune activation and systemic inflammation can continue despite successful ART and CD4 recovery, thereby sustaining cardiovascular risk in PLWH. Alternatively, this association may represent a legacy effect, whereby individuals with the greatest CD4 recovery are also those who initiated care with the lowest nadir CD4 counts and the greatest degree of pre-treatment vascular injury [19].

The significance of the 0.11 m/s annual acceleration factor reaches beyond physiology into health service delivery. As shown in the broader regional context, the trend of managing HIV alongside NCDs like hypertension is becoming the norm rather than the exception. For the Zambian public health sector, this implies a mounting economic burden and a logistical challenge in maintaining long-term adherence. For the younger cohort (under 50), our results are equally sobering. While their current rate of stiffening is slower, the early presence of NCD markers suggests they face decades of dual-medication therapy. The risk of "treatment fatigue" and the potential for polypharmacy to diminish quality of life cannot be overlooked.

Strengths/Limitations

A major strength of this study is the utilization of a robust, 7-year longitudinal dataset detailing the hemodynamic profiles of PLWH. Several limitations were noted in this study. First, ePWV is an estimation rather than directly measured using carotid-femoral tonometry. The Mattace-Raso equation was validated in a general population and its performance in sub-Saharan African HIV cohorts has not been formally established. Second, the analysis is restricted to 7 years due to declining CD4 data availability beyond year 7 (64% complete); the trajectory beyond this window cannot be reliably characterised. Third, study year was used as a proxy for calendar time rather than actual date-derived follow-up years, which may introduce imprecision. Fourth, the sample size was modest ($n=106$), limiting statistical power for subgroup analyses. Fifth, education and employment data had high rates of non-documentation ($>60\%$), precluding their use as covariates. Sixth, informative censoring cannot be excluded; participants with fewer visits may have died, transferred, or been lost to follow-up, potentially introducing survivor bias. Seventh, hypertension baseline captured no variation (all participants coded as not hypertensive at baseline), suggesting this variable did not reliably capture hypertension history and was excluded from models. Eighth, regimen class showed no discriminatory value given the near-universal NNRTI

distribution. Ninth, smoking status was not routinely recorded at baseline or during follow-up and could not be included as a covariate. Consequently, we cannot rule out residual confounding from tobacco use on the observed ePWV trajectories.

CONCLUSION

Ultimately, our findings advocate for a paradigm shift in HIV care at first-level hospitals in Zambia.

If the vascular aging gap widens progressively for patients enrolling into care after age 50, then cardiovascular screening and aggressive management of both blood pressure and BMI must be integrated into routine HIV care well before this trajectory of accelerated stiffening takes hold. The male-driven nature of trajectory divergence and the independent contributions of BMI and CD4 count provide additional targets for sex-specific and metabolic cardiovascular prevention strategies. Decelerating the accelerated vascular stiffening through early, targeted intervention remains the most viable strategy for reducing the long-term burden of cardiovascular events in this population.

Declarations

Data availability

The data that support the findings of this study are available from the corresponding author, L.K., upon reasonable request.

Acknowledgements

AI technology was used for language editing and to improve the overall flow of the manuscript.

Competing interests

The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article.

Author Contribution

LK conceived the study and designed the conceptual framework. KM, SM, and BM were responsible for data curation, project administration, and clinical investigations at the field site. LK and TC developed the methodology and performed the formal analysis and statistical modeling. LK drafted the original manuscript. KM, WM, and FG provided senior supervision throughout the project lifecycle. All authors contributed to the critical review and editing of the manuscript, and all have read and approved the final version for submission.

Funding Declaration

The authors received no financial support for the research, authorship, and/or publication of this article

References

1. Mweemba C, et al. Estimating district HIV prevalence in Zambia using small-area estimation methods (SAE). *Popul Health Metr.* 2022;20(1):8.
2. Roomaney RA, van Wyk B, Pillay-van V, Wyk. Aging with HIV: Increased Risk of HIV Comorbidities in Older Adults. *Int J Environ Res Public Health*, 2022. 19(4).
3. Fourie CMT, Schutte AE. Early vascular aging in the HIV infected: Is arterial stiffness assessment the ideal tool? *Virulence*. 2017;8(7):1075–7.
4. Koethe JR, et al. From Wasting to Obesity: The Contribution of Nutritional Status to Immune Activation in HIV Infection. *J Infect Dis.* 2016;214(Suppl 2):S75–82.
5. Masenga SK, et al. Patho-immune Mechanisms of Hypertension in HIV: a Systematic and Thematic Review. *Curr Hypertens Rep.* 2019;21(7):56.
6. Shah ASV, et al. Global Burden of Atherosclerotic Cardiovascular Disease in People Living With HIV: Systematic Review and Meta-Analysis. *Circulation*. 2018;138(11):1100–12.
7. 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(13):1318–27.
8. Fourie CMT, et al. Vascular function and cardiovascular risk in a HIV infected and HIV free cohort of African ancestry: baseline profile, rationale and methods of the longitudinal EndoAfrica-NWU study. *BMC Infect Dis.* 2020;20(1):473.
9. Zhong, Qi, et al. Carotid–Femoral Pulse Wave Velocity in the Prediction of Cardiovascular Events and Mortality: An Updated Systematic Review and Meta-Analysis. *Angiology*. 2017;69(7):617–29.
10. Greve SV, et al. Estimated carotid-femoral pulse wave velocity has similar predictive value as measured carotid-femoral pulse wave velocity. *J Hypertens.* 2016;34(7):1279–89.
11. UNAIDS. *PEOPLE AGED 50 YEARS AND OLDER*. 2014.
12. Drago F, et al. Characterizing blood pressure trajectories in people living with HIV following antiretroviral therapy: A systematic review. *HIV Med.* 2023;24(11):1106–14.
13. Kaluba L et al. Immune activation and arterial stiffness in lean adults with human immunodeficiency virus on antiretroviral therapy. *S Afr J HIV Med*, 2021. 22(1).
14. Karand JC, et al. Sex-dependent correlates of arterial stiffness in Tanzanian adults. *Trop Med Int Health.* 2021;26(11):1494–502.
15. Lu Y, et al. Global distributions of age- and sex-related arterial stiffness: systematic review and meta-analysis of 167 studies with 509,743 participants. *EBioMedicine.* 2023;92:104619.
16. Martínez-Ayala P, et al. Effect of antiretroviral therapy on decreasing arterial stiffness, metabolic profile, vascular and systemic inflammatory cytokines in treatment-naïve HIV: A one-year prospective study. *PLoS ONE.* 2023;18(3):e0282728.

17. Chikopela T, et al. Arterial stiffness is associated with oxidative stress and endothelial activation among persons with treated HIV in Zambia. *South Afr J HIV Med.* 2021;22(1):1298.
18. Lekakis J, et al. Association of highly active antiretroviral therapy with increased arterial stiffness in patients infected with human immunodeficiency virus. *Am J Hypertens.* 2009;22(8):828–34.
19. Dirajlal-Fargo S, Funderburg N. HIV and cardiovascular disease: the role of inflammation. *Curr Opin HIV AIDS.* 2022;17(5):286–92.

Figures

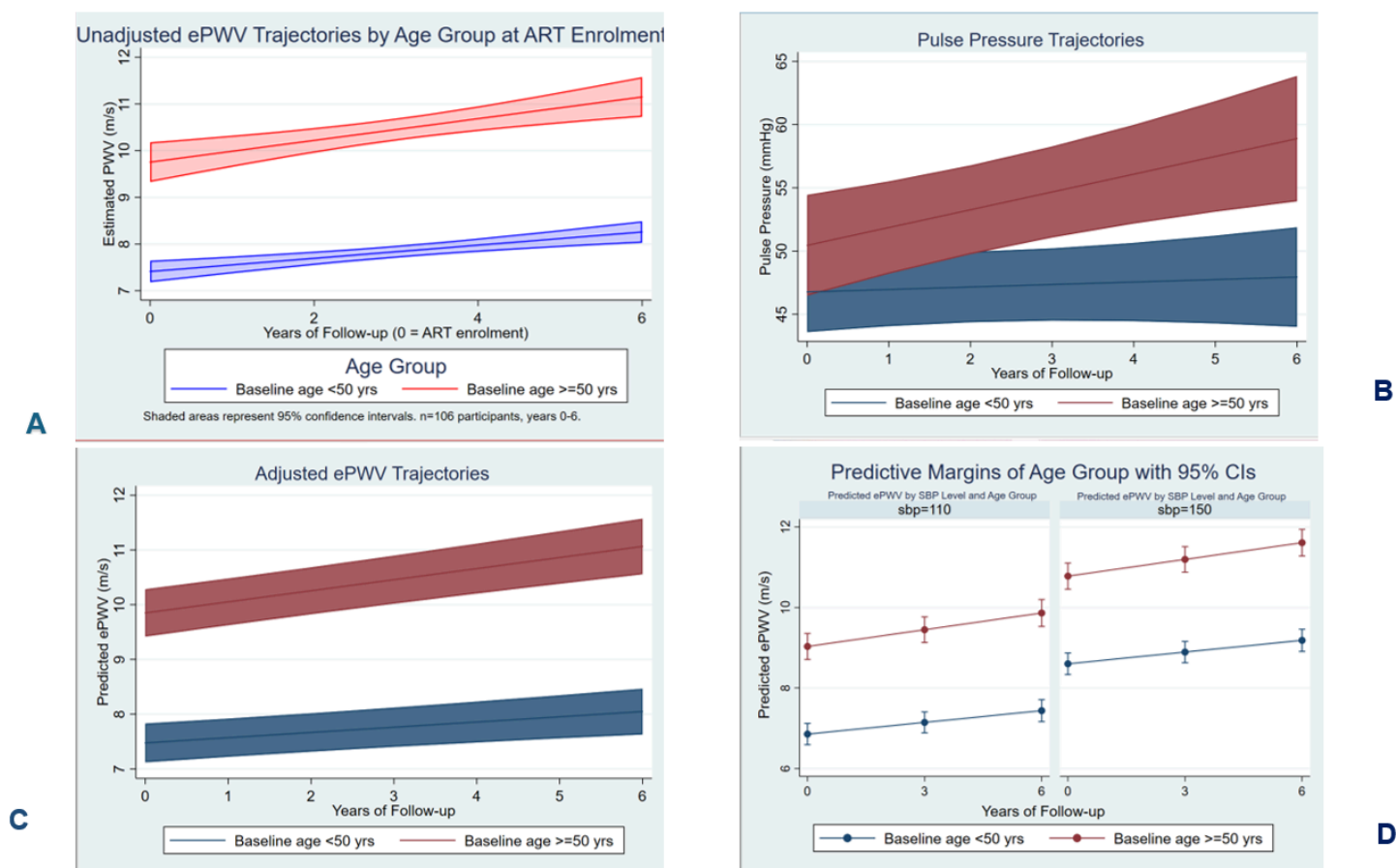


Figure 1

a. Unadjusted ePWV Trajectories by Age Group at ART Enrolment over 7 Years of Follow-up; b. Pulse Pressure Trajectories by Age Group over 7 Years of Follow-up; c. Adjusted ePWV Trajectories by Age Group (adjusted for BMI, sex, CD4); d. Predicted ePWV at Fixed SBP Levels (110 mmHg vs 150 mmHg) by Age Group and Year of Follow-up. Shaded areas = 95% confidence intervals. n=106 participants, study years 0–6.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SUPPLEMENTARYDATA.docx](#)