Measurement of retinal vessels as a biomarker of cerebrovascular ageing in older HIV positive men compared to controls

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Running title: Retinal vessel diameters in HIV

ABSTRACT

Background: To compare retinal vascular measurements, biomarkers of cerebral small vessel disease (SVD), in HIV positive men aged 50 years and above with similarly-aged HIV negative men and younger HIV positive men.

Methods: We recruited white, non-diabetic men to a cross-sectional substudy of a larger cohort including three demographically-matched groups. Optic disc centred 45° colour fundus photographs were used to calculate central retinal arterial and venous calibre and the arterial-venous ratio (AVR). We used univariate and multivariable linear regression to compare retinal vessel measurements in the three groups and to identify factors associated with AVR.

Results: All HIV positive men were virologically suppressed. In a multivariable model, study group was not associated with AVR (adjusted β 0.010 for HIV positive men <50 [n=39] compared to HIV positive men aged ≥50 years [n=120], 95% CI -0.018 to 0.038, p=0.47; adjusted β 0.00002 for HIV negative men ≥50 years [n=52], 95% CI -0.022 to 0.022, p=0.99). Factors associated with lower AVR were systolic BP (adjusted β -0.009 per +10 mmHg, 95% CI -0.015 to -0.003, p=0.002), history of stroke or transient ischemic attack (adjusted β -0.070, 95% CI -0.12 to -0.015, p=0.01), and recent recreational drug use (adjusted β -0.037, 95% CI -0.057 to -0.018, p=0.0002).

Conclusion: There were no differences in retinal vascular indices between HIV positive men aged ≥50 years and HIV negative men aged ≥50 years or HIV positive men aged <50 years, suggesting that HIV is not associated with an increased burden of cerebral SVD.

Key words: HIV; retinal vessels; cerebral small vessel diseases.

ORIGINAL ARTICLE

INTRODUCTION

Life expectancy of people living with HIV (PLWH) now approaches that of the general population in many countries,[1] and an increasing proportion of PLWH are over the age of 50 years.[2, 3] As the cohort ages, PLWH are at greater risk of comorbidities of older age such as cardiovascular disease, dementia, hypertension and type 2 diabetes. Cerebral small vessel disease (SVD) is associated with circulating inflammatory mediators and chronic immune activation in HIV negative populations.[4, 5] Corresponding elevations of systemic inflammatory markers are reported in HIV even after virological suppression has been achieved.[6, 7] It is plausible that HIV infection in middle and older age is associated with an increased risk of cerebral SVD. Cognitive impairment is reported to be highly prevalent in PLWH,[8-10] and SVD is one potential mechanism for this.

While epidemiological evidence suggests higher rates of large vessel ischaemic stroke in PLWH,[11-18] SVD is likely to involve different pathological processes, and there have been fewer studies of SVD in HIV. One study of Dutch HIV positive men aged over 45 and HIV negative controls reported a greater burden of white matter hyperintensities of presumed vascular origin on brain magnetic resonance imaging [19] whereas two similar studies have found no association between HIV and neuroimaging measures of SVD,[20, 21] and an autopsy study of SVD found similar negative results.[22] The retina shares common embryological and physiological features with the central nervous system, and retinal vascular photography provides a cheaper, quicker and non-invasive way of indirectly measuring SVD. In cross-sectional studies in the general HIV-negative population, retinal vascular abnormalities (measured quantitatively by vessel diameters or qualitatively by as retinopathy) have been found to be independently associated with the extent of white matter hyperintensities [23, 24] and the presence of dementia in

people with hypertension.[25] In cohorts, similar markers are independently associated with progression of cerebrovascular SVD [26] and ventricular enlargement.[27] All of these neuropathological phenomena are associated with vascular cognitive impairment.

To date, one published study has compared retinal vascular measures between PLWH and HIV negative controls.[28] The study was conducted in Cape Town, South Africa and recruited mainly young adult Xhosa women (75% female, median age 40 years, 19% of the study sample aged over 50). There were no overall differences in arteriolar or venular diameter between PLWH and controls. A US-based study also looked at retinal measures in PLWH but did not include a seronegative control group.[29] There is a need for studies employing retinal vascular measurements in other ethnic groups and in older, male participants who make up a larger proportion of PLWH in high-income countries and who are potentially at greatest risk of SVD. This study aimed to determine the association between HIV status, age and retinal vascular measurements in a United Kingdom-based sample of men living with HIV aged 50 years and over, and comparable groups of HIV negative men of similar age and HIV positive men aged under 50.

METHODS

Study population

We conducted a cross-sectional ophthalmic substudy within the Pharmacokinetic and Clinical Observations in People over Fifty (POPPY) study, a multicentre UK and Ireland cohort study exploring a range of age-associated measures of health, service use and pharmacokinetic parameters in PLWH (ClinicalTrials.gov Identifier: NCT01737047). POPPY comprises three demographically matched groups (PLWH aged $\geq$50 years; PLWH aged <50 years; HIV negative persons aged $\geq$50 years) in a 2:1:1 ratio and includes both white and black African participants. White, male, non-diabetic participants were recruited between May 2014 and July 2016 in

London and Brighton, UK, in the same proportions as the parent cohort. Participants gave written, informed consent and the study was approved by the National Research Ethics Service Committee London – Camden & Islington, reference 14/LO/0316.

Sample size calculations were based on observations from a previous study,[28] where the arteriolar diameter in participants over 50 was mean 159.13, standard deviation (SD) 17.63 in PLWH and mean 166.24, SD 18.56 in HIV negative participants. Assuming that similar differences existed in the target population, and maintaining the 2:1:1 ratio, we aimed to include 156 HIV positive men aged ≥50 years and 78 in each of the other two groups. This would achieve 80% power and an alpha level of 5% (STATA Statistics and Data Analysis software version 14.1, StataCorp, Texas, USA).

Data collection

Optic disc-centred 45 degree colour fundus photographs were taken at three study sites and uploaded to a central site (Moorfields Eye Hospital). In the first instance this was done with undilated pupils, and image quality was assessed in real time to determine the need for tropicamide-induced dilatation and repeat photography. Techniques for measurement and calculation of CRAE and central retinal vein equivalent (CRVE) and the arterial-venous ratio (AVR) have been reported previously.[30, 31] Diameters of all vessels crossing a zone 0.5 to 1 disc diameter from the disc margin were assessed by the same certified and validated grader with image analysis software. The calibres of the six largest arterioles and the six largest venules were combined to estimate the calibre of the central retinal artery and vein. The AVR, a ratio of these two measures, was calculated for each eye.

The POPPY cohort database provided clinical information relating to previous cardiovascular disease and risk factors, current and previous antiretroviral therapy use, latest CD4+ and CD8+ lymphocyte counts, anthropometric data, syphilis and hepatitis C virus serology, recreational

drug use and serum lipids. Framingham 10-year cardiovascular disease risk was calculated using the *framingham* STATA module.[32, 33]

Statistical analysis

After excluding images of insufficient quality, retinal measurements from one randomly-chosen eye per participant were compared between the three groups (HIV positive ≥50, HIV positive <50, HIV negative ≥50) using linear regression. PLWH who did not have a suppressed HIV-1 viral load (<50 copies/mL) on antiretroviral therapy were excluded from analysis. Factors associated with AVR were determined in bivariate models. Variables considered included study group as three categories, linear continuous variables (body mass index, waist circumference, diastolic and systolic blood pressure, and concentrations of total, high density lipoprotein and low density lipoprotein cholesterol) and binary variables (smoking status, self-reported history of ischaemic heart disease, stroke or transient ischaemic attack [TIA], injection drug use, psychoactive recreational drug use in the past 6 months, hepatitis C antibody status, and previous syphilis). Study group and factors found to have at least a modest association ($p<0.2$) with AVR in bivariate models were analysed in multivariable linear regression models.

Additional linear regression analyses including only HIV positive participants explored associations between AVR and HIV-specific factors (linear continuous variables of CD4+ and CD8+ lymphocyte counts and CD4:CD8 ratio, time since HIV diagnosis, time between diagnosis and first initiation of antiretroviral therapy, and total duration of antiretroviral therapy, and binary categorisation of current use of all antiretroviral drug classes as well as abacavir and tenofovir), adjusted for study group (older or younger than 50 years). Again, a multivariable model was constructed of all variables found to be at least weakly associated ($p<0.2$) with AVR in bivariate models.

RESULTS

Participant characteristics

In total 228 people were photographed. Of these, grading was successfully done on images from 211 participants (411 eyes; 120 HIV positive aged ≥50 years; 39 HIV positive <50 years; 52 HIV negative ≥50 years). Reasons for exclusion of the other 17 were missing data (n=6), incorrect field definition used during photography (n=8), images too dark (n=2), and severe tilting of the optic disc (n=1).

There were some differences between the three groups, such as a higher rate of current smoking in PLWH aged <50, more PLWH reporting a lifetime history of injection drug use, and more PLWH in the older group reporting a history of stroke or TIA, or being seropositive for previous or current syphilis and hepatitis C (Table 1). There were no significant differences in blood pressure, anthropometry or serum lipid concentrations between groups. Differences in 10-year cardiovascular risk were commensurate with age. Recent recreational drug use was high in all three groups (>25% in the past 6 months). The most frequently reported recreational drugs were cannabis (16%), ketamine (15%), methamphetamine ("crystal meth", 13%) and amphetamine (13%). The number of HIV positive patients receiving each antiretroviral therapy drug class did not differ between the older and younger groups (total number on nucleoside reverse transcriptase inhibitors, 138 [86.8%]; non-nucleoside reverse transcriptase inhibitors, 82 [51.6%]; protease inhibitors, 76 [47.8%]; integrase inhibitors, 14 [8.8%]).

Prior ocular conditions in the final sample included cytomegalovirus retinitis (n=1), acanthamoeba infection resulting in a prosthesis on that side (n=1), hyaline asteroidosis (n=1) and cataracts (n=2), all of which were in PLWH aged ≥50 years. An additional 17 patients (8.1%) with drusen were identified (11 PLWH aged ≥50 years, 1 PLWH aged <50 years, and 5 HIV negative aged ≥50 years).

Comparison of retinal vascular measurements between study groups

Among the 211 participants, the mean AVR was 0.72 (standard deviation [SD] 0.07), mean CRAE was 141 (SD 20.2) and mean CRVE was 197 (SD 28.1), with no differences on any variable between the three groups (p>0.3), or between the two groups aged ≥50 years (HIV positive and HIV negative; p>0.2) (Fig. 1). The difference between the mean AVR in HIV positive men aged ≥50 and the HIV negative men of the same age was small: 0.716 compared to 0.719, a difference of 0.0026 (95% confidence interval [CI] -0.018 to 0.023, p=0.81) or one quarter of the standard error of the difference. Where good quality images were obtained from both eyes (n=202), AVR was correlated between left and right eyes (R^2 = 0.161 by linear regression) and there was no evidence of an overall difference between left and right on paired t-test (mean 0.73, SD 0.08 [left] versus mean 0.73, SD 0.07 [right], p=0.88).

In bivariate models (Fig. 2, broken lines), factors associated with lower AVR were higher systolic BP (β -0.0009 per +10 mmHg, 95% CI -0.0014 to -0.0003, p=0.004), higher diastolic BP (β -0.001 per +10 mmHg, 95% CI -0.0024 to -0.0003, p=0.01) and recent recreational drug use (β -0.031, 95% CI -0.051 to -0.011, p=0.002). Variables that were weakly associated with AVR, which were incorporated into the multivariable model, were a history of stroke or TIA (β -0.042, 95% CI -0.095 to 0.01, p=0.11) and a history of syphilis (β -0.015, 95% CI -0.034 to 0.005, p=0.14).

A multivariable model incorporating these variables, as well as study group, was then analysed (Fig. 2, solid lines). Systolic BP was included but diastolic BP was omitted because of collinearity (systolic BP had the stronger association of the two variables). Neither comparison group was associated with AVR when compared to HIV positive men aged ≥50 years (adjusted β 0.010 for HIV positive men <50 years, 95% CI -0.018 to 0.038, p=0.47; adjusted β 2.0×10^{-5} for HIV negative men ≥50 years, 95% CI -0.022 to 0.022, p=0.99). Factors associated with lower AVR in the multivariable model were systolic BP (adjusted β -0.009 per +10 mmHg, 95% CI -0.015 to -

0.003, p=0.002), history of stroke or TIA (adjusted β -0.070, 95% CI -0.12 to -0.015, p=0.01), and recent recreational drug use (adjusted β -0.037, 95% CI -0.057 to -0.018, p=0.0002). Similar analyses (not shown) failed to show any association between the three study groups and either CRAE or CRVE.

Association between HIV-related factors and retinal vascular measurements

In the analysis of only PLWH (Fig. 3), adjusted for age group, there was an association between lower AVR and higher current CD4+ lymphocyte count (adjusted β -0.004 per +100 cells/mm^3, 95% CI -0.008 to 0, p=0.05) but no association with current CD8+ count, CD4:CD8 ratio, any specific antiretroviral drug or class, or any of the time spans analysed (years on or off antiretroviral therapy, or total time since diagnosis).

DISCUSSION

In this study of 159 HIV positive and 52 HIV negative men, we found no association between HIV status and retinal vascular measurements. This is in keeping with the findings of a similar study in a South African population.[28] As expected, we found associations with hypertension and stroke history, as well as an association with recent recreational drug use. Within the group of PLWH, there was a smaller AVR in those with higher absolute $CD4^+$ lymphocyte count which may indicate the influence of immune activation, although $CD4^+$:$CD8^+$ cell ratio was not associated. We did not observe any association between the duration of antiretroviral therapy and retinal arteriolar narrowing [28], although this was observed in the South African study. PLWH receiving ART in South Africa differ markedly in their clinical features from the UK HIV positive population, and the healthcare systems providing HIV care have evolved in different ways, so there are several plausible explanations for the differences in our observations, including possible collinearity between age and duration of treatment in the South African patient sample.

Retinal photography with measurement of vascular calibre is widely used in epidemiological studies and clinical monitoring. Abnormal retinal diameters and qualitative features of retinopathy are associated with an increased risk of numerous conditions in middle-aged or older HIV negative people, including neuroimaging features of SVD, cognitive impairment, stroke, cardiovascular disease, incident diabetes, metabolic syndrome and all-cause mortality.[23-27, 34-40] In a large Dutch study (n=5540), a decrease in AVR of 1 standard deviation (0.06 in that study) was associated with a 14% increase in the risk of stroke.[40] This compares to an 8% increase in risk conferred by a 10 mm Hg increase in systolic BP in white Americans and 24% increase in stroke risk from the same increase in BP in black Americans.[41]

In studies employing other measures of cerebral SVD, there is mixed evidence for whether the extent and severity of cerebrovascular disease is increased in PLWH. Earlier studies of mainly untreated HIV positive individuals using older neuroimaging techniques found little evidence of an association between HIV and white matter hyperintensities of presumed vascular origin.[42, 43] Furthermore, an autopsy study of HIV positive, hepatitis C virus positive and uninfected individuals found no association between cerebral SVD and HIV status.[22] More recently, two other studies measuring the total volume of white matter hyperintensities, a standard quantitative measure of cerebral SVD,[44] found no overall difference between HIV positive and HIV negative participants.[20, 21] But two recent neuroimaging studies that have found an association between HIV status and small vessel disease include a recent Dutch study of men over 45 years of age, in which the total volume of white matter hyperintensities was greater in PLWH than in well-matched HIV negative study participants,[19] and a larger French study using the Fazekas and Schmidt neuroradiological rating scale,[45] which reported a higher prevalence of cerebral SVD in PLWH than in HIV negative controls [unpublished conference abstract].[46] The control group in the latter study may not have been well-matched, however, thus introducing bias.

Our study focused on a specific demographic group, which may limit the generalisability of our findings to other settings. We chose to restrict on the basis of ethnic group and gender to eliminate the well-described and powerful confounding effects of these variables on vascular disease markers; the study would likely have been insufficiently powered to perform gender and ethnicity subgroup analyses. We chose an older male cohort because such individuals are at greater risk of vascular disease, and we targeted the predominant demographic and transmission risk group (white men who have sex with men) attending our local clinics and living with HIV in the UK and many high-income settings. The study by Pathai *et al* included a study sample that was different to ours, being of younger age, entirely Xhosa ethnic group and predominantly female, and they also found no association between HIV status and retinal vascular calibres.[28] It is a strength of our study that the HIV negative controls had similar measured characteristics to the older HIV positive sample, and their attendance for sexual health and HIV testing at the same centres as the HIV positive groups should have reduced the effect of unmeasured confounders.

The study's sample size was smaller than planned and the participants were in middle age and therefore at lower risk of cerebrovascular disease than an older-aged group. However the difference observed between the HIV positive and HIV negative groups aged ≥50 was very small compared to the overall distribution of values, and despite the small sample the evidence for there being no true difference in retinal calibres on the basis of HIV status is strong.

The results of this study contribute to the accumulated evidence so far in this field. In summary, this evidence does not consistently find HIV to be a risk factor for cerebral SVD. Of the PLWH aged ≥50, 24% had a 10-year risk of cardiovascular disease (Framingham model) of above 10% (the threshold above which the UK National Institute for Health and Care Excellence

recommends statin therapy for primary prevention).[47] Our findings do not diminish the need for assessment and optimisation of individual patients' cardiovascular risks.

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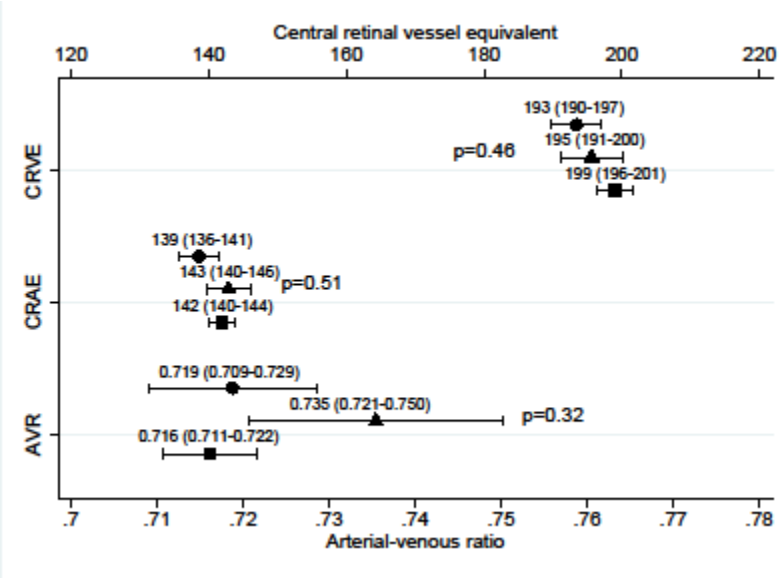
FIGURE LEGENDS

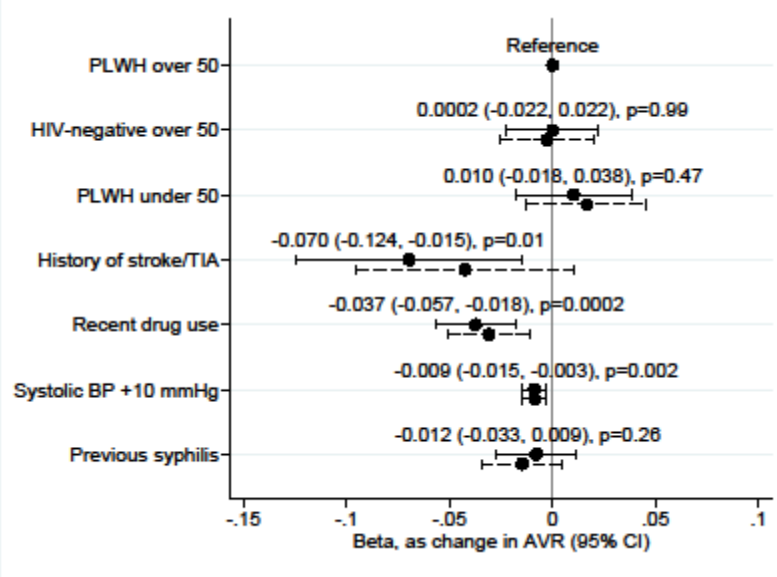
Figure 1. Retinal vascular indices, showing mean and standard error for all three groups.



Footnotes: PLWH aged 50 years and above, squares (n=120); PLWH under 50 years, triangles (n=39); HIV negative aged 50 years and above, circles (n=52). Lower values of AVR and CRAE, and higher values of CRVE, are considered to be pathological.

AVR, arterial-venous ratio; CRAE, central retinal artery equivalent; CRVE, central retinal vein equivalent.

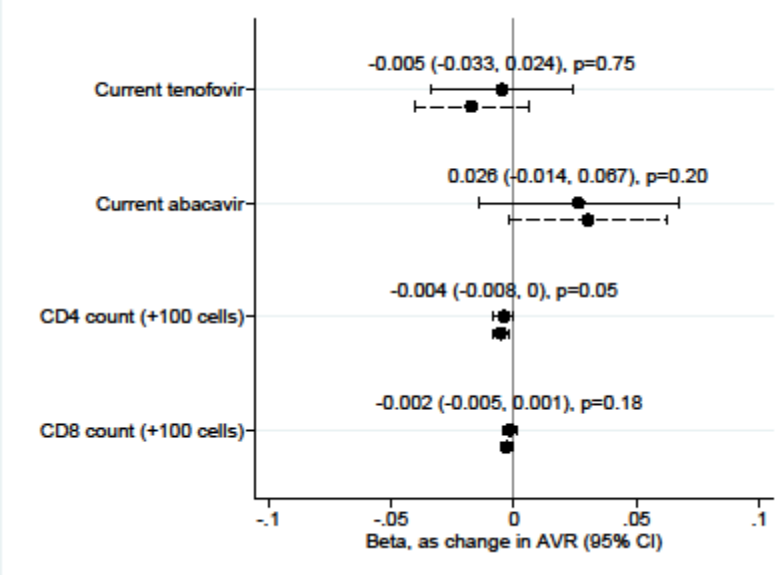
Figure 2. Factors associated with arterial-venous ratio, showing unadjusted (broken lines) and adjusted (solid lines) coefficients.



Footnotes: Multivariable estimates and 95% confidence intervals are shown. Leftward deflections in coefficient (negative values) indicate an association with lower AVR and more pathological change. Variables that were assessed in unadjusted models but are not shown here because p>0.2 were body mass index, waist circumference, lipids, smoking status, self-report of previous ischaemic heart disease, lifetime history of injection drug use and hepatitis C antibody status.

AVR, arterial-venous ratio; BP, systolic blood pressure; PLWH, people living with HIV; TIA, transient ischaemic attack.

Figure 3. HIV-related factors associated with arterial-venous ratio, showing partially adjusted (broken lines) and fully adjusted (solid lines) coefficients



Footnotes: Models include only HIV positive participants and are adjusted for study group (aged above or below 50). Estimates and 95% confidence intervals are shown. Leftward deflections in coefficient (negative values) indicate an association with lower AVR and more pathological change. Variables that were assessed in partially adjusted models but are not shown here because p>0.2 were CD4:CD8 ratio, years since HIV diagnosis, years between diagnosis and first initiation of antiretroviral therapy, total years of antiretroviral therapy, and current use of each antiretroviral drug class.

AVR, arterial-venous ratio.