Title: Unstructured treatment interruption: an important risk factor for arterial stiffness in adult Malawian antiretroviral (ART) patients

Running title: ART interruption - arterial stiffness

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Abstract:

Objectives: To evaluate the impact of unstructured ART treatment interruption (TI) on arterial stiffness in adult Malawians ≥35 years on ART.

Design: The number of TI events ≥30 days and ≥90 days during ART treatment were quantified in patients ≥35 years using retrospective routinely collected clinic data. TI data were linked to patient carotid femoral pulse wave velocity (PWV) with a threshold clinically relevant to CVD risk (PWV >10 m/s).

Methods: PWV was measured in patients (on ART ≥18 months), during routine ART clinic visits in Blantyre, Malawi between November 2014 and July 2015. Multivariable logistic regression was used to estimate the risk of PWV>10 m/s associated with TI, controlling for demographic and cardio-metabolic risk factors.

Results: In 220 patients (median age; range), 123(55.9%) had ≥1 TI event >30 days; 61 (27.7%) had ≥ 1 TI event > 90 days. Median length of TI events was 75 days (range: 31 days to 8 years). Overall, 31 (14%) patients had a PWV>10 m/s. In multivariable analysis, there was greater than a 2-fold increased risk of PWV>10 m/s *per* TI event >90 days (aOR: 2.6 95% CI: 1.1, 6.3)(Table 1), compared to patients with no TI event.

Conclusion: TI in ART patients ≥35 years is a common and important risk factor for arterial stiffness, and therefore the link between TI and CVD in this setting where traditional risks factors are less prevalent needs to be explored further.

Key words: Antiretroviral therapy, treatment interruption, Africa, HIV, cardiovascular disease

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Introduction

There is compelling evidence that Sub-Saharan Africa (SSA) is on the verge of a cardiovascular disease (CVD) epidemic, led by rising hypertension and obesity rates in urban populations[1-3]. In countries with a high HIV burden, this CVD epidemic will overlap with an HIV infected population where successful scale-up of ART has led to rising HIV prevalence among middle aged adults (≥35 years). ART patients are potentially an important risk group for CVD in SSA. HIV has been associated with an approximate 50% increased risk of CVD compared to HIV-uninfected populations [4], which is attributable to both ART and HIV *per se*[4-6]. Key causal mechanisms underlying this increased risk relate to arterial inflammation and atherosclerosis in association with HIV-driven immune activation[7, 8]. Whilst on-going immune activation occurs in about 20% of patients with suppressed viral load [9], unstructured ART treatment interruption (TI) leading to systemic inflammation may cause a rise in cardiovascular risk biomarkers and therefore an increased risk of CVD. In randomized trials, structured ART treatment interruption (structured TI) was significantly associated with elevated levels of markers of systemic inflammation and endothelial activation [10, 11] in adults initiating ART in Thailand and South Africa; secondary analysis of SMART trial data from the US showed structured TI resulted in a 57% increase in CVD events [12].

TI is therefore a potentially important modifiable risk factor for CVD in ART patient populations in SSA. However, currently there are very limited data regarding the relationship between TI and CVD risk in ART patients in SSA, where the prevalence of co-factors, including background immune activation from other infectious diseases, obesity, diet and smoking may differ from other world regions. Carotid-femoral pulse wave velocity (PWV) is a non-invasive measure of arterial stiffness. In meta-analysis, PWV> 10m/s was associated with an approximate doubling of risk of CVD[13]. Here we examine the impact of TI on arterial stiffness in adults ≥35 years with HIV infection and therefore CVD risk in a typical urban ART Clinic in Malawi.

Methods

Adult patients receiving ART at Queen Elizabeth Central Hospital (QECH) in Blantyre, Malawi were recruited from November 2014 to July 2015 for measurement of PWV and retrospective, longitudinal analysis of TI. QECH is a large teaching hospital for Malawi's

Southern Region. Since 2004, the QECH ART clinic has offered ART treatment according to Malawi Ministry of Health guidelines free-of-charge. In 2014, the clinic initiated approximately 1500 adults onto ART, and had 12000 adults under follow-up. Since 2008, the clinic has maintained an electronic medical system (EMS) into which clinicians prospectively enter demographic, clinical and ART patient-level data; data on patient visits before 2008 have been retrospectively captured and validated [14]. From 2003 until 2006, eligibility criteria for ART initiation in adults (≥15 years) were diagnosis of a WHO stage 3 or 4 defining illness, or a CD4 count of <200 cells/µl, or a total lymphocyte count <1200 cells/µl with a clinical diagnosis of WHO stage 2 or greater. The CD4 count threshold was raised to <250 cells/µl in 2006 and to <350 cells/µl in 2011. HIV infection in pregnancy, regardless of CD4 count was added as a criterion for ART initiation in 2011 (Option B+). ART visits occur monthly for 3 months following ART initiation and thereafter every 3 months or as clinically indicated. ART is dispensed at each routine clinic visit. Inclusion criteria were: age ≥35 years, enrolled in ART for ≥18 months, ART initiation and all follow-up at the QECH ART clinic, attending a routine ART appointment, not critically ill at the time of enrolment and not pregnant. Blood pressure, weight, height and waist circumference were measured at a single study visit. PWV was measured (sternal notch to femoral cuff) according to expert consensus guidelines on the Vicorder system [15]; PWV > 10 m/s was set as the threshold of clinically significant CVD risk [15]. Fasting glucose, total and HDL cholesterol and HIV viral load were measured. Study visit data were linked to patient TI clinical history data from the EMS. Ethical approval was obtained from the University of Malawi College of Medicine Ethics Committee (Protocol # P.07/14/1598).

Statistical Analysis

We used logistic regression to estimate risk of PWV >10 m/s associated with TI, where risk was expressed as Odds Ratios (OR) with associated 95% confidence intervals (95% CIs) . TI events were defined a patient being at least 31 days and 91 days overdue for a scheduled ART visit; ART can only be obtained from these visits. Demographic and clinical factors were examined in univariate logistic models of PWV> 10 m/s; factors included age, sex, education level, years on ART, ART regimen (initial and current), body mass index, blood pressure, total cholesterol, high density lipoprotein (HDL) cholesterol, fasting glucose, CVD behavioural risk

factors, year of ART initiation, current HIV viral load and TI. Factors significant at a univariate p-value of <0.20 were candidate factors in the multivariable model. Backwards selection of factors significant at p<0.05 was performed to develop a final multivariable model, with age, sex and years on ART included *a priori*. All statistical analysis was carried out in SAS Version 9.3 (Cary, NC).

Results

We recruited 230 ART patients ≥35 years from the QECH ART clinic; median length on ART was 6.5 years (range: 1.8, 13.1). Three patients were not linked to ART EMS clinic data, 1 patient declined PWV measurement, and 6 patients were missing co-factor data; our final analysis included 220 patients. Patient median age was 44 years (range 35 to 80), 60% were female and 36% had completed secondary education; 97% had been initiated on first line ART consisting of D4T,3TC and nevirapine (Table 1). Patients had a low prevalence of reported alcohol consumption (6.4%) and cigarette smoking (1.8%). Prevalence of overweight or obesity was 22%. Hypertension was prevalent in the patient cohort (28%); a further 29% of patients had pre-hypertensive blood pressure levels[16] (Table 1). Two individuals reported prior clinical CVD event (these were myocardial infarction and stroke).

In total, 123(55.9%) patients had ≥ 1 TI event >30 days; of these, 26.4% had a TI event within 12 months prior to recruitment. Sixty-one (27.7%) of patients had ≥ 1 TI event >90 days (Figure 1). The median length of individual TI events (> 30 days) was 75 days (range: 31 days to 8 years). Years on ART was the only factor associated with having ≥ 1 TI event >30 days (Adjusted Odds Ratio [aOR]: 2.1 95% CI: 1.4, 3.1) in a multivariable analysis controlling for age, gender, education, marital status and year of ART initiation– data not shown).

In total, 31 (14.1%) of patients had PWV>10 m/s; median PWV was 8.0 (range: 4.2, 12.4). In multivariable analysis, we found greater than a 2-fold increased risk of PWV>10 m/s *per* TI event >90 days (aOR: 2.6 95% CI: 1.1, 6.3)(Table 1). A single TI event >30 days was not significantly associated with PWV >10 m/s, however ≥2 TI events >30 days was marginally associated with a 3 times the risk of PWV>10 m/s (aOR: 3.6 95% CI: 0.9, 13.8, p-value=0.06). Older age, and hypertension were strongly associated with PWV>10 m/s (Table 1). Detectable viral load, current ART regimen, years on Stavudine containing ART regimen, years on any ART regimen, total cholesterol, HDL cholesterol, blood glucose and body mass

index were not significantly associated with PWV >10 m/s. Patients with PWV >10 m/s were 3.5 times more likely to have experienced symptoms consistent with CVD (chest pain, shortness of breath, oedema, hemiplegia), though this was not statistically significance (p-value=0.08, multivariable analysis controlling for age, gender and mean systolic blood pressure – data not shown).

We re-ran our main multivariable logistic models of PWV> 10 m/s in two strata of time since most recent TI '≥ 36 months ago' and '< 36 months ago', using patients with no TI as the comparator group in both models. In the strata '>36 months ago', the aOR for ≥ 1 TI event > 90 days was 10.8 (95% CI; 1.7, 68.7) compared to an aOR of 2.3 (95% CI: 0.45, 10.6) in the strata '<36 months ago'.

Discussion

We report a high prevalence of TI in an adult long-term ART patient cohort ≥35 years attending routine ART clinic appointments in Malawi. Similarly high levels of TI (of >30 days) have been observed in other African ART patient cohorts, including South Africa (41%)[17] and Ivory Coast (53.4%)[18]. In the context of these frequent gaps, we found a significant association with arterial stiffness (PWV >10 m/s) which implies a high risk of CVD.

We found a greater than 2-fold increased risk of PWV>10 m/s per TI event >90 days, when controlling for other important CVD risk factors. This finding is consistent with evidence from intervention studies in industrialised countries demonstrating that structured TI is associated with higher levels of soluble markers of systemic inflammation and endothelial dysfunction and greater risk of CVD clinical events [10-12]. Clinical trials of structured TI on CVD risk report on effects only up to 36 months. We found that TI-associated risk of arterial stiffness persisted beyond 36 months and may worsen over time.

There were some limitations in our study. Our data derived from a small, observational study and may thus be subject to confounding by unmeasured factors. For example, inflammatory illnesses such as tuberculosis may have contributed to some TI events. However, the strength of the effect of TI on arterial stiffness, the fact that this effect had a dose-response and the biological evidence supporting this effect leads us to conclude that confounding of this type would have minimal impact on our findings. Although retrospective, TI data were collected independently in real time and thus are likely to be largely unbiased. We note that our study sample represents patients that survived at least

18 months on ART, and therefore is not generalizable to patients in the period of early ART. However, SMART trial patients in the structure TI arm experienced a continuous rise in CVD, renal and hepatic disease events starting by about 6-months post-randomization[19], which suggests that TI-associated CVD risk begins early on.

In conclusion, we have identified an important, prevalent and potentially modifiable CVD risk factor in an African ART patient population, where traditional CVD risk factors are considerably less prevalent than in industrialized countries. Factors associated with TI in African ART patient cohorts include male gender [17], lower education level [20], adverse drug effects[21], longer time on ART[17] and higher CD4+ T-cell count at ART initiation[17, 20, 22]. Structural factors including travel costs, inconvenient clinic hours and discontinuous drug supply have also been identified as causes [18, 21, 23]. Appropriate medical, structural or behavioural interventions to reduce TI-associated arterial stiffness should be tested to avoid the potential epidemic of CVD in this growing population of middle-aged ART patients in SSA.

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Conflict of interest: The authors have no conflict of interest to declare.

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Table 1. Risk factors for arterial stiffness (PWV> 10 m/s) in 220 adult ART patients aged > 35 years, Blantyre, Malawi

	Total	PWV>10 m/s	PWV≤10 m/s	Univariate				Multivariable			
	N(%)	N(%)	N(%)	OR	95% CI		p-value	OR	95% CI		p-value
Female	133 (60)	22 (17)	111 (83)								
Male	87 (40)	11 (13)	76 (87)	0.73	0.33,	1.59	0.429	0.79	0.30,	2.11	0.643
Age											
35-44	111 (50)	8 (7)	103 (93)								
45-54	68 (31)	9 (13)	59 (87)	1.96	0.72,	5.36	0.188	2.18	0.68,	6.97	0.188
55-64	33 (15)	10 (30)	23 (70)	5.60	1.99,	15.74	0.001	2.91	0.83,	10.24	0.097
65+	8 (4)	6 (75)	2 (25)	38.62	6.68,	223.29	<0.001	14.07	1.94,	101.93	0.009
Education level											
Non/Primary	58 (26)	9 (16)	49 (84)								
Some Secondary	83 (38)	13 (16)	70 (84)	1.01	0.40,	2.55	0.981				
Secondary or higher	79 (36)	11 (14)	68 (86)	0.88	0.34,	2.29	0.794				
Baseline WHO stage											
Stage 1 or 2	86 (44)	9 (10)	77 (90)								
Stage 3 or 4	110 (56)	19 (17)	91 (83)	1.79	0.76,	4.18	0.181				
Current ART regimen											
TDF/3TC/EFV (5A)	142 (62)	18 (13)	124 (87)								
AZT/3TC/NVP (2A)	59 (26)	11 (19)	48 (81)	1.58	0.69	3.58					
d4T/3TC/EFV (3A)	14 (6)	4 (29)	10 (71)	2.76	0.78	9.71					
Other (1A/4A/6A/7A)	14 (6)	4 (29)	10 (71)	2.76	0.78	9.71	0.228				
Years on ART[1]	6.7	(1.8, 13.1)		1.20	0.99,	1.39	0.066	1.18	0.94,	1.49	0.159
Years on Stavudine containing ART regimen[1]	3.8	(0, 10.4)		1.02	0.87	1.20	0.771				
Undetectable viral load	198 (90)	31 (16)	167 (84)								
Detectable viral load	21 (10)	2 (10)	19 (90)	0.57	0.13,	2.56	0.461				
Number of unstructured ART treatment gaps >30 days[2]											
0	97 (44)	12 (12)	85 (88)								
1	82 (37)	8 (10)	74 (90)	0.77	0.30,	1.97	0.581	0.92	0.29,	2.95	0.887
2 or more	41 (19)	13 (32)	28 (68)	3.29	1.35,	8.03	0.009	4.28	1.17,	15.73	0.029
Number of unstructured ART treatment gaps >90 days[2]											
0	159 (72)	16 (10)	143 (90)								
1	51 (23)	13 (25)	38 (75)	3.06	1.35,	6.90	0.007	3.43	1.09,	10.79	0.035
2 or more	10 (5)	4 (40)	6 (60)	5.96	1.52,	23.37	0.011	9.49	1.09,	82.90	0.042
Normotensive [3]	94 (43)	1 (1)	93 (99)								
Pre-hypertensive	64 (29)	8 (13)	56 (88)	13.29	1.62,	109.05	0.016	12.87	1.49,	111.24	0.020
Hypertensive	62 (28)	24 (39)	38 (61)	58.74	7.67,	449.77	<0.001	44.30	5.13,	382.57	0.001
Blood glucose ≤7.0 mmol/L	208 (95)	29 (14)	179 (86)								
Blood glucose >7.0 mmol/L[4]	12 (5)	4 (33)	8 (67)	3.09	0.87,	10.91	0.080				
Tot. cholesterol >5.2 mmol/L	164 (75)	20 (12)	144 (88)								
Tot. cholesterol ≤5.2 mmol/L	56 (25)	13 (23)	43 (77)	2.18	1.00,	4.73	0.049	2.03	0.74,	5.54	0.168
HDL cholesterol ≥ 1.03 mmol/L	136 (60)	19 (14)	117 (86)								
HDL cholesterol < 1.03 mmol/L	91 (40)	18 (20)	73 (80)	1.50	0.75,	3.08	0.248				
Body Mass Index											
<18.5	34 (15)	6 (18)	28 (82)								
18.5-24.99	144 (63)	23 (16)	121 (84)	0.89	0.33,	2.38	0.812				
25-29.999	38 (17)	7 (18)	31 (82)	1.06	0.31,	3.51	0.932				
>30	13 (6)	1 (8)	12 (92)	0.39	0.04,	3.58	0.404				

1. Median and range
2. Parsimonious models included either 'treatment gap >30 days' or 'treatment gap >90 days'. OR estimates reported here are from parsimonious containing 'treatment gap >90 days'; in parsimonious model with 'treatment gap >30 days' change in ORs is <10%, except for total cholesterol which fell to 1.64
3. Pre-hypertensive blood pressures was defined as systolic 120–139 mmHg and/or a diastolic blood pressure of 80–89 mmHg)
4. or on blood glucose lowering medication