Impact of Low CD4 Count and HIV Persistence on Endothelial Function in Patients with Low Plasma RNA

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Background

HIV-positive individuals are at high risk for myocardial infarction and sudden cardiac death. Chronic inflammation is a contributing factor to increased cardiovascular risk in HIV-infected individuals, even among those with low plasma viral RNA levels. Endothelial impairment is proatherogenic and brachial artery flow-mediated vasodilation (FMD) has been established as a highly sensitive and specific assessment of vascular endothelial function. Hyperemic velocity, a measure of microvascular function, is the stimulus for FMD, a measure of macrovascular function. Hyperemic velocity of blood flow stimulates nitrous oxide release and arterial flow mediated vasodilation. Impaired hyperemic velocity has recently been determined as more predictive of cardiovascular events than FMD in the general population. The contribution of HIV persistence and traditional cardiovascular risk factors to microvascular and macrovascular dysfunction has not previously been evaluated.

Methodology

Patient selection - We performed a cross-sectional study of 70 HIV-infected adults from the SCOPE cohort at San Francisco General Hospital. Criteria:

- Treated HIV patients with viral suppression defined as stable ART for ≥ 12 months and HIV RNA levels <75 copies/mL for ≥ 12 months
- Untreated HIV patients with HIV RNA <1000 copies/mL for ≥ 12 months
- No changes in medications within 12 weeks prior to study.

Flow mediated vasodilation (FMD)–Measurements included brachial artery diameter at baseline and during reactive hyperemia after 5 minutes of forearm occlusion by blood pressure cuff. Maximal diameter increase was captured after 1 minute of reactive hyperemia. FMD was measured as percentage of increase from baseline (Figure 1). Vascular response and dilation was measured by change in baseline resting brachial diameter and velocity after 0.4 mg sublingual nitroglycerin. Maximal brachial artery dilation was measured 3 minutes post nitroglycerin administration.

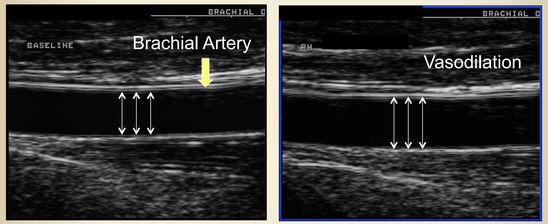
Hyperemic velocity - Hyperemic velocity was measured as peak flow velocity-time integral (VTI) of the first complete velocity envelope after blood pressure cuff release (Figure 2). Doppler ultrasound studies were performed with a 10MHz linear array probe and the GE VividSeven Imaging System. Imaging was performed and analyzed using software by Information Integrity Inc. Iowa City, Iowa. A single certified technician, blinded to HIV and treatment status, performed ultrasound testing and image analyses.

HIV persistence – Samples were obtained on the morning of ultrasound study. HIV persistence was determined using ultrasensitive plasma HIV RNA, lower limit of detection <0.3 copies/ mL, single copy assay (Palmer, Westmead, NSW, Australia), total proviral DNA in PBMCs in copies/million CD4+ T-cells (BSRI), and cell-associated RNA in PBMCs using TMA assay signal/cutoff per million CD4+ T-cells (BSRI).

Statistical analysis

Multivariate Poisson regression models were used to determine predictors of hyperemic velocity and FMD in relation to viral persistence. Multivariate models were adjusted for demographic factors. Multiple imputation using the Markov chain Monte Carlo method was used in the event of missing values in the final models. Inflammatory and coagulation factors were evaluated as probable predictors of hyperemic velocity and FMD, including hs-CRP, D-dimer, IL-6, TNF-α, sCD14, and sCD163.

Figure 1: Brachial Artery Flow Mediated Vasodilation



Macrovascular function assessed by measurement of brachial artery vasodilation before and after restoration of blood flow (reactive hyperemia).



Figure 2: Hyperemic Velocity

Microvascular function assessed by measurement of the peak velocity of restored brachial blood flow.

Results

Table 1: Baseline Characteristics

	Tx Suppressed (n=51)	Elite Controllers (n=19)
Traditional risk factors:		
Age (years)[1]	53 ±7.2	43 ±9.2
Male/ female	48/3	18/1
History of CAD	0 (0%)	0 (0%)
Current cigarette smoking	12 (24%)	9 (47%)
Diabetes	10 (19%)	2 (11%)
Hypertension	22 (43%)	2 (11%)
Hyperlipidemia	21 (41%)	2 (11%)
History of IV Drug Use	3 (6%)	3 (16%)
CKD (eGFR<59)	3 (6%)	1 (5%)
HCV co-infection	8 (16%)	1 (5%)
Laboratory values:		
Total cholesterol (mg/dL)[2]	189 (165-214)	162 (122-194)
LDL-C (mg/dL)[2]	114 (92-131)	90 (66.5-113)
HDL-C (mg/dL)[2]	44.5 (37.75-55)	43 (32-52)
Triglycerides (mg/dL)[2]	134 (96-208)	87 (72-122)
hs-CRP (mg/L)[2]	1.8 (0.7-2.78)	1.55 (0.58-3.9)
D-dimer (μg/mL)[2]	0.2 (0.12-0.36)	0.38 (0.29-0.57)
Interleukin-6 (pg/mL)[2]	1.79(1.52-2.74)	1.72 (1.36-4.68)
TNF-α (pg/mL)[2]	4.37 (3.37-6.31)	4.12 (2.82-4.25)
sCD14 (ng/mL)[2]	1902 (1652-2099)	1711 (1413-2010)
sCD163 (ng/mL)[2]	1189 (1019-1423)	1311 (1264-2493)
Hemodynamic values:		
SBP (mm Hg)[2]	117 (107-124)	116 (109-125)
DBP (mm Hg)[2]	75 (69-81)	71 (65-80)
Heart rate (BPM)[2]	62 (57.5-72)	73 (57-82.5)

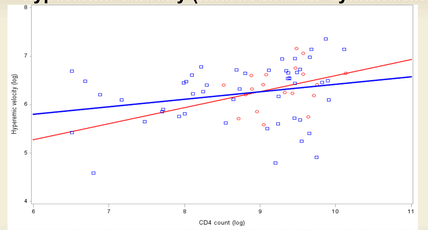
[1]Average values shown ± standard deviation
[2]Median (IQR)

Table 2: HIV and Vascular Characteristics

	Tx Suppressed (n=51)	Elite Controllers (n=19)
HIV Characteristics:		
HIV duration (yrs)[2]	18 (13-22)	6 (2-13)
HAART duration (yrs)[2]	7.44 (3.75-11.92)	0
HIV RNA viral load (copies/mL)[2]	40 (40-40)	40 (40-61)
CD4 count (cells/mm^3)[2]	591 (260-738)	642 (476-762)
Nadir CD4 count (cells/mm^3)[2]	189 (72-348)	476 (352-657)
Plasma RNA Single Copy Assay (copies/mL)[2]	0.5 (0.3-1.7)	33.25 (0.3-855)
Cell-associated RNA[2,**]	1.6 (1.4-2.2)	0.91 (0.6-1.9)
Proviral DNA (copies/million CD4+ T-cells)[2]	1.5 (0.98-2.5)	1.9 (1.72-2.4)
Vascular Characteristics:		
Baseline brachial artery diameter (mm)[2]	4.5 (4.2-4.95)	4.3 (4.1-4.7)
Hyperemic Velocity VTI (cm)[2]	85.7(53.8-102.1)	84.9 (72.8-99.2)
FMD (%)[2]	3.9 (2.7-5.2)	5.38 (4.1-4.7)

[2]Median (IQR)
** $\log_{10}$ S/Co per million CD4+ T-cells

Figure 3: Lower CD4 count was associated worsened hyperemic velocity (microvascular dysfunction)



After adjusting for age and gender, worsened hyperemic velocity was independently associated with lower proximal CD4+ T-cell count in treated suppressed (RR 1.04, p=0.045) and elite controllers (RR 1.10, p=0.011).

Results

We studied two "aviremic groups": those doing well on long-term ART and elite controllers. Subject demographic and clinical characteristics are displayed in Table 1. After adjustments for age and gender, worsened hyperemic velocity was independently associated with lower CD4+ T-cell count (RR 1.05 per 100 CD4+ T-cells, p=0.009) but not measures of HIV persistence (plasma HIV RNA, cell-associated RNA, cell-associated DNA). When analyzed in treated suppressed only, the association between worsened hyperemic velocity and lower CD4+ T-cell count remained (RR 1.04, p=0.045) and also remained when analysis was performed in elite controllers only (RR 1.10, p=0.011) (Figure 3). After adjusting for age and gender, impaired FMD was associated with lower eGFR (RR 1.72, p=0.003), smoking (RR 1.26, p=0.035) and higher cell-associated RNA (RR 0.95, p=0.05). Inflammatory and coagulation markers were not significantly associated with HV or FMD. Among treated and suppressed individuals only, lower eGFR and smoking remained significantly associated with impaired FMD while the association with cell-associated RNA weakened.

Conclusions

Among HIV-infected individuals with undetectable plasma RNA levels using conventional assays, a lower current CD4+ T-cell count, but not measures of viral persistence, was independently associated with impaired microvascular function, as defined by hyperemic velocity. In contrast, cell-associated HIV RNA was associated with impaired macrovascular function, as measured by FMD, suggesting that HIV may preferentially affect the microvasculature, leading to worsened FMD. Further studies using other measures of HIV persistence, including *in situ* studies using vascular tissue samples, will be necessary to more fully define the role of low-level virus production and/or replication have on vascular function. Therapies targeted at further reduction of HIV persistence may provide additional benefit on reduction of cardiovascular risk.

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