

Background
Although dyslipidaemia, inflammation and endothelial dysfunction have been linked to increased cardiovascular disease (CVD) observed with HIV-1 infection and antiretroviral therapy (ART), the role of platelets is yet to be determined. We hypothesised that HIV infection would disrupt platelet reactivity.

Methods
We compared platelet reactivity in 20 fasted HIV+ subjects and 20 matched HIV- controls by measuring time-dependent platelet aggregation (by light absorbance) upon exposure to increasing concentrations of platelet agonists ADP, collagen, epinephrine and thrombin receptor activating peptide (TRAP). We analysed relationships between platelet aggregation and demographic, treatment-related and inflammatory parameters using regression with data presented as median [IQR] unless otherwise stated.

Results
Groups were matched for age (HIV+ mean [SD] 34 [9] yrs versus 34 [8] yrs for controls) and gender (both groups 65% male). In the HIV+ group, mean [SD] CD4+ T-cell count was 529 [204] cells/μL. HIV RNA was 50 [507] copies/mL with 80% on ART. In the HIV- group, both ADP and TRAP induced less platelet aggregation at sub-maximal concentrations in a pattern suggesting non-competitive inhibition (ADP 70 [13%] versus 77 [10%] aggregation at 10μM, $P=0.035$; TRAP 75 [15%] versus 82 [12%] at 10μM, $P=0.011$ and 79 [11%] versus 86 [14%] at 20μM, $P=0.012$). In contrast collagen and epinephrine affected platelet aggregation in a pattern suggesting competitive inhibition. Collagen induced less aggregation at mid-range concentrations (5 [13%] versus 23 [6%] at 0.07μg/mL, $P=0.007$ and 45 [27%] versus 73 [19%] at 0.14μg/mL, $P=0.016$) with the concentration of collagen required to induce 50% aggregation (EC_{50}) higher in the HIV+ group (5.11 [0.89]μg/mL versus 0.28 [0.22]μg/mL, $P=0.002$). The EC_{50} for epinephrine was lower in the HIV+ group (4.19 [1.47]μM versus 19.7 [109.58]μM, $P=0.014$). In multivariate regression, CD8% and being HIV+ were independently associated with ADP-induced and TRAP-induced platelet aggregation respectively while higher neutrophil count and lower diastolic blood pressure were independently associated with collagen-induced platelet aggregation.

Conclusions
This is the first study to show platelet dysfunction at multiple levels in HIV+ subjects with both clinical and HIV parameters associated. Further research into underlying mechanisms and examining the effect of ART is needed to determine how platelet dysfunction links to CVD in HIV+ patients.

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A Case- Control Assessment of Platelet Function in HIV-1 Positive and HIV Negative Individuals

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Introduction

Dyslipidaemia, inflammation and endothelial dysfunction, observed in HIV-infected patients, have all been linked to increased cardiovascular disease (CVD).

Platelet activation and aggregation plays a pivotal role in development of arterial thrombus, a cardinal event in myocardial infarction (MI). Relationships between platelet dysfunction, HIV and antiretroviral therapy (ART) have not been clearly defined.

Aims

To assess platelet function in HIV-1 infected individuals in comparison to HIV negative controls.

Hypothesis

We hypothesised that HIV-infected patients would have abnormal platelet reactivity.

Methods

Platelet reactivity was compared in 20 fasted HIV+ and 20 matched HIV- subjects by measuring time-dependent platelet aggregation upon exposure to increasing concentrations of the following platelet agonists:

- Adenosine diphosphate (ADP)
- Collagen
- Epinephrine
- Thrombin receptor activating peptide (TRAP)

Between-group differences in platelet aggregation with exposure to maximal and sub-maximal agonist concentrations were estimated and relationships between platelet reactivity and demographic, treatment, haematological, immunological and inflammatory parameters were investigated using multiple regression.

Results

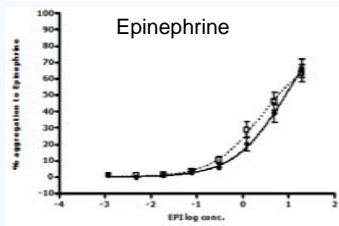
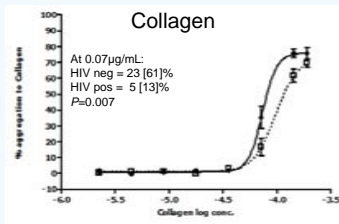
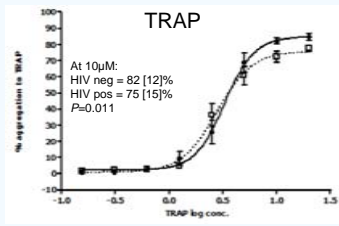
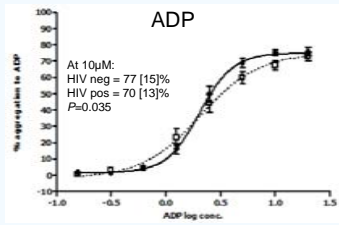
Cases and controls were well matched for age and gender (Table 1). Cases tended to be lighter, have higher systolic and diastolic blood pressure, lower total and differential white cell count than controls. More cases smoked and had nearly double HOMA IR compared to controls.

	Cases	Controls
<i>n</i>	20	20
Age (yrs) (mean, [SD])	34 [9]	34 [8]
Male (<i>n</i> [%])	13 (65)	13 (65)
Caucasian (<i>n</i> [%])	19 (95)	13 (65)
BMI (kg/m ²)	22 [20-26]	25 [22-26]
Systolic BP (mmHg)	129 [119-137]	123 [112-128]
Diastolic BP (mmHg)	78 [75-85]	73 [68-77]
Cholesterol (mmol/L)	4.2 [3.3-4.7]	4.7 [4.1-5.6]
HDL (mmol/L)	1.2 [0.9-1.28]	1.5 [1.3-1.7]
LDL (mmol/L)	2.6 [1.8-3]	2.7 [2.1-4.0]
Platelet count (10 ⁹ /L)	227 [158-265]	263 [220-312]
CD4 count (cells/μL)	285 [177-454]	824 [667-1056]
HIV RNA (copies/mL)	50 [50-2686]	-
On ART (<i>n</i> [%])	16 [80]	-
On PI (<i>n</i> [%])	8 [40]	-
On NNRTI (<i>n</i> [%])	8 [40]	-
On AZT (<i>n</i> [%])	3 [15]	-
AIDS (<i>n</i> [%])	5 [25]	-
CVD risk factors		
Hypertension (<i>n</i> [%])	1 [5]	0 [0]
Diabetes (<i>n</i> [%])	0 [0]	0 [0]
History of IHD (<i>n</i> [%])	0 [0]	0 [0]
FH-IHD (<i>n</i> [%])	5 [25]	5 [25]
Smoker (<i>n</i> [%])	10 [50]	5 [25]
Ex-smoked (<i>n</i> [%])	2 [10]	4 [20]
Hep C pos (<i>n</i> [%])	2 [13]	0 [0]

Table 1. Demographic and cardiovascular risk factors. Data are median [interquartile range] unless otherwise stated. Hypertension noted as positive if blood pressure exceeded 140/90mmHg. Family history noted as positive if one or more individuals, aged <65 years, of the subject's biological siblings, parents or children suffered from diabetes or cardiovascular disease.

Results cont'd

In the HIV+ group, both ADP and TRAP induced less platelet aggregation at sub-maximal concentrations in a pattern consistent with non-competitive inhibition.



Figures 1-4. Platelet aggregation in response to increasing concentrations of 1) ADP, 2) TRAP, 3) Collagen and 4) Epinephrine.

Results cont'd

In contrast, collagen and epinephrine affected platelet aggregation in a pattern consistent with competitive inhibition with collagen inducing less aggregation at mid-range concentrations.

In multivariate regression, a higher CD8% and being HIV+ were independently associated with less ADP-induced and TRAP-induced platelet aggregation respectively while higher neutrophil count and lower diastolic BP were associated with higher collagen EC_{50} .

	Univariate Analysis		Multivariate Analysis	
	r ²	P	r ²	P
ADP at 10μM				
- CD8%	-0.16	0.01	0.163	0.01
TRAP at 10μM				
- HIV infection	-0.79	0.012	0.156	0.012
Collagen at 71.1μg/ml				
- Diastolic BP	-0.83	0.008	0.355	0.001
- Neutrophil count	0.13	0.022		0.003
Collagen logEC₅₀				
- Diastolic BP	0.15	0.018	0.381	0.002
- Neutrophil count	-0.79	0.016		0.001
Epinephrine logEC₅₀				
- HIV infection	-0.76	0.02	0.146	0.02

Table 3 Association of agonist-induced platelet aggregation with clinical and laboratory parameters.

Conclusions

Platelet dysfunction was observed at multiple levels in HIV-infected subjects with both clinical and HIV parameters associated.

Further research into underlying mechanisms and examination of the effect of ART is required to determine what role platelets play in CVD in HIV-infected patients.

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