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Platelet count kinetics following interruption of antiretroviral treatment

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Abstract

Objectives—To investigate the mechanisms of platelet kinetics in the Strategies for Management of Antiretroviral Therapy (SMART) study that demonstrated excess mortality with CD4 guided episodic antiretroviral therapy (ART) drug conservation compared with continuous treatment viral suppression. Follow-up analyses of stored plasma samples demonstrated increased activation of both inflammatory and coagulation pathways after stopping ART.

Design—SMART patients from sites that determined platelets routinely.

Methods—Platelet counts were retrospectively collected from 2206 patients from visits at study entry, and during follow-up. D-dimer levels were measured at study entry, month 1, and 2.

Results—Platelet levels decreased in the drug conservation group following randomization, but remained stable in the viral suppression group [median (IQR) decline from study entry to month 4: –24 000/μl (–54 000 to 4000) vs. 3000 (–22 000 to 24 000), respectively, $P < 0.0001$] and the

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Conflicts of interest

There are no conflicts of interest.

rate of developing thrombocytopenia ($<100\,000/\mu\text{l}$) was significantly higher in the drug conservation vs. the viral suppression arm (unadjusted drug conservation/viral suppression [HR (95% CI) = 1.8 (1.2–2.7)]. The decline in platelet count among drug conservation participants on fully suppressive ART correlated with the rise in D-dimer from study entry to either month 1 or 2 ($r = -0.41$; $P = 0.02$). Among drug conservation participants who resumed ART 74% recovered to their study entry platelet levels.

Conclusion—Interrupting ART increases the risk of thrombocytopenia, but reinitiation of ART typically reverses it. Factors contributing to declines in platelets after interrupting ART may include activation of coagulation pathways or HIV-1 replication itself. The contribution of platelets in HIV-related procoagulant activity requires further study.

Keywords

antiretroviral therapy; D-dimer; HIV; platelets; strategies for management of antiretroviral therapy

Introduction

In the Strategies for Management of Antiretroviral Therapy (SMART) study, HIV-1-infected patients were randomly assigned to either continuous (viral suppression arm) or CD4 guided episodic use of antiretroviral therapy (ART; drug conservation arm). Unexpectedly, the drug conservation strategy was associated with an increased risk of all-cause mortality, largely non-AIDS-related causes, including cardiovascular, renal, hepatic, and non-AIDS cancers [1]. To better understand these findings, biomarker studies conducted on stored plasma samples have demonstrated levels of hsCRP, IL-6, and D-dimer [2] markedly predict risk for mortality in SMART. In addition, stopping ART lead to increases in these same biomarkers that correlated with the rise in HIV replication, suggesting that HIV infection lead to activation of both the inflammatory and coagulation systems [3]. These observations suggest that HIV-induced activation of coagulation pathways might have contributed to the pathologic processes underlying the increased mortality. The findings from the SMART study suggested that interruption of ART and corresponding increase in HIV-1 viral load may directly upregulate coagulation pathways. Most data on HIV-1-induced thrombocytopenia have been obtained prior to the widespread use of ART in patients with early HIV infection [4–6] and there is limited information on how ART affects the incidence and risk factors for thrombocytopenia in treated vs. untreated patients [7,8]. In this analysis of the SMART study we investigated the effect of interruption of ART, and the corresponding increases in HIV-1 viral load and D-dimer, on platelet count kinetics.

Methods

Detailed information on the study population and methods of the SMART study has already been published [1]. In short, 5472 HIV-1-infected participants with CD4^+ cell count more than 350 cells/ μl were enrolled at 318 sites in 33 countries. Patients were randomized to one of two ART arms; the viral suppression (viral suppression) arm involved continuous use of ART while the drug conservation arm (drug conservation) involved CD4^+ guided interruptions of therapy when CD4^+ cell counts were more than 350 cells/ μl and reinitiation of therapy when CD4^+ cell counts were less than 250 cells/ μl . All the necessary institutional/

ethical review board approvals were obtained. The study was approved by the institutional review board at each site, and written informed consent was obtained from all participants. The [ClinicalTrials.gov](https://clinicaltrials.gov) identifier is: NCT00027352 and the EudraCT number is: 2004–000441-38.

Enrolment was discontinued in January 2006 because of the observed increased risk of opportunistic disease or death in the drug conservation arm compared with the viral suppression arm.

Study population

This report consists of 2206 patients (1090 from the drug conservation arm and 1116 from the viral suppression arm) participating in the SMART study who were enrolled at study sites wherein platelet counts had been consistently collected. Platelet counts were retrospectively collected at study entry, month 1, 2, 4, 6, 8, 10, 12 and every 4 months thereafter. As it was not possible to retrospectively collect data on all patients, sites with limited data collected on participants who had died or been lost to follow-up were excluded from this report (defined as <20% of dead/lost to follow-up participants), as were sites with less than 80% completeness of platelet levels at randomization. This decision to remove entire sites based on summary characteristics of data from the site, rather than removing individual patients based on patient characteristics was taken a priori. This resulted in all participants randomized at 157 sites (out of 318) being included in this analysis. As randomization was done in blocks at the site level, treatment allocation can be considered as being random in this subset of the entire study population.

D-dimer levels were available at study entry for all SMART participants with a stored plasma sample available ($N = 1998$ in this report). D-dimer, IL-6, and hsCRP levels at months 1 or 2 of follow-up were available for 75 drug conservation and 99 viral suppression patients from previous reports [2,9]. Serious non-AIDS events were defined as major cardiovascular disease (CVD) events (myocardial infarction, stroke, or coronary artery disease requiring surgery), end-stage renal disease, cirrhosis, non-AIDS malignancies, or death from non-AIDS-related causes. Plasma HIV-1 RNA was collected prospectively at each visit and was required by the protocol. HIV-RNA levels were measured at the site in real time and used as standard of care. D-dimer levels were measured from stored specimens at Laboratory for Clinical Biochemistry Research at the University of Vermont after termination of the study [2].

Statistical methods

Thrombocytopenia was defined as having a follow-up platelet count less than 100 000 μl . Participants were censored at the date of the last available platelet count and time-to-event analyses were restricted to those with a platelet count at least 100 000 μl at study entry. The rates of developing thrombocytopenia per 100 person-years were calculated and hazard ratios for the comparison of the drug conservation and viral suppression groups were estimated from Cox models. Adjusted models included the following study entry covariates: age, sex, race, ART and HIV-1 RNA, CD4^+ cell count, hepatitis coinfection, prior CVD, smoking, diabetes, blood pressure lowering drugs, lipid lowering drugs, total/HDL

cholesterol, BMI, and study entry platelet level. The proportional hazards assumption was tested in a univariate model by including an interaction term between treatment group and log transformed follow-up time.

Spearman rank correlation coefficients were used to assess associations of changes in platelet counts in the drug conservation group with study entry D-dimer, changes in D-dimer levels, and changes in hemoglobin levels and HIV-RNA after log₁₀ transformation.

Predictors for recovery time of platelets to the study entry level from initiation of ART in the drug conservation group were assessed by Cox models. Time zero was redefined to be the time of initiation of ART for the first time after randomization to SMART. The outcome was defined as the first platelet count after initiation of ART that was at least study entry platelet level. Predictors included change in platelets from study entry to ART initiation, time from randomization to ART initiation, HIV-RNA prior to initiation of ART and type of ART initiated.

The risk of serious non-AIDS events in the drug conservation group associated with at least 25% decline in platelets during the first 4 months of SMART was assessed by Cox regression including an indicator for those with and without a 25% decline in platelet count from study entry to month 4. Time zero was defined as the date of the month 4 visit and the model was adjusted for change in CD4⁺ and log₁₀ HIV-1 RNA to month 4, age, sex, race, nadir CD4⁺, hepatitis coinfection, smoking, diabetes, and BMI.

Results

Study population

The median age was 43 years, 27.5% were women and 85.9% of patients were on ART when entering the study. There were no significant differences between viral suppression and drug conservation arms in respect to: age, sex, race, ART and HIV-1 RNA, CD4⁺ cell count, nadir CD4⁺ cell count, prior AIDS, hepatitis coinfection, smoking, diabetes, blood-pressure-lowering drugs, lipid lowering drugs, total/HDL cholesterol, BMI, and hemoglobin (Table 1). However, the study cohort differed significantly from remaining SMART participants for whom platelet counts were not available. The study population included in this study had fewer of black race (26.1 vs. 31.2%; $P < 0.001$), lower median BMI (24.6 vs. 25.1; $P < 0.001$), higher percentage of patients on ART (85.9 vs. 82.5%; $P < 0.001$) and with HIV RNA 400 or less (74.2 vs. 70.0%; $P < 0.001$), and a lower percentage with prior AIDS (22.1 vs. 25.6%; $P = 0.003$), hepatitis coinfection (15.7 vs. 17.9%; $P = 0.03$), current smokers (37.3 vs. 42.7%; $P < 0.001$), prevalence of diabetes (5.8 vs. 7.9%; $P = 0.003$), and use of blood pressure (16.7 vs. 20.1%; $P = 0.04$), or lipid lowering drugs (14.6 vs. 16.7%; $P = 0.04$).

Stopping/deferring antiretroviral therapy (drug conservation group) results in significantly higher rates of thrombocytopenia than continuing/starting therapy (viral suppression group)

The median (IQR) platelet count was 236 000/μl (199 000–281 000) at study entry, decreased temporarily within the first year of the study in the drug conservation arm, but

remained stable in the viral suppression arm [median (IQR) decline from study entry to month 4: $-24\ 000/\mu\text{l}$ ($-54\ 000$ to 4000) vs. 3000 ($-22\ 000$ to $24\ 000$), respectively, $P < 0.0001$] (Table 2, Fig. 1). There were 62 thrombocytopenia events in the drug conservation group (rate = 2.6 per 100 person-years) and 36 events in the viral suppression group (rate = 1.4 per 100 person-years). The rate of developing thrombocytopenia ($<100\ 000/\mu\text{l}$) was significantly higher in the drug conservation arm vs. viral suppression arm [unadjusted drug conservation/viral suppression hazard ratio (95% CI) = 1.8 (1.2–2.7)]. The excess risk in the drug conservation vs. viral suppression group was evident during the first 4 months [adjusted hazard ratio (aHR) drug conservation/viral suppression = 9.0 (95% CI 2.7–29.5)], but not thereafter [aHR = 1.1 (0.7–1.8); interaction drug conservation/viral suppression vs. time: $P = 0.0006$]. Of the 62 drug conservation patients who developed thrombocytopenia, 45 initiated after the first date with platelets less than $100\ 000$, 12 initiated before the first date with platelets less than $100\ 000$ and five did not initiate ART during follow-up. Of the 45 that initiated after developing thrombocytopenia, the majority initiated due to protocol instructed reasons (CD4 cell count <250 or Data Safety Monitoring Board recommendation). There were eight patients for whom the site wrote on the form that thrombocytopenia was a reason for initiation. Male sex ($P = 0.01$), nonblack race ($P = 0.02$), coinfection ($P = 0.006$), and higher study entry platelet level ($P < 0.001$) were all associated with greater declines in platelets.

Changes in D-dimer levels were inversely correlated with changes in platelet counts

The early decrease in platelet numbers was neither associated with levels of D-dimer at study entry, nor with change in the hemoglobin levels [correlation = 0.02 ($P = 0.66$) and -0.06 ($P = 0.06$), respectively]. However, for drug conservation patients with D-dimer values available at baseline and either month 1 or month 2 ($n = 75$), the rank correlation coefficient between change in D-dimer and change in platelets was -0.24 ($P = 0.04$). The association was even stronger [rank correlation coefficient -0.41 ($P = 0.02$)] for drug conservation patients who were on ART and had a suppressed viral load at study entry ($n = 33$) (Fig. 2a). For drug conservation patients, the correlations between change in platelets and changes in IL-6 and hsCRP were -0.23 ($P = 0.05$) and -0.06 ($P = 0.59$), respectively. When restricting to those on ART with a suppressed viral load, the correlations are -0.29 ($P = 0.11$) for IL-6 and -0.01 ($P = 0.97$) for hsCRP.

Changes in HIV-1 RNA levels inversely correlated with changes in platelet count in drug conservation patients with initially suppressed viral loads

When restricted to drug conservation participants with HIV-1 RNA 400c/ml or less on ART at entry ($n = 738$), changes in HIV-1 RNA levels after interruption of ART were inversely correlated with changes in platelet count (Fig. 2b). The correlation between 4-month change in $\log_{10}$ transformed HIV-1 RNA and 4-month change in $\log_{10}$ transformed platelet count was $r = -0.34$ ($P < 0.001$).

Recovery of platelet counts following (re)initiation of antiretroviral therapy was dependent on size of prior decline in platelet count as well as HIV-1 RNA levels at time of (re)initiation

Of 900 patients in the drug conservation arm (re)initiating ART, 663 had platelet information available thereafter and had a study entry platelet level greater than the value prior to initiation. For these 633 participants, the median (IQR) change from study entry platelet level to level prior to initiation was $-47\ 000$ ($-79\ 000$, $-27\ 000$) and 489 (74%) recovered to their study entry levels during follow-up. Estimated percents recovered by 4, 8 and 12 months after ART reinitiation were 45, 60 and 69%, respectively. Those with the larger declines in platelet counts from study entry to ART initiation [aHR per $\log_{10}$ higher = 184 (56–610)] and those with higher HIV-1 RNA levels at time of (re)initiation [aHR per $\log_{10}$ higher = 1.3 (1.2–1.5)] recovered their platelet counts faster. Those who initiated ART later in the study took longer to recover their platelet counts (Table 3).

Thrombocytopenia and risk of subsequent clinical disease

In the drug conservation arm 199 of the 1010 participants with month 4 platelet levels (20%) had a platelet decline of at least 25% during the first 4 months. A 25% decline in platelet level at 4 months was not associated with subsequent risk of serious non-AIDS events [aHR = 0.8 (0.4–1.7), $P = 0.52$]. Developing thrombocytopenia was not associated with subsequent risk of serious non-AIDS events [aHR = 0.9 (0.6–2.6); $P = 0.92$].

Discussion

Here we describe platelet kinetics, and the relationship to HIV replication and changes in D-dimer levels, in the context of an ART interruption study (SMART). A significant decrease in the platelet count as well as an increased rate of thrombocytopenia (defined as $<100.000/\mu\text{l}$) was observed among 1090 participants randomized to ART interruption (drug conservation arm) in the SMART Study, whereas patients assigned to continuous ART use (viral suppression arm) had stable platelet counts. Inverse correlations were seen between changes in platelets and changes in D-dimer or HIV-1 RNA levels, and were strongest among those with viral suppression at study entry who then stopped ART. Most patients recovered to platelet levels seen at study entry after reinitiating ART.

The prevalence of HIV-1-induced thrombocytopenia varies greatly between different studies, with reports between 1.1 and 54.7%, but is generally more than 10% [10]. HIV-related thrombocytopenia is likely caused by accelerated platelet destruction, splenic platelet sequestration, and variably impaired platelet production [11]. Accelerated clearance can be caused by immune-complex-based disease, antiplatelet glycoprotein antibodies, and/or anti-HIV-1 antibodies that cross-react with platelet membrane glycoproteins [12]. Impaired platelet production is probably caused by HIV-1 directly infecting megakaryocytes through the viral CXCR4 coreceptor, inhibiting mature megakaryocytes from effectively producing platelets [13]. HIV-1 can also impair megakaryocyte maturation as shown by reduced formation megakaryocytopoietic colony forming units in HIV-1-infected individuals [14].

The predominant mechanism(s) contributing to HIV-related platelet reductions may differ by HIV disease stage. In the early phase of disease with low viral loads, an immune

thrombocytopenic purpura (ITP) like mechanism with presence of antiplatelet antibodies and increased platelet destruction dominates. In advanced HIV-1 infection with high viral loads and low CD4 cell counts ($<200/\mu\text{l}$) thrombocytopenia is mainly caused by decreased platelet production [15,16].

HIV-1-induced thrombocytopenia is usually responsive to standard ITP treatments (prednisone, intravenous immunoglobulin, anti RhD, splenectomy). Moreover, reducing viral load using ART has also proven efficient [12,17].

Our study is one of the few available wherein incidence and risk factors of incident thrombocytopenia have been systematically compared in the context of continuing/starting and stopping ART within a randomized trial. The findings are consistent with the Agence nationale de recherches sur le sida et les hépatites virales (ANRS) 106-window trial analyzing 391 patients randomized to continuous or intermittent therapy for 96 weeks, showing that thrombocytopenia ($<150 \times 10^3$ platelets/ μl) was more prevalent in the intermittent therapy arm compared to the CT arm (25.4 vs. 9.8%, respectively, $P < 0.001$). Decreased platelet counts were correlated with changes in CD4 T-cell counts and plasma HIV-1 RNA levels ($P < 0.001$ for both) [18]. This study had a relatively small sample size and used a very sensitive endpoint (thrombocytopenia defined as $<150 \times 10^3$ platelets/ μl) that is clinically not thought of as thrombocytopenia. Whereas in the SMART study, the much larger sample size and the more accurate endpoint (thrombocytopenia defined as 100×10^3 platelets/ μl) more convincingly showed the correlation between ART interruption and thrombocytopenia as well as allowing for the study of risk factors in more detail.

An elevated D-dimer reflects activation of the coagulation and fibrinolytic systems and is well known to be associated with increased risk of CVD and mortality from any cause [19–21]. Activation of the coagulation system may in certain conditions also cause thrombocytopenia, best described in syndromes such as disseminated intravascular coagulation, paroxysmal nocturnal haematuria [22], heparin-induced thrombocytopenia [23], thrombotic thrombocytopenic purpura and hemolytic uremic syndrome [24]. These conditions are all characterized by endothelial and platelet activation as well as increased risk of thrombosis in spite of low platelet counts. Also in ITP, a paradoxically increased risk of thrombosis has been described [25]. In-vivo platelet activation, circulating platelet-leukocyte-monocyte aggregates, platelet-derived microparticles, and immature reticulated platelets have all been implicated in the mechanism of a prothrombotic state in patients with ITP [26,27]. Reticulated platelets are immature platelets with increased mass and a greater prothrombotic potential compared with mature platelets [28–30]. In our study, we confirmed the inverse association between D-dimer, HIV-1 RNA levels, and platelet counts in the context of interrupting HIV treatment, suggesting that HIV-1 viremia might activate platelets and coagulation factors.

In the SMART study, reinitiation of ART was not associated with an improvement in inflammatory parameters (hsCRP, IL-6) [2]. However, we have noted that platelet counts increased in most patients with HAART reintroduction, returning to their study entry levels. This lack of association does not support the hypothesis that unspecific inflammation is the sole force driving thrombocytopenia. As seen before, our data point to uncontrolled HIV-1

replication itself (most probable) or an activation in the coagulation pathways as pivotal underlying factors in the genesis of platelet count decreases.

Our study has some limitations that should be noted. Platelet levels were collected retrospectively at sites, only where available. Thus, we did not have platelet data for all SMART participants. By excluding sites that were unable to report platelet data on participants who had died or were lost to follow-up, we exclude 229 participants who experienced serious non-AIDS events. This resulted in our having limited power to study the effect of platelet kinetics on clinical event risk.

In summary, we have shown a significant decrease in platelet counts as well as a higher rate of thrombocytopenia (defined as $<100,000/\mu\text{l}$) after treatment interruption in SMART, which was related to increased viral replication and D-dimer levels. Most patients recovered to entry platelet levels after antiretroviral treatment reinitiation. Our data give support to uncontrolled HIV-1 replication and/or activation of coagulation pathways as the main factors underlying platelet count decreases.

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Appendix

The SMART Study Group: SMART was initiated by the Terry Bein Community Programs for Clinical Research on AIDS (CPCRA) and implemented in collaboration with Investigators in international coordinating centers in Copenhagen (Copenhagen HIV Programme), London (Medical Research Council, Clinical Trials Unit), Sydney (National Centre in HIV Epidemiology and Clinical Research) and Washington (CPCRA). Participating staff are listed below.

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DC Group:	1090	978	1007	1010	1001	961	895	911
VS Group:	1116	965	999	1005	994	955	917	925

Fig. 1.
Mean (± 2 SE) log platelet change from study entry.

Fig. 2. Kinetic changes in platelet count according to change in D-dimer and HIV-RNA
 (a) Drug conservation group, change in D-dimer level by change in platelet level participants on ART at study entry. (b) Median (IQR) change in platelet levels to month 4 by Month 4 HIV-RNA: drug conservation group on ART with HIV-RNA 400 or less at study entry.

Table 1

Study population characteristics by treatment group.

	DC	VS	<i>P</i>
<i>N</i>	1090	1116	
Median (IQR) age (years)	43 (37, 50)	43 (38, 50)	0.24
Sex (% female)	28.5	26.5	0.29
Percentage of black race (%)	26.1	26.0	0.93
Percentage on ART (%)	86.1	85.8	0.89
Percentage with HIV RNA < 400 (%)	74.0	74.4	0.82
Median (IQR) CD4 cell count	600 (480, 766)	597 (462, 780)	0.59
Percentage with prior AIDS (%)	22.8	21.5	0.48
Percentage with viral hepatitis coinfection (%)	16.1	15.3	0.64
Percentage of current smokers (%)	35.8	38.7	0.15
Percentage with diabetes (%)	5.6	6.0	0.68
Percentage on blood-pressure-lowering drugs (%)	17.7	15.7	0.20
Percentage on lipid-lowering drugs	13.6	15.6	0.18
Median (IQR) total/HDL cholesterol	4.7 (3.7, 5.9)	4.6 (3.6, 5.9)	0.44
Median (IQR) BMI	24.7 (22.4, 27.7)	24.5 (22.0, 27.4)	0.06

Table 2

Study entry and follow-up laboratory measurements by treatment group.

	DC group	VS group	DC vs. VS	Total
N	1090	1116		2206
Study entry platelet count; median (IQR) (/μl)	237000 (199000, 285000)	234000 (198000, 278000)	$P = 0.21$	236000 (199000, 281000)
Study entry D-dimer level; median (IQR) (μg/ml) ^a	0.20 (0.13, 0.38)	0.20 (0.12, 0.34)	$P = 0.11$	0.20 (0.13, 0.36)
Study entry hemoglobin level; median (IQR) (g/dl) ^b	14.3 (13.1, 15.3)	14.3 (13.2, 15.3)	$P = 0.96$	14.3 (13.2, 15.3)
Change in hemoglobin level from 0–4 months; median (IQR) (g/dl) ^c	−0.10 (−0.70, 0.50)	0.00 (−0.60, 0.50)	$P = 0.20$	
Change in D-dimer level from 0 to 1 or 2 months; median (IQR) (μg/ml) ^d	0.07 (−0.05, 0.27)	0.00 (−0.11, 0.07)	$P < 0.0001$	
Change in platelet level from 0–4 months; median (IQR) (/μl) ^e	−24000 (−54000, 4000)	3000 (−22000, 24000)	$P < 0.0001$	
Rate (per 100 person-years) of developing thrombocytopenia during first 4 months	8.26	0.83	$P = 0.0001$	

DC, drug conservation; VS, viral suppression.

^a Available for 990 DC and 998 VS participants.^b Available for 1089 DC and 1115 VS participants.^c Available for 1010 DC and 1004 VS participants.^d Available for 191 DC and 198 VS participants.^e Available for 1010 DC and 1005 VS participants.

Table 3

Percentage of participants recovering to study entry platelet level by duration of ART interruption.

Duration of ART interruption	<i>N</i> at risk (estimated % recovered to baseline level) by month		
	4 Months	8 Months	12 Months
<4 months	216 (55%)	97 (66%)	71 (73%)
4–8 months	150 (52%)	72 (68%)	45 (76%)
8–12 months	102 (39%)	61 (57%)	41 (64%)
12+ months	195 (32%)	123 (49%)	79 (64%)
Overall	663 (45%)	353 (60%)	236 (69%)

ART, antiretroviral therapy.