Long-Term Trends of Coagulation Parameters in People Living With HIV Treated With Combined Antiretroviral Therapy

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Abstract

Trends of coagulation parameters during long-term treatment with combination antiretroviral therapy (cART) are unclear. We followed 40 male subjects living with human immunodeficiency virus (HIV). Plasma levels of procoagulant parameters, factor VIII, von Willebrand factor and D-dimer, and anticoagulant parameter Protein S (PS), were measured before start and 3 months, 1 year, and 9 years after. Analyses were adjusted for cardiovascular risk factors (age, smoking, and hypertension) at baseline. At baseline, procoagulant parameters were markedly elevated and PS was in the lower range of normal. CD4/CD8-ratio improved during the complete follow-up period. In the first year, procoagulant parameters were decreasing, but at year 9 an increase was observed. After correction for cardiovascular risk factors, this increase was no longer present. PS remained stable during the first year and slightly increased from one to 9 years. This study indicates that decreasing immune activation by cART reverses the procoagulant state in HIV partially during the first year. These parameters increase in the long term despite an on-going decrease in immune activation. This increase might be related to established cardiovascular risk factors.

Keywords

HIV, blood coagulation, thrombosis, anti-HIV agents, aging

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Introduction

Similar to other inflammatory and infectious conditions, the acute phase of human immunodeficiency virus (HIV) infection is characterized by a procoagulant state.[1,2] This can be at least partially attributed to immune activation, which in turn activates the coagulation system.[3] Combination antiretroviral therapy (cART) effectively suppresses viral replication and drastically diminishes immune activation.[4] Jong et al demonstrated that this procoagulant state reverses partially in the first 6 months of cART.[5]

Although better immune control by cART has improved life expectancy drastically, people living with HIV (PLWH) are at an increased risk of noncommunicable disease, including venous[6,7] and arterial thrombosis.[8] A better understanding of the pathophysiological mechanisms of this risk might guide potential preventive strategies. The SMART trial,[4] in which patients were randomized between a viral suppression aimed strategy by continuous use of cART or episodic use based on certain CD4+ T-cell count thresholds, showed that the latter was associated with an increased risk of all-cause mortality, mostly from non-AIDS-related causes.[4] Exploration of biomarkers showed that this could be attributed to increases in IL-6 and D-dimer levels, indicating immune and coagulation activation, which could be dampened by a strict cART

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regimen. Interestingly, IL-6 and D-dimer levels in participants of the SMART trial were almost twice as high compared to uninfected individuals from the general population, irrespective of randomization allocation. This was also found in a subgroup analysis of participants with successful suppression of HIV replication (≤400 copies/mL), suggesting residual immune and subsequent coagulation activation despite effective cART.[9]

Currently, longitudinal data on the coagulation status in PLWH after the first year of cART are lacking. Improved immunological control by cART might reverse the procoagulant state completely in the long term. On the other hand, residual immune activation despite effective cART, as suggested by the SMART trial, might result in a persistent procoagulant state. Alternatively, a procoagulant state could also be maintained by vascular damage related to aging.[10] This process occurs universally and might be more pronounced in PLWH, as HIV infection is associated with accelerated aging, putatively by residual immune activation, more comorbidities, and a higher prevalence of smoking compared to the general population.[11–13] In this study, we describe the course of coagulation parameters in PLWH on long-term cART.

Methods

Study Population

In 2009 to 2010, we recruited a cohort of adult cART naive HIV-1 infected subjects visiting the outpatient clinics or admitted to the infectious diseases ward of the University Medical Centre Groningen (UMCG), in whom cART was initiated according to international treatment guidelines. Exclusion criteria were HIV-2 infection, pregnancy, and use of combined oral contraception.

All participants provided informed consent. The study was approved by the medical ethics committee of the University Medical Center Groningen (METc 2010/189), the Netherlands, in accordance with the Declaration of Helsinki (updated version 2013). The study was prospectively registered at the Dutch Trial Register (www.trialregister.nl, NL7884).

Study Design

Initially, 3 study visits were planned: before start of cART (baseline), and 3 months and 1 year after start of cART during regular follow-up visits with the treating HIV physician. At baseline, data were collected from all participants, including demographics, general medical history, risk factors for venous and arterial thrombosis, history regarding venous and arterial thrombosis, medication use, and smoking by semistandardized interviews. Data on HIV status, including HIV RNA load, CD4+, and CD8+ T-cell count were collected from medical records. At each study visit, blood samples were collected for the measurement of coagulation parameters.

In the period of 2018 to 2019, all participants were reinvited for a long-term follow-up visit (median 9 years after initiation of cART). This extension was approved by the medical ethics committee of the UMCG (METc 2019/086). As the study was initially designed with one year of follow-up, all participants were asked for informed consent again. Data on the occurrence of venous and arterial thrombosis, comorbidities, and medication use since the last study visit were collected by semistandardized interviews. Most recent data on current HIV status, including HIV RNA load, CD4+, and CD8+ T-cell count were collected from medical reports. Furthermore, blood sampling for the measurement of coagulation factors was repeated once.

Blood Sampling

Blood was drawn by venous puncture from the antecubital vein. For all coagulation tests, blood was collected into a 6.0 mL citrated Vacutainer with: 10 volume of 0.109 mol/L trisodium citrate. Platelet-poor plasma was prepared by centrifugation at 2500× *g* for 15 min, aliquoted and immediately frozen at −80 °C, and analyzed after rapidly thawing at 37 °C. Details on the used assays are provided in the Supplementary Appendix.

Outcomes

Plasma factor VIII activity (FVIII), von Willebrand factor ristocetin activity (vWF), and D-dimer levels were measured as markers of a procoagulant state. Plasma protein S (PS), total activity, and activity of the free fraction were measured as markers of an anticoagulant state. These parameters were selected based on available literature at the time of the initial study design demonstrating an association of these parameters with CD4+ T-cell count[2,14] or HIV viral load.[15] Plasma CD4 + T-cell count and the CD4/CD8 ratio were measured as markers of immune activation. All outcomes were measured at baseline (ie, before initiation of cART), and 3 months, 1 year, and 9 years after start of cART.

Statistical Analysis

We described the absolute course of the coagulation parameters and markers of immune activation over the aforementioned periods. In addition, we analyzed changes in the coagulation parameters over the follow-up period. We compared 3 different follow-up periods (ie, baseline to 3 months, 3 months to 1 year, and 1 year to 9 years), because of the nonlinear trends of coagulation parameters over time. The sample size did not allow for nonlinear regression techniques.

Coagulation parameters were missing in 8 subjects at 3 months and another 8 subjects did not participate in the follow-up measurements (Figure 1). The missingness at 3 months was due to logistical reasons and was considered completely at random. We did not impute these missing outcome values and performed a complete case analysis for each follow-up period.

We calculated the crude change for each follow-up period and a corresponding 95% confidence interval with a one-sample Student *t* test. In the analysis of changes of the procoagulant factors (FVIII, vWF, and D-dimer) for 1 year versus 9 years, traditional cardiovascular risk factors can act as confounding

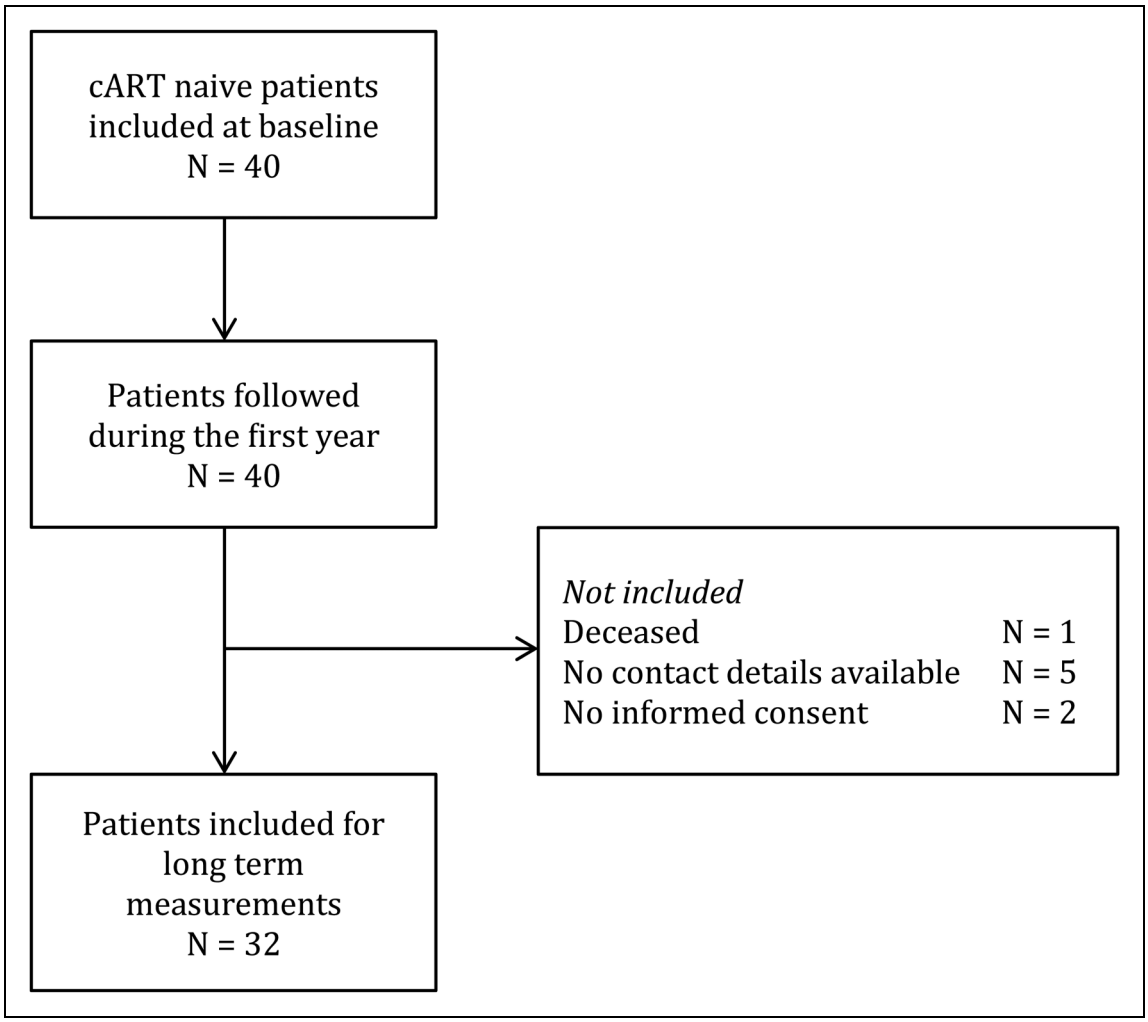

Figure 1. Patient flow.

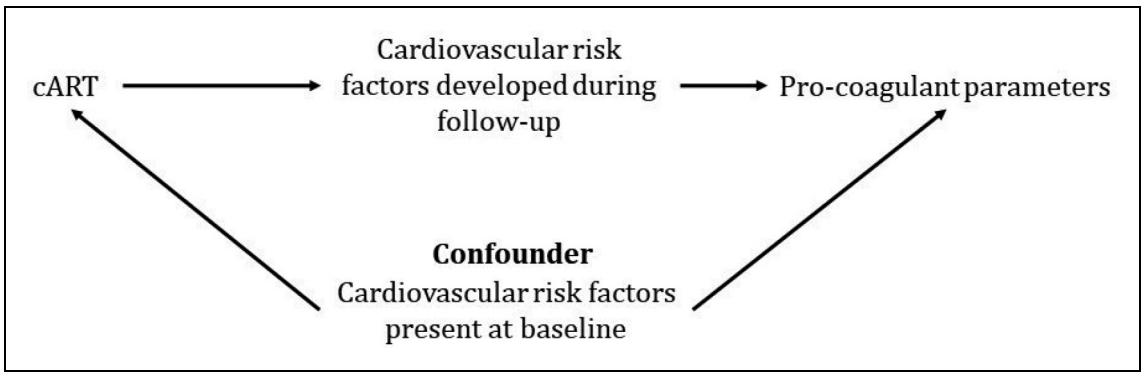

Figure 2. Directed acyclic graph displaying the relationship between combination antiretroviral therapy (cART) in people living with HIV (PLWH), procoagulant parameters, cardiovascular risk factor a baseline, and emerging cardiovascular risk factor.

factors, but this only applies to cardiovascular risk factors that are unrelated to the HIV infection or cART. This is explained in the directed acyclic graph depicted in Figure 2. HIV infection and cART itself are risk factors for developing cardiovascular risk factors. These cardiovascular risk factors emerging during follow-up are in the causal path of the association between cART and procoagulant factor levels, and therefore, do not fulfill the definition of a confounding factor. For this reason, we only consider cardiovascular risk factors present at baseline as confounding factors. Because of sample size considerations, we only corrected for age, smoking, and hypertension in multivariable linear regression. A second model only

including smoking and hypertension was evaluated too, as it seems implausible that age at inclusion itself would affect the change in (or slope of) coagulation parameters. Lastly, the association between changes in the coagulation parameters per unit change of CD4+ T-cell count and CD4/CD8-ratio was analyzed in linear regression for each follow-up period.

Descriptive variables are presented as means with a standard deviation (±SD) or as medians with an interquartile range (IQR) in the case of a normal and non-normal distribution, respectively. All analyses were performed in R 3.6.2 (R Core Team).

Sensitivity Analyses

Two sensitivity analyses were performed in which (1) 2 subjects with a history of arterial and/or venous thrombosis at baseline and (2) 2 patients with the incomplete viral response (ie, detectable viral load) at 9 years of cART were excluded.

Results

Patient Characteristics

A total of 40 male cART naive patients with HIV were included, in whom cART was initiated at median 11 (IQR 3-40) months after HIV diagnosis. All patients contributed one year of follow-up. Eight patients were lost to follow-up after this first year and were thus not available for long-term measurements (Figure 1). All others were included in the long-term analysis.

Baseline characteristics at study entry are summarized in Table 1. One patient had a history of venous thrombosis for which he was no longer treated with therapeutic anticoagulation. Two patients were treated with antiplatelet therapy, both because of a history of myocardial infarction. Lastly, one patient with atrial fibrillation was treated with a vitamin K antagonist.

The median follow-up time was 9 (IQR 9-10) years. In 29 of the 32 patients, who underwent long-term measurements, cART therapy was never interrupted. In the other 3 patients, cART therapy was temporarily discontinued for a few months because of severe abdominal infection with absorption problems, compliance issues, and a severe allergic reaction, respectively. However, all patients had been treated uninterruptedly for at least 4 years at the moment of long-term measurements. At 9 years since the start of cART, HIV viral load was at clinically non-relevant levels in all patients and CD4+ T-cell count had increased to 700 ± 250 cell/mm^3 and CD4/CD8 ratio to 1.00 ± 0.43. During follow-up, one patient suffered from an ischemic stroke and one from a myocardial infarction for which they both received antiplatelet therapy. Furthermore, one patient was diagnosed with liver cirrhosis developed from nonalcoholic fatty liver disease without impaired liver synthesis function.

Trend of Coagulation Factors

In Figure 3, the absolute trend of the coagulation parameters, CD4+ T-cell count, and CD4/CD8 ratio are displayed at baseline and 3 months, 1 year, and 9 years after start of cART.

Table 1. Patient Characteristics at Baseline.

	N = 40
Male sex (n)	40
Age mean (SD)	44 (12)
BMI (kg/m^2) mean (SD)	24 (3)
Months since HIV diagnosis median (IQR)	11 (3-40)
HIV RNA load (copies/mL) median (IQR)	87,100 (38,850-359,000)
CD4+ T-cell count (cells/mm^3) mean(SD)	260 (130)
CD4/CD8 ratio mean(SD)	0.27 (0.14)
Chronic hepatitis B (n)	2
Chronic hepatitis C (n)	1
Smoking (n)	
Never	13
Current	17
Former	10
Comorbidities (n)	
Venous thrombosis	1
Arterial thrombosis	2
Hypertension	7
Hypercholesterolemia	2
Diabetes mellitus	1
Malignancy	2[a]
Respiratory disease	3
Antithrombotics (n)	
Antiplatelet therapy	2
Vitamin K antagonist	1
Years of follow-up median (IQR)	9 (9-10)

Abbreviations: BMI, body mass index; HIV, human immunodeficiency virus; IQR, interquartile range; SD, standard deviation.
[a]Kaposi sarcoma and non-Hodgkin lymphoma.

Overall, during the entire follow-up period, CD4+ T-cell count and CD4/CD8 increased, indicating decreasing immune activation due to the HIV infection. Table 2 contains a summary of the absolute changes for the 3 different follow-up periods (ie, baseline vs 3 months, 3 months vs 1 year and 1 year vs 9 years).

At baseline, before start of cART, there were increased levels of procoagulant parameters (ie, FVIII, vWF, and D-dimer) and less pronounced decreased anticoagulant PS levels, indicating a procoagulant hemostatic balance (Table 1). During the first 2 follow-up periods (ie, baseline vs 3 months and 3 months vs 1 year), we observed a partial restoration of the hemostatic balance. The procoagulant parameters (ie, D-dimer, FVIII, and vWF) decreased during the first year of cART treatment (Figure 3 and Table 2). This decrease was most pronounced during the first 3 months of cART treatment. During the same periods, the total and free fraction of PS increased (Figure 3 and Table 2).

By contrast, between 1 and 9 years after start of cART, an increase in the procoagulant parameter levels was found with a simultaneous less pronounced increase in anticoagulant PS levels (Figure 3 and Table 2). During this period, there was a continuing decrease in immune activation (ie, increase in CD4+ T-cell count and CD4/CD8 ratio) (Figure 3). After adjustment for smoking and hypertension, this increase in procoagulant parameters was no longer present (Table 2). As expected, the impact of age on inclusion was limited.

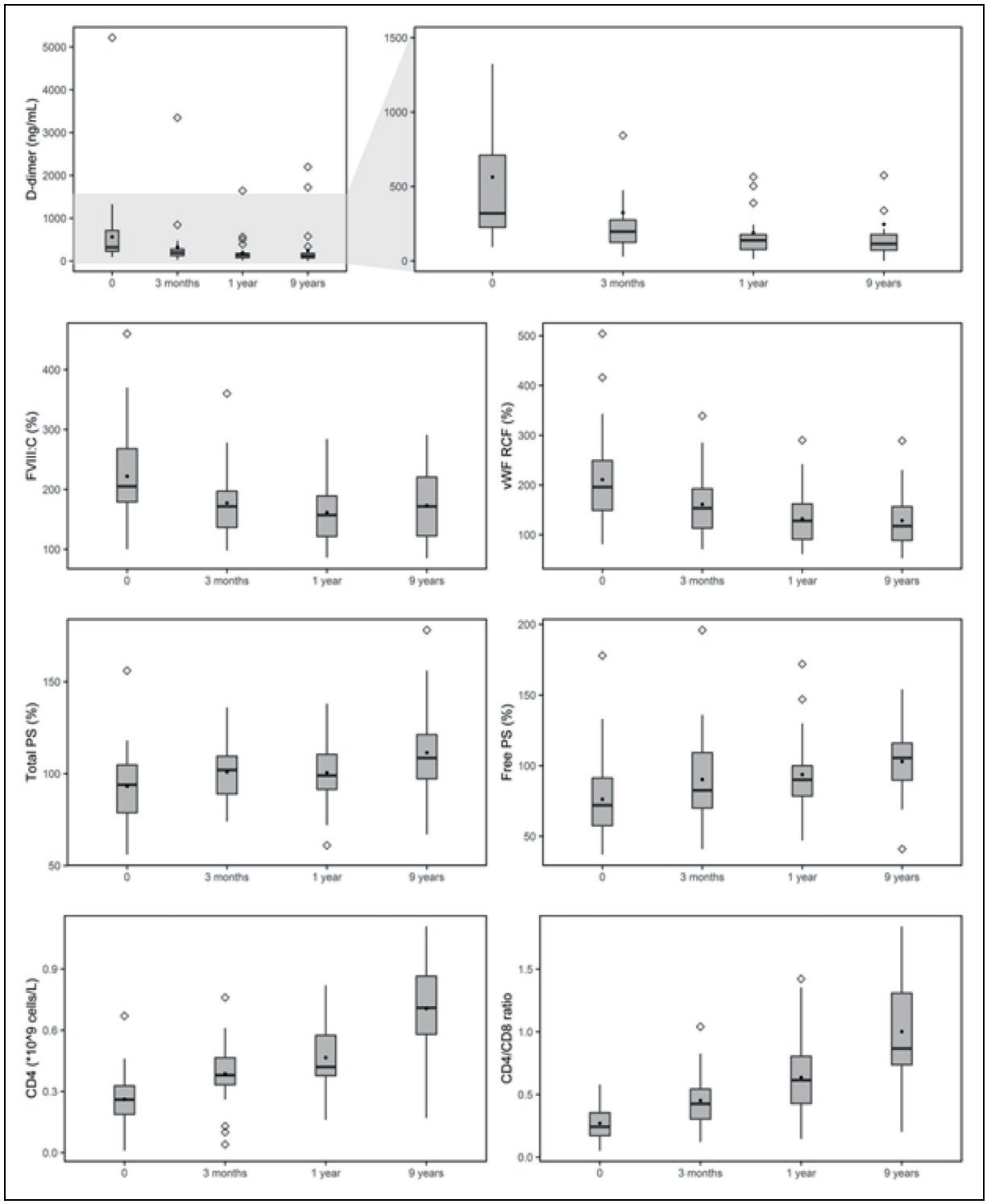

Figure 3. Trends of coagulation factors and immune activation over time. The boxes represent the interquartile ranges with the medians at the horizontal central lines. The upper and lower whiskers are defined as 1.5× second and third quartiles, respectively. Mean values at each time point are displayed as black dots within the boxes.

Association Between Immune Activation and Coagulation Parameters

Table 3 displays association of the absolute changes in the coagulation parameters and the markers for immune activation (ie, CD4+ T cell count and CD4/CD8 ratio) for the 3 different follow-up periods. There was an inverse association, although not significant for all parameters, between immune activation and the procoagulant parameters during the periods baseline versus 3 months and

Table 2. Analysis of Changes in Coagulation Parameters.

	Parameter	Crude[a]	Model 1[b]	Model 2[c]
Baseline versus 3 months	FVIII (%)	−37.5 (−53.9, −21.2)		
	vWF (%)	−52.7 (−77.9, −27.6)		
	D-dimer (ng/mL)	−239.5 (−376.9, −102.2)		
	Total PS (%)	7.6 (3.6, 11.6)		
	Free PS (%)	13.3 (3.4, 23.1)		
3 months versus 1 year	FVIII (%)	−12.7 (−26.1, 0.7)		
	vWF (%)	−24.0 (−36.0, −11.9)		
	D-dimer (ng/mL)	−114.9 (−231.4, 1.6)		
	Total PS (%)	−0.1 (−6.2, 6.0)		
	Free PS (%)	1.9 (−4.6, 8.5)		
1 year versus 9 years	FVIII (%)	15.6 (4.5, 26.7)	−6.5 (−23.5, 10.5)	−6.0 (−22.8, 10.8)
	vWF RCF (%)	1.2 (−13.3, 15.7)	−10.4 (−33.6, 12.7)	−10.5 (−33.2, 12.1)
	D-dimer (ng/mL)	102.4 (−33.3, 238.1)	−9.1 (−254.3, 236.2)	−22.3 (−268.6, 224.0)
	Total PS (%)	12.2 (4.2, 20.2)		
	Free PS (%)	13.2 (6.2, 20.2)		

Abbrevations: FVIII, factor VIII; PS, protein S; vWF, von Willebrand factor.
Crude and adjusted absolute changes in the coagulation parameters plasma activity or levels for baseline vs. 3 months, 3 months vs. 1 year, 1 year vs. 9 years respectively with 95% confidence intervals, estimated with one sample T-test and multivariable linear regression model.
[a]adjusted for mean-centered age, smoking and hypertension
[b]adjusted for smoking and hypertension

Table 3. Association Between Change in Immune Reconstitution and Change of Coagulation Factor Levels.[a]

	ΔCD4 + T-cell[b]	ΔCD4/CD8-ratio[c]
Baseline to 3 months		
ΔFVIII (%)	−30.9 (−47.0, −14.8)	−8.4 (−22.4, 5.6)
ΔvWF (%)	−46.6 (−70.0, −23.2)	−20.0 (−39.6, −0.2)
ΔD-dimer (ng/mL)	−166.5 (−306.8, −26.3)	−10.4 (−121.4, 100.7)
ΔTotal protein S (%)	−0.24 (−5.2, 4.7)	2.9 (−3.3, 3.9)
ΔFree protein S (%)	9.6 (−0.55, 19.7)	2.2 (−5.5, 9.9)
3 months to 1 year		
ΔFVIII (%)	−12.4 (−24.4, −0.34)	−5.3 (−12.9, 2.3)
ΔvWF (%)	−2.0 (−13.5, 9.6)	−4.4 (−11.3, 2.5)
ΔD-dimer (ng/mL)	−98.7 (−204.3, 6.9)	2.0(−6.6, 70.3)
ΔTotal proteinS (%)	0.53 (−5.3, 6.4)	−0.26 (−3.8, 3.3)
ΔFree protein S (%)	−2.8 (−8.8, 3.3)	1.9 (−1.8, 5.6)
1 year to 9 years		
ΔFVIII (%)	0.24 (−6.0, −6.5)	3.7 (0.17, 7.3)
ΔvWF (%)	−1.0 (−9.2, 7.1)	1.8 (−3.2, 6.8)
ΔD-dimer (ng/mL)	27.6 (−48.4, 103.6)	−17.1 (−64.0, 29.8)
ΔTotal protein S (%)	−1.9 (−6.2, 2.5)	−0.58 (−3.3, 2.1)
ΔFree protein S (%)	2.2 (−1.5, 5.9)	−0.85 (−3.2, 1.5)

Abbreviations: FVIII, factor VIII; PS, protein S; vWF, von Willebrand factor.
[a]The association between changes in the coagulation parameters per unit change of CD4+ T-cell count and CD4/CD8-ratio with a 95% confidence interval obtained from a linear regression analysis for each follow-up period is provided.
[b]Per $0.1*10^9$ cells/L increase.
[c]Per 0.1 increase.

3 months versus 1 year after start of cART. For the period 1 year versus 9 years after start of cART, this association was no longer present. There was no clear association between immune activation and PS, both total and free fraction, over all follow-up periods.

Sensitivity Analyses

The results of the sensitivity analyses were in line with the main analyses described above (Supplementary Appendix).

Discussion

In this cohort of cART naive HIV-infected subjects, we describe the long-term course of pro- and anticoagulant parameters after initiation of cART. Before start of cART, a procoagulant state was present with increased levels of procoagulant parameters (ie, FVIII, vWF, and D-dimer) and decreased levels of anticoagulant parameters (total and free PS), as described in other studies.[16–18] In line with a previously

published cohort with 6 months of follow-up,[16] this procoagulant balance was reversed partially during the first year and occurred with decreasing immune activation. At 9 years, we found yet again a trend of increasing procoagulant parameter levels despite continuously decreasing immune activation, which after adjustment for confounding factors (ie, smoking and hypertension at baseline) led to a neutral or slight decreasing trend. This suggests that in the long-term established cardiovascular risk factors become a determinant for a procoagulant state, instead of HIV itself.

This study adds to the current literature by providing a description of the long-term coagulation status in PLWH and an exploration of the underlying mechanisms. However, some aspects regarding the validity of this study should be taken into account when interpreting the results. The limited sample size yielded wide confidence intervals in the long-term analysis: the confidence interval for change over 8 years after adjustment for baseline risk factors includes 10 units of FVIII, which is in line with increases in the general population without adjustment for risk factors. Furthermore, the limited sample size did not allow us to consider a comprehensive set of cardiovascular risk factors potentially affecting procoagulant parameter trends (eg, diabetes mellitus, hypercholesterolemia, and body mass index).

For the question whether HIV itself accelerates the increase of procoagulant parameters, our study is clear: there is limited evidence for this. Another study in patients with Von Willebrand's disease supports this finding, even though it concerned a cross-sectional cohort. Higher vWF levels with increasing age in type I patients appeared to be associated with comorbidities at inclusion, rather than age itself.[19] However, we cannot exclude that HIV interacts with other risk factors to accelerate aging processes. For instance, acute HIV infection might accelerate the development of cardiovascular risk factors, such as hypertension and diabetes mellitus. Ideally, a control group would be available where we would be able to adjust for baseline characteristics and to make longitudinal comparisons. However, we are not aware of such data existing, as most population trends are derived from cross-sectional measurements, in which temporality is problematic.

Future research should aim to provide more precise estimates of trends in coagulation parameters in PLWH in a longitudinal cohort, preferably with a control group from the general population. A larger cohort would also allow us to evaluate the impact of other established cardiovascular risk factors. Furthermore, the interaction of HIV with the effect of these cardiovascular risk factors on (mainly) procoagulant parameter trends should be the subject of research too.

Until then, it is difficult to say what clinical implications our findings have. The SMART trial showed that a procoagulant state is associated with higher all-cause mortality.[4] The next step would be to investigate to what extent a persistent procoagulant state is associated with thrombotic events. Subsequently, specific strategies could be applied to treat a persistent procoagulant state. This could vary from methods to more effectively treat residual immune activation, better lifestyle management, or treatment directly targeting the coagulation system (ie, thromboprophylaxis).

In conclusion, during the first year, the procoagulant state in HIV is reversed partially under the influence of cART. In the long term, procoagulant parameters increase again despite an ongoing decrease in immune activation. Our findings suggest that this increase is determined by established cardiovascular risk factors, which may have been acquired during the untreated phase of HIV. Future research is needed to evaluate whether this increase in procoagulant parameters develops more steeply in PLWH than in the general population, preferably with controlled analyses. For the time being, clinicians should aim for optimal cardiovascular risk management in patients with HIV.

Authors' Note

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Declaration of Conflicting Interests

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Supplemental Material

Supplemental material for this article is available online.

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