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ORIGINAL ARTICLE

von Willebrand factor propeptide-to-antigen ratio in HIV-infected pregnancy: Evidence of endothelial activation

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Abstract

Background: Endothelial activation has been proposed as a potential mechanism for the increased risk of venous thromboembolism (VTE) in human immunodeficiency virus (HIV)–infected pregnancy.

Objectives: To assess the state of endothelial activation in HIV-infected pregnancy by measuring the von Willebrand factor (VWF) propeptide-to-antigen ratio, as an index of acute endothelial activation.

Methods: VWF antigen and VWF propeptide were measured in HIV-negative participants (n = 85), HIV-infected virologically suppressed participants, (n = 89) and HIV-infected participants with HIV viral load (VL) of >50 copies/ml (n = 63) in each trimester. Results were correlated with multimer patterns and a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS-13) antigen, activity, and antibody levels.

Results: VWF propeptide-to-antigen ratio was increased, in the first, second, and third trimester, in the HIV-infected virologically suppressed group (1.7 ± 0.7, 1.7 ± 0.4, 1.6 ± 0.5) and the HIV-infected group with VL > 50 copies/ml (1.9 ± 0.9, 1.7 ± 0.9, 1.6 ± 1.1) compared to the HIV-negative group (1.4 ± 0.6, 1.3 ± 0.4, 1.2 ± 0.3, $P < .05$). Increased high molecular weight multimers were observed in the HIV-infected groups, despite only a mild reduction in ADAMTS-13 activity compared to the HIV-negative group ($P < .001$). No correlation was observed between VWF antigen or VWF propeptide and ADAMTS-13 activity.

Conclusion: HIV-infected virologically suppressed pregnant participants showed persistent endothelial activation. Future research should focus on whether endothelial activation contributes to the excess risk of pregnancy-related VTE.

KEYWORDS
ADAMTS-13, antiretroviral therapy, endothelial activation, human immunodeficiency virus, pregnancy, von Willebrand factor antigen, von Willebrand factor propeptide

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1 | INTRODUCTION

Expanded antiretroviral therapy (ART) coverage has reduced the risks associated with human immunodeficiency virus (HIV) infection in pregnant women. However, non-infectious complications have emerged as an important cause of morbidity and mortality. Epidemiologic studies performed over the last decade have described an increased incidence of placental vascular complications, preterm delivery, and venous thromboembolism (VTE) in association with HIV and/or its treatment.[1-5] Recently, we have shown that this increased VTE risk can be attributed to a higher prevalence of obstetric and venous risk factors.[6] However, evidence from observational studies of VTE in non-pregnant populations suggests that HIV and its treatment play an important role, independent of traditional VTE risk factors.[7,8] Accordingly, HIV encoded proteins (gp120, Tat, and Nef) cause activation of pathways of inflammation and coagulation, resulting in endothelial activation.[9,10] Long-term ART results in a modest improvement in endothelial activation. However, normalization has not been consistently achieved.[11] It has been proposed that persistent endothelial activation may predispose virologically suppressed HIV-infected women to adverse pregnancy events such as VTE.[12]

The significance of endothelial activation has been increasingly shown in the pathogenesis of cardiovascular disease and VTE in non-pregnant HIV-infected populations.[8,13-16] Several soluble markers of endothelial activation have been investigated. These include: D-dimer, von Willebrand factor (VWF), soluble thrombomodulin, E-selectin, vascular cell adhesion molecule-1, and intercellular adhesion molecule-1. In HIV-infected pregnant women, endothelial activation has also been described. Increased D-dimer levels were found in ART-naïve HIV-infected pregnant women, albeit a small number ($n = 11$), compared to HIV-negative pregnant controls.[17]

The VWF propeptide-to-antigen ratio represents another important indicator of endothelial activation that has been associated with vascular disorders of pregnancy.[18] VWF propeptide (97 kDa containing 741 amino acids) and VWF antigen (270 kDa containing 2050 amino acids) are secreted following endothelial activation in equimolar amounts.[19] Different rates of clearance produce a molar ratio between VWF propeptide and VWF antigen of 1.3 in healthy subjects.[20] Normal pregnancy progression is associated with an increase in the half-life of VWF antigen (12–24 h), which is evidenced by a physiological decline in the plasma ratio.[21,22] In contrast, acute endothelial activation is characterized by an increase of the VWF propeptide-to-antigen ratio.[18] Studies to date, however, have not investigated the VWF propeptide-to-antigen ratio in HIV-infected pregnant women in the era of widespread access to ART.

To assess the state of endothelial activation in HIV-infected pregnancy, the present study aimed to determine the VWF propeptide-to-antigen ratio, as an index of acute endothelial activation, in each trimester and its relationship with HIV viral load (VL). Results were correlated with multimer patterns and ADAMTS-13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) antigen, activity, and antibody levels.

Essentials

- von Willebrand factor (VWF) propeptide-to-antigen ratio is a useful marker of endothelial activation.
- VWF propeptide-to-antigen ratio was measured in human immunodeficiency virus (HIV)-infected pregnant participants in each trimester.
- VWF propeptide-to-antigen ratio was increased in HIV-infected compared to HIV-negative participants.
- HIV participants showed evidence of persistent endothelial activation, despite virological suppression.

2 | MATERIALS AND METHODS

2.1 | Study design and population

Initially a pilot study was conducted between 1 April and 31 October 2019 to estimate an adequate sample size. This was followed by a prospective cross-sectional study over a 1-year period from 1 May 2020 to 30 April 2021. This is part of a research protocol, studying pregnancy-related thrombosis in HIV infection at the Charlotte Maxeke Johannesburg Academic Hospital Antenatal Clinic, Johannesburg, South Africa. This hospital is one of the specialist referral centers for HIV infected patients in Johannesburg with a reported antenatal HIV seroprevalence of ~18%. Written informed consent was obtained from all study participants, and the study protocol was approved by the Institutional Review Board (M-181018).

The study included three groups: HIV negative, HIV infected with virological suppression (<50 copies/ml), and HIV infected with VL > 50 copies/ml. Participants >18 years of age, with a singleton pregnancy, representative of the population attending the Antenatal Clinic, were recruited at the first antenatal care visit. Consecutive HIV-infected participants with virological suppression were enrolled in each trimester. For each HIV-infected participant with virological suppression, the first presenting HIV-negative participant and HIV-infected participant with VL > 50 copies/ml were selected as comparators. The three study groups were matched according to gestation and ethnicity. The gestational age, based on early ultrasound, was continuously monitored to ensure an even distribution of first trimester (6–12 weeks), second trimester (13–26 weeks), and third trimester (27–36 weeks) patients. Women with conditions known to be associated with an increased risk for VTE were excluded, namely: active infection/inflammation, active cancer, inherited thrombophilia, or a personal history of VTE. In addition, participants who developed thrombotic micro-angiopathies, pre-eclampsia, and macerated stillbirth

(intra-uterine fetal death >28 weeks) during the study period were excluded.

2.2 | Study protocol

2.2.1 | Data collection

Antenatal visits were scheduled at 2- to 4-week intervals from enrolment until delivery, including a visit at 6 weeks' postpartum. Data collected on predesigned data collection sheets included demographics, obstetric history, delivery characteristics, and symptomatic VTE during pregnancy and up to 6 weeks' postpartum. VTE was evaluated by compression ultrasound or ventilation/perfusion lung scan or computed tomography pulmonary angiogram. All study participants were screened for HIV infection prior to enrolment. Retesting was performed in the HIV-negative participants at follow-up antenatal visits. In the HIV-infected participants, data on ART (regimen, date of commencement, and adherence) and VL and CD4 cell counts measured on presentation were collected. First-line treatment consisted of a fixed-dose combination tablet containing tenofovir (TDF), emtricitabine and efavirenz or TDF, lamivudine (3TC), and dolutegravir. Second-line treatment consisted of zidovudine, 3TC, and aluvia (lopinavir/ritonavir). Apart from ART, the HIV-negative and HIV-infected participants received the same level of antenatal care.

2.2.2 | Blood sampling and laboratory methods

Blood sampling was performed at enrolment in each participant. Blood was collected in 3.2% sodium citrate (ratio of one volume trisodium citrate to nine volumes of blood; Becton-Dickinson) and dipotassium (K_2) ethylenediaminetetraacetic acid (EDTA). Platelet-poor plasma was obtained by centrifugation at 3500 x g for 15 min. Aliquots were stored at −80°C for analysis at the National Health Laboratory Service, South Africa. Daily quality control was performed prior to analysis.

2.2.3 | Hematological testing

Full blood count parameters were analyzed on the Sysmex XN 9000 hematology analyzers (Sysmex Corporation). ABO blood groups were not determined as a prior study indicated that ABO blood type does not significantly influence VWF level changes in pregnancy.[21]

2.2.4 | Endothelial cell activation testing

VWF antigen was measured with the STA-Liatest immunoturbidimetric assay (Diagnostica Stago) on the STA-R Max® automated coagulation analyzer (Diagnostica Stago). VWF propeptide was assayed with an in-house ELISA kit by the University of the Free State using antibodies from Cell Sciences as previously described.[23]

VWF multimer analysis was performed using gel electrophoresis on sodium dodecyl sulfate (SDS) 0.65% agarose gel with diluted plasma samples (1:40 for VWF Ag levels of 51%–143% and 1:80 for VWF Ag levels of >143%) as previously described.[24] Multimer patterns were further evaluated quantitatively by densitometry scanning using gene tools analysis software (Syngene). Assessment of multimer patterns were performed by two independent observers. Normal pooled plasma (NPP) was included in each run as a reference.

ADAMTS-13 antigen, activity, and antibody testing were performed using respective Technozym ADAMTS-13 fluorescence ELISA kits (Technoclone) according to the manufacturer's recommendations. The increase in fluorescence due to cleavage of the enzyme-linked peptide substrate, FRETS-VWF73 by ADAMTS-13 was measured at 37°C using spectrofluorometry (Synergy HT Reader, Biotek).

2.3 | Statistical methods

A target sample size of at least 25 participants per study group from each trimester of pregnancy was calculated from the initial pilot study based on an analysis of variance (ANOVA) overall fixed effect size of 0.30 with no interactions with a power of 90% and an $\alpha = .05$. Clinical and laboratory data were analyzed using Statistica 13.2 software and SAS 9.1 software. Normally distributed, continuous data were presented as mean ± standard deviation (SD) and variables with non-Gaussian distribution as median (interquartile range [IQR]). Variables with a non-Gaussian distribution were log transformed and presented as mean ± SD. Comparisons for continuous measurements were performed using a parametric one-way ANOVA test with Scheffe's post hoc test or non-parametric Kruskal-Wallis test between the study participants subdivided according to the trimester of pregnancy and the study group. Comparisons for categorical measurements were performed using Chi-square test or Fisher's exact test when necessary. Pearson's correlation coefficients were used to measure the linear associations between normally distributed, continuous variables. Significance level was set at <0.05.

3 | RESULTS

3.1 | Patient characteristics

During the 22-month study period, 260 pregnant participants, of African ethnicity, were eligible for enrolment in the study. Twenty-three were excluded and therefore the final analysis consisted of 237 participants (Figure 1). The mean ± SD gestational age was

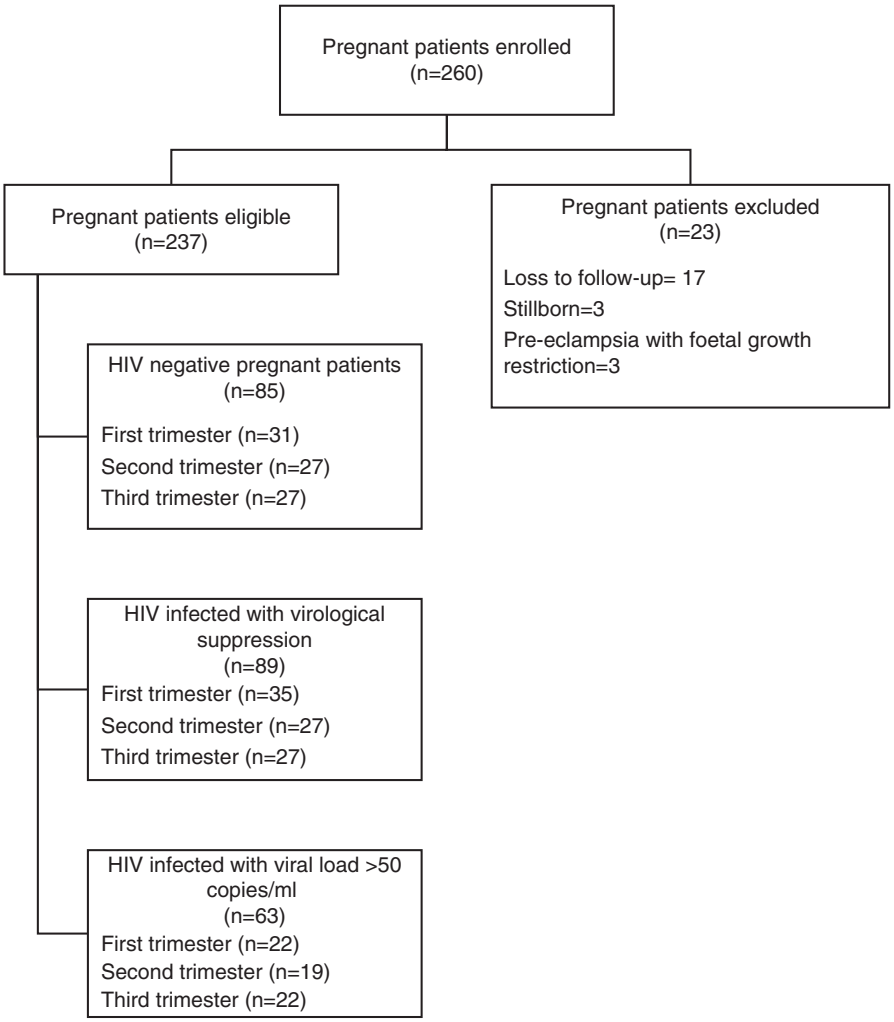

FIGURE 1 Flow diagram of study patients

9 ± 3 weeks for the first trimester participants, 23 ± 4 weeks for the second trimester participants, and 34 ± 2 weeks for the third trimester participants.

Table 1 shows the demographics and clinical and laboratory characteristics of the three study groups. The mean age of the study participants was 33 ± 6 years. The HIV-infected group with virological suppression was associated with an increased maternal age and subsequently parity compared to the HIV-negative group ($P < .001$ and $P < .020$, respectively). White blood cell count and hemoglobin levels were significantly lower in the HIV-infected groups compared to the HIV-negative group ($P < .012$ and $P < .001$, respectively). There were 218 (91.98%) live births. There was a statistically significant difference in the gestational age at delivery and the birth weights between the HIV-infected group with virological suppression and the HIV-negative group ($P < .040$ and $P < .001$, respectively). There were no cases of VTE.

Baseline HIV characteristics differed significantly between the HIV groups with virological suppression and VL > 50 copies/ml (Table 2). The HIV-infected study group with virological suppression consisted of 89 participants with a median [IQR] CD4 count of 551 [259] x 10^6/L on ART for a median of 5.0 [7.0] years. The majority ($n = 86$, 96.62%) were on first-line treatment. In the HIV-infected study group with VL > 50 copies/ml, there were 63 HIV- infected pregnant participants with a median VL of 527 [7377] copies/ml and median CD4 count of 306 [280] x 10^6/L. Of these, 39 (61.90%) were newly diagnosed with HIV infection. A further 24 (38.10%) presented with VL > 50 copies/ml on ART for a median of 5^4 years.

3.2 | Laboratory characteristics

3.2.1 | VWF antigen and VWF propeptide

Log VWF antigen and propeptide levels, measured in each trimester, showed a significant increase with pregnancy progression (Table S1 in supporting information). Among the three study groups, a significant difference in the overall plasma concentrations of log VWF antigen and propeptide, within each trimester, was observed (Table 3). This resulted in a significant difference in the overall VWF propeptide-to-antigen ratio among the three study groups within each trimester. Subgroup analysis with multiple pairwise comparisons showed no statistically significant decreases between the HIV-infected group with virological suppression and the HIV-infected group VL > 50 copies/ml.

TABLE 1 Demographics and clinical, laboratory, and delivery characteristics of the three study groups

Characteristic	HIV negative ($n = 85$)	HIV and VL <50 copies/ml ($n = 89$)	HIV and VL >50 copies/ml ($n = 63$)	*P* value
Demographics				
Age at study entry (years), mean ± SD	31.0 ± 6.0	34.0 ± 6.0***	32.0 ± 6.0	<.001
Parity, median [IQR]	1.0 [1.0]	2.0 [2.0]*	2.0 [2.0]	.008
BMI (kg/m^2), mean ± SD	28.3 ± 5.6	27.6 ± 5.8	27.4 ± 6.0	.545
Smoking history >10/day (*n*, %)	0 (0)	0 (0)	0 (0)	-
Laboratory characteristics				
White cell count (x10^9/L), mean ± SD (ref: 3.9–12.6)	7.9 ± 2.0	7.7 ± 2.4**	6.7 ± 2.1*	.012
Hemoglobin (g/L), mean ± SD (ref: 116–164)	124.5 ± 12.8	119.3 ± 14.7	114.5 ± 16.6**	<.001
Platelet count (x 10^9/L), mean ± SD (ref: 186–454)	254.7 ± 74.0	249.2 ± 66.0	261.3 ± 99.3	.719
Delivery characteristics of live births				
Termination of pregnancy (*n*, %)	0 (0)	5 (5.6)	0 (0)	.058
Live births (*n*, %)	78 (91.8)	79 (88.8)	61 (96.8)	
Intra-uterine fetal death (*n*, %)	7 (8.2)	5 (5.6)	2 (3.2)	
Gestational age delivery (weeks), mean ± SD	39.0 ± 2.0	37.0 ± 4.0*	38.0 ± 2.0	.040
Birthweight (g), mean ± SD	3284.3 ± 535.3*	2942.2 ± 585.6***	3027.9 ± 494.8	<.001
Outcome				
VTE (*n*, %)	0 (0)	0 (0)	0 (0)	-

Note: Intra-uterine fetal death was defined as the death of fetus at >10 week and <28 weeks of gestation. *$P < .05$ versus HIV negative; **$P < .01$ versus HIV negative; ***$p < .001$ versus HIV negative.

Abbreviations: BMI, body mass index; HIV, human immunodeficiency virus; IQR, interquartile range; SD, standard deviation; VL, viral load; VTE, venous thromboembolism.

TABLE 2 HIV characteristics of the HIV-infected study groups

Characteristic	HIV and VL < 50 copies/ml ($n = 89$)	HIV and VL > 50 copies/ml ($n = 63$)	*P* value
HIV characteristics			
Years from diagnosis, median [IQR]	5.0 [7.0]	0.3 [3.0]	<0.001
CD4 cell count (x106/L), median [IQR]	551 [259]	306 [280]	<0.001
HIV viral load (copies/ml), median [IQR]	<50	527 [7377]	<0.001
ART, (*n*, %)			
First-line therapy[a]	86 (96.6)	9 (14.3)	<0.001
Second-line therapy[b]	3 (3.4)	15 (23.8)	
No therapy	0 (0)	39 (61.9)	

Abbreviations: ART, antiretroviral therapy; HIV, human immunodeficiency virus; IQR, interquartile range.

[a]First-line consisted of tenofovir, emtricitabine and efavirenz or tenofovir, lamivudine and dolutegravir.

[b]Second-line consisted of zidovudine, lamivudine, and aluvia.

3.2.2 | VWF multimer analysis

VWF multimer analysis was performed in a subgroup of HIV-infected participants with virological suppression ($n = 63$) and HIV-infected pregnant participants with VL > 50 copies/ml ($n = 36$). A pattern of increased high molecular weight multimers (HMWM) and loss of the low molecular weight multimers (LMWM) was observed in 30 (47.6%) HIV-infected pregnant participants with virological suppression and 17 (47.2%) HIV-infected pregnant participants with VL > 50 copies/ml. This was not statistically significantly different between the HIV-infected study groups ($p = 1.00$; Figures S1 and S2 in supporting information).

3.2.3 | ADAMTS-13 activity and antibodies

There were no differences in ADAMTS-13 antigen, activity, and antibody levels measured in each trimester (Table S1). ADAMTS-13

TABLE 3 Comparison of von Willebrand factor–associated parameters between HIV negative, HIV infected with virological suppression, and HIV infected with viral load (VL) > 50 copies/ml study groups

Parameter	Reference Ranges	Gestational age	HIV negative	HIV and VL < 50 copies/ml	HIV and VL > 50 copies/ml	*P* value
VWF antigen (U/dl) Log VWF antigen	92.8–150.6	First trimester	123.0 [64.9] 4.7 ± 0.4	141.9 [86.6] 5.0 ± 0.4*	147.7 [77.2] 5.0 ± 0.4	.131 .021
	98.7–174.4	Second trimester	167.9 [47.8] 5.1 ± 0.3	186.8 [62.2] 5.2 ± 0.3*	175.9 [97.1] 5.2 ± 0.3	.764 .042
	134.0–245.6	Third trimester	208.0 [65.1] 5.3 ± 0.3	225.3 [98.4] 5.4 ± 0.4	228.7 [82.8] 5.4 ± 0.4	.283 .038
VWF propeptide (U/dl) Log VWF propeptide	116.9–169.4	First trimester	162.0 [60.7] 5.1 ± 0.4	219.0 [100.6] 5.4 ± 0.4***	218.9 [101.3] 5.4 ± 0.4***	<.001 <.001
	116.0–154.9	Second trimester	209.8 [103.6] 5.3 ± 0.4	301.9 [129,3] 5.8 ± 0.4***	269.3 [204.7] 5.6 ± 0.4	.009 <.001
	120.8–188.0	Third trimester	230.7 [135.5] 5.4 ± 0.6	289.0 [219.3] 5.7 ± 0.7*	262.3 [277.4] 5.6 ± 0.8*	.019 .001
VWF pp to Ag ratio	1.04–1.43	First trimester	1.4 ± 0.6	1.7 ± 0.7	1.9 ± 0.9*	.030
	0.88–1.27	Second trimester	1.3 ± 0.4	1.7 ± 0.4	1.7 ± 0.9	.033
	0.69–1.00	Third trimester	1.2 ± 0.3	1.6 ± 0.5	1.6 ± 1.1	.035
ADAMTS-13 antigen (%)	50–150	First trimester	67.0 ± 23.7	61.0 ± 23.0	63.6 ± 24.1	.591
	50–150	Second trimester	65.6 ± 19.0	67.0 ± 24.2	67.8 ± 21.8	.940
	50–150	Third trimester	67.3 ±14.4	69.4 ± 24.1	67.3 ± 20.8	.908
ADAMTS-13 activity (%)	50–150	First trimester	84.5 ± 31.4	55.1 ± 25.2***	53.6 ± 23.3***	<.001
	50–150	Second trimester	92.9 ± 27.3	62.7 ± 30.1***	64.6 ± 29.2**	<.001
	50–150	Third trimester	96.1 ± 24.4	71.5 ± 29.9**	63.4 ± 26.5***	<.001
ADAMTS-13 autoantibodies n, (%) [6]	0–15	First trimester	4 (12.9)	3 (8.6)	5 (22.7)	.296
	0–15	Second trimester	4 (14.8)	4 (14.8)	5 (26.3)	.551
	0–15	Third trimester	5 (18.5)	3 (11.1)	5 (22.7)	.534

Note: *$P < .05$ versus HIV negative; **$P < .01$ versus HIV negative; ***$P < .001$ versus HIV negative.

Abbreviations: ADAMTS-13, a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; HIV, human immunodeficiency virus; VL, viral load; VWF Ag, von Willebrand factor antigen; VWF pp, von Willebrand factor propeptide.

activity levels were statistically significantly decreased, when measured in each trimester, between the HIV-infected virologically suppressed group and the HIV-infected group with VL > 50 copies/ml, compared to the HIV-negative group (Table 3). There was no difference, however, in ADAMTS-13 antigen levels or the presence of circulating antibodies among the three study groups.

3.2.4 | Correlations

ADAMTS-13 antigen levels correlated positively with log VWF antigen ($r = .20, P < .002$) and log VWF propeptide levels ($r = .20, P < .012$), respectively. No correlation was observed with ADAMTS-13 activity and log VWF antigen or propeptide levels.

4 | DISCUSSION

The endothelium is the critical regulator of the hemostatic balance in pregnancy. Endothelial activation or dysfunction, directly by HIV infection and/or pro-inflammatory cytokines, has been proposed as a potential mechanism for the increased risk of VTE among HIV-infected pregnant women.[7,8,25] In the present study, we demonstrated an increase in the overall VWF propeptide, VWF antigen, and VWF propeptide-to-antigen ratio in HIV-infected participants with VL > 50 copies/ml as well as HIV-infected participants with virological suppression compared to matched HIV-negative participants. The increased VWF propeptide-to-antigen ratio corresponds to the acute release of VWF antigen and VWF propeptide predominantly from activated endothelial cells as demonstrated by *in vivo* studies with l-deamino-8-D-arginine vasopressin (DDAVP) and endotoxin.[20] This finding of an increased VWF propeptide-to-antigen ratio represents a potentially useful measure of acute endothelial activation associated with HIV infection.[18]

Earlier studies have proposed VWF propeptide, which regulates the secretion of VWF, as a more reliable index of endothelial activation compared to VWF antigen alone.[26] There is also increasing evidence to suggest that VWF propeptide plays an important role in inflammatory and cell adhesion processes.[27,28] The current study

adds to the limited data on the effect of maternal HIV infection on the expression of VWF propeptide. Increased VWF propeptide has also been reported in pregnancies complicated by pre-eclampsia and hemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome. Notably, an increase of VWF propeptide of up to 3-fold has been described in HELLP syndrome, consistent with the underlying mechanisms of acute endothelial dysfunction and increased maternal inflammation.[18] In the current study, a modest increase in VWF propeptide was found in the HIV-infected participants, compared to the HIV-negative group. This can be attributed to the study design, which excluded participants with factors associated with the increased release of VWF propeptide, such as vascular complications in the index pregnancy. Future studies are needed to determine whether the significantly increased VWF propeptide-to-antigen ratio, suggestive of acute endothelial activation, contributes to the excess risk of pregnancy-related VTE and placental vascular complications in HIV.

This study confirmed a previous report of endothelial cell activation in HIV-infected pregnant women with VL > 50 copies/ml.[17] Importantly, this study also demonstrated a significantly increased VWF propeptide-to-antigen ratio in HIV-infected participants on long-term suppressive ART, compared to HIV-negative participants. Nonetheless, no significant decline in the VWF propeptide-to-antigen ratio was observed in this group, compared to HIV-infected participants with VL > 50 copies/ml. Similarly, persistent endothelial activation has been reported in non-pregnant HIV-infected populations on long-term ART.[29,30] In particular, large observational studies have illustrated a decline in D-dimer levels with virological suppression.[31-34] However, D-dimer levels remained significantly elevated compared to HIV-negative controls[13,35] and pre-HIV levels.[36,37] Several mechanisms for ongoing endothelial activation on suppressive long-term ART have been proposed. These include: loss of the gastro-intestinal mucosal integrity and microbial translocation of bacterial lipopolysaccharide and bacterial DNAs.[38-40] These microbial products aid in the recruitment of tissue factor–bearing monocytes to the persistently activated endothelium.[9]

Moreover, the multimer pattern is critical for the function of VWF. This is illustrated by a relative loss of HMWM from the third trimester of pregnancy[21] in an attempt to balance the hypercoagulability of pregnancy. A modest elevation in factor VIII levels has also been described in physiological pregnancy.[41] In contrast, in the present study, increased HMWM were detected in approximately half of a subgroup of HIV-infected participants. Moreover, there was no significant difference in the pattern of increased HMWM between participants with virological suppression and VL > 50 copies/ml. Under physiological conditions, these larger circulating multimers, which bind and aggregate platelets, are rapidly proteolyzed by the VWF cleaving metalloprotease, ADAMTS-13, into smaller, less prothrombotic multimers.[42] In this study, the acquired increase in HMWM, however, was observed in the presence of a mild reduction in ADAMTS-13 activity, which did not achieve clinical significance. Likewise, antibodies directed against ADAMTS-13 were not significantly increased in HIV-infected participants compared to HIV-negative participants. Therefore, the increased HMWM cannot be explained by a deficiency in ADAMTS-13 activity alone. Studies in patients with sepsis have proposed that ultra-large multimers accumulate because of the inhibition of ADAMTS-13 by inflammatory cytokines, in particular interleukin-6. Nonetheless, a deficiency and/or antibodies directed against ADAMTS-13 have not been consistently demonstrated in HIV infection.[43,44] Earlier studies of HIV-related thrombotic thrombocytopenic purpura reported ADAMTS-13 levels that were not significantly reduced to cause overt pathology.[45,46] More recently, elevated VWF levels in HIV-infected men at various stages of infection were also not associated with lower ADAMTS-13 levels.[47] Rather, endothelial activation appears to be the predominant mechanism for increased circulating large multimers in HIV infection in pregnant and non-pregnant populations.[12]

Our results must be interpreted in the light of certain limitations. First, the study was not statistically powered to draw conclusions regarding the direct relationship between the VWF propeptide-to-antigen ratio and the risk of pregnancy-related VTE. The study enrolled participants at low risk for VTE and accordingly there were no cases of VTE recorded during the study period. Second, multimer analysis was not performed in the HIV-negative participants. Rather NPP was included in each run of HIV-infected participants as a control. Third, the presence of ultra-large multimers could not be visualized using 0.65% agarose gels. A final study limitation is that assays of VWF activity and FVIII activity were not performed due to sample volume constraints. Nonetheless, to the best of our knowledge, this is one of the first studies to investigate the VWF propeptide-to-antigen ratio as a measure of endothelial cell activation in HIV-infected pregnant participants.

In conclusion, virological suppression was not associated with a significant reduction in the VWF propeptide-to-antigen ratio, which is suggestive of persistent endothelial activation on long-term ART. Additionally, increased HMWM among the HIV-infected groups was observed in the presence of a modest reduction of ADAMTS-13 activity. This, too, suggests the importance of endothelial activation in the pathogenesis of thrombosis in HIV-infected pregnant women. Future longitudinal studies should focus on whether persistent endothelial activation contributes to the increased risk of thrombosis in pregnancy and the postpartum period.

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CONFLICTS OF INTEREST

The authors have declared no conflicts of interest with respect to the authorship and/or publication of this article.

AUTHOR CONTRIBUTIONS

Conceptualization: E. Schapkaitz. Statistical analysis: E. Schapkaitz and E. Libhaber. Supervision: E. Libhaber, B. F. Jacobson, H. R. Buller. Writing of original draft: E. Schapkaitz. Review and editing: E. Schapkaitz, E. Libhaber, B. F. Jacobson, H. R. Buller, M. Meiring.

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REFERENCES

1. Stringer EM, Kendall MA, Lockman S, et al. Pregnancy outcomes among HIV-infected women who conceived on antiretroviral therapy. *PLoS ONE*. 2018;13(7):e0199555.
2. Uthman OA, Nachega JB, Anderson J, et al. Timing of initiation of antiretroviral therapy and adverse pregnancy outcomes: a systematic review and meta-analysis. *Lancet HIV*. 2017;4(1):e21-e30.
3. Theron G, Brummel S, Fairlie L, et al. Pregnancy outcomes of women conceiving on antiretroviral therapy (ART) compared to those commenced on ART during pregnancy. *Clin Infect Dis*. 2021;73(2):e312-e320.
4. Howard JFB, Rokx C, Smit C, et al. Incidence of a first venous thrombotic event in people with HIV in the Netherlands: a retrospective cohort study. *Lancet HIV*. 2019;6(3):e173-e181.
5. Jansen JM, Lijfering WM, Sprenger HG, van der Meer J, van Pampus MG. Venous thromboembolism in HIV-positive women during puerperium: a case series. *Blood Coagul Fibrinolysis*. 2008;19(1):95-97.
6. Schapkaitz E, Libhaber E, Rhemtula H, et al. Pregnancy-related venous thromboembolism and HIV infection. *Int J Gynaecol Obstet*. 2021; Online ahead of print.
7. Musselwhite LW, Sheikh V, Norton TD, et al. Markers of endothelial dysfunction, coagulation and tissue fibrosis independently predict venous thromboembolism in HIV. *Aids*. 2011;25(6):787-795.
8. van den Dries LW, Gruters RA. von Willebrand Factor is elevated in HIV patients with a history of thrombosis. *Front Microbiol*. 2015;6:180.
9. Funderburg NT, Mayne E, Sieg SF, Asaad R, et al. Increased tissue factor expression on circulating monocytes in chronic HIV infection: relationship to in vivo coagulation and immune activation. *Blood*. 2010;115(2):161-167.
10. Wada NI, Jacobson LP, Margolick JB, et al. The effect of HAART-induced HIV suppression on circulating markers of inflammation and immune activation. *Aids*. 2015;29(4):463-471.
11. van der Heijden WA, Wan J, Van de Wijer L, et al. Plasmatic coagulation capacity correlates with inflammation and abacavir-use during chronic HIV-infection. *J Acquir Immune Defic Syndr*. 2021;87(1):711-719.
12. Jong E, Louw S, van Gorp EC, Meijers JC, ten Cate H, Jacobson BF. The effect of initiating combined antiretroviral therapy on endothelial cell activation and coagulation markers in South African HIV-infected individuals. *Thromb Haemost*. 2010;104(6):1228-1234.
13. Neuhaus J, Jacobs DR Jr, Baker JV, et al. Markers of inflammation, coagulation, and renal function are elevated in adults with HIV infection. *J Infect Dis*. 2010;201(12):1788-1795.
14. Ford ES, Greenwald JH, Richterman AG, et al. Traditional risk factors and D-dimer predict incident cardiovascular disease events in chronic HIV infection. *Aids*. 2010;24(10):1509-1517.
15. Affi R, Gabillard D, Dunyach-Remy C, et al. Association of plasma soluble vascular cell adhesion molecule-1 and sCD14 with mortality in HIV-1-infected west african adults with high CD4 counts. *J Acquir Immune Defic Syndr*. 2021;86(1):138-145.
16. Siedner MJ, Bwana MB, Asiimwe S, et al. Inflammatory biomarkers prior to antiretroviral therapy as prognostic markers of 12-month mortality in South Africa and Uganda. *Aids*. 2019;33(13):2043-2048.
17. de Andrade CM, Duarte G, Quintana SM, Montes MB, Toloi MR. Effect of antiretroviral therapy on hemostasis in Brazilian pregnant women with HIV infection. *Blood Coagul Fibrinolysis*. 2007;18(8):769-774.
18. Hulstein JJ, van Runnard Heimel PJ, Franx A, et al. Acute activation of the endothelium results in increased levels of active von Willebrand factor in hemolysis, elevated liver enzymes and low platelets (HELLP) syndrome. *J Thromb Haemost*. 2006;4(12):2569-2575.
19. Wagner DD. Cell biology of von Willebrand factor. *Annu Rev Cell Biol*. 1990;6:217-246.
20. Borchiellini A, Fijnvandraat K, ten Cate JW, et al. Quantitative analysis of von Willebrand factor propeptide release in vivo: effect of experimental endotoxemia and administration of 1-deamino-8-D-arginine vasopressin in humans. *Blood*. 1996;88(8):2951-2958.
21. Drury-Stewart DN, Lannert KW, Chung DW, et al. Complex changes in von Willebrand factor-associated parameters are acquired during uncomplicated pregnancy. *PLoS ONE*. 2014;9(11):e112935.
22. Sie P, Caron C, Azam J, et al. Reassessment of von Willebrand factor (VWF), VWF propeptide, factor VIII: C and plasminogen activator inhibitors 1 and 2 during normal pregnancy. *Br J Haematol*. 2003;121(6):897-903.
23. Maleka R, Meiring M. Verification of a rapid von Willebrand factor propeptide assay. *J Med Lab Sci Technol South Africa*. 2020;2(2):36-40.
24. Meiring M, Badenhorst PN, Kelderman M. A rapid and cost-effective method to visualize von Willebrand factor multimers in plasma. *Medical Technology SA*. 2005;19(2):16-18.
25. Nossent AY, Van marion V, Van tilburg NH, et al. von Willebrand factor and its propeptide: the influence of secretion and clearance on protein levels and the risk of venous thrombosis. *J Thromb Haemost*. 2006;4(12):2556-2562.
26. van Mourik JA, Boertjes R, Huisveld IA, et al. von Willebrand factor propeptide in vascular disorders: A tool to distinguish between acute and chronic endothelial cell perturbation. *Blood*. 1999;94(1):179-185.
27. Takagi J, Sekiya F, Kasahara K, Inada Y, Saito Y. Inhibition of platelet-collagen interaction by propolypeptide of von Willebrand factor. *J Biol Chem*. 1989;264(11):6017-6020.
28. Taooka Y, Chen J, Yednock T, Sheppard D. The integrin alpha9beta1 mediates adhesion to activated endothelial cells and transendothelial neutrophil migration through interaction with vascular cell adhesion molecule-1. *J Cell Biol*. 1999;145(2):413-420.
29. Mosepele M, Mohammed T, Mupfumi L, et al. HIV disease is associated with increased biomarkers of endothelial dysfunction despite viral suppression on long-term antiretroviral therapy in Botswana. *Cardiovasc J Afr*. 2018;29(3):155-161.
30. Graham SM, Rajwans N, Tapia KA, et al. A prospective study of endothelial activation biomarkers, including plasma angiopoietin-1 and angiopoietin-2, in Kenyan women initiating antiretroviral therapy. *BMC Infect Dis*. 2013;13:263.
31. Hamlyn E, Stohr W, Cooper DA, et al. The effect of short-course antiretroviral therapy initiated in primary HIV-1 infection on interleukin-6 and D-dimer levels. *Aids*. 2015;29(11):1355-1361.
32. Castillo-Mancilla JR, Morrow M, Boum Y, et al. Brief report: higher ART adherence is associated with lower systemic inflammation in treatment-naive ugandans who achieve virologic suppression. *J Acquir Immune Defic Syndr*. 2018;77(5):507-513.
33. Kiefer EM, Hoover DR, Shi Q, Dusingize JC, Sinayobye JD, Anastos K. Longitudinal evaluation of markers of inflammation in HIV-positive and HIV-negative Rwandan women. *HIV Med*. 2018;19(10):734-744.

34. Teasdale CA, Hernandez C, Zerbe A, Chege D, Hawken M, El-Sadr WM. Changes in D-dimer after initiation of antiretroviral therapy in adults living with HIV in Kenya. *BMC Infect Dis*. 2020;20(1):508.
35. Subramanya V, McKay HS, Brusca RM, et al. Inflammatory biomarkers and subclinical carotid atherosclerosis in HIV-infected and HIV-uninfected men in the Multicenter AIDS Cohort Study. *PLoS ONE*. 2019;14(4):e0214735.
36. Freiberg MS, Bebu I, Tracy R, et al. D-dimer levels before HIV seroconversion remain elevated even after viral suppression and are associated with an increased risk of non-AIDS events. *PLoS ONE*. 2016;11(4):e0152588.
37. Baker JV, Sharma S, Grund B, et al. Systemic inflammation, coagulation, and clinical risk in the START trial. *Open Forum Infect Dis*. 2017;4(4):ofx262.
38. Shivakoti R, Gupte N, Kumar NP, et al. Intestinal barrier dysfunction and microbial translocation in human immunodeficiency virus-infected pregnant women are associated with preterm birth. *Clin Infect Dis*. 2018;67(7):1103-1109.
39. Lopez M, Figueras F, Coll O, et al. Inflammatory markers related to microbial translocation among HIV-infected pregnant women: a risk factor of preterm delivery. *J Infect Dis*. 2016;213(3):343-350.
40. Brenchley JM, Price DA, Schacker TW, et al. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. *Nat Med*. 2006;12(12):1365-1371.
41. Thornton CA, Bonnar J. Factor VIII-related antigen and factor VIII coagulant activity in normal and pre-eclamptic pregnancy. *Br J Obstet Gynaecol*. 1977;84(12):919-923.
42. Gerritsen HE, Robles R, Lammle B, Furlan M. Partial amino acid sequence of purified von Willebrand factor-cleaving protease. *Blood*. 2001;98(6):1654-1661.
43. Bernardo A, Ball C, Nolasco L, Moake JF, Dong JF. Effects of inflammatory cytokines on the release and cleavage of the endothelial cell-derived ultralarge von Willebrand factor multimers under flow. *Blood*. 2004;104(1):100-106.
44. Kremer Hovinga JA, Zeerleder S, Kessler P, et al. von Willebrand factor and related parameters in severe sepsis and septic shock. *J Thromb Haemost*. 2007;5(11):2284-2290.
45. Benjamin M, Terrell DR, Vesely SK, et al. Frequency and significance of HIV infection among patients diagnosed with thrombotic thrombocytopenic purpura. *Clin Infect Dis*. 2009;48(8):1129-1137.
46. Gunther K, Garizio D, Nesara P. ADAMTS13 activity and the presence of acquired inhibitors in human immunodeficiency virus-related thrombotic thrombocytopenic purpura. *Transfusion*. 2007;47(9):1710-1716.
47. Graham SM, Chen J, Le J, et al. Von Willebrand factor adhesive activity and ADAMTS13 protease activity in HIV-1-infected men. *Int J Med Sci*. 2019;16(2):276-284.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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