

HIV INFECTION, ANTIRETROVIRAL THERAPY AND THE HAEMOSTASIS OF PREGNANCY



UNIVERSITY OF THE
WITWATERSRAND,
JOHANNESBURG

Elise Schapkaitz

**A thesis submitted to the Faculty of Health Sciences, University of the Witwatersrand,
in fulfilment of the requirements for the degree of
Doctor of Philosophy**

Johannesburg, August, 2023

DECLARATION

I declare that this thesis is my own work. It is being submitted for the degree of Doctor of Philosophy at the University of the Witwatersrand, Johannesburg.

This thesis is submitted in the optional format, approved by the faculty, of published work with a supporting introduction, a literature review, and conclusion.

It has not been submitted before for any degree or examination at any other University.

A handwritten signature in cursive script, reading "Elise Schapkaitz", written over a horizontal line.

Elise Schapkaitz

14 day of August 2023 in Johannesburg

DEDICATION

To Brad, Saul, Yoni and Livi

LIST OF PUBLICATIONS

The work presented in this thesis has been published in the following peer reviewed manuscripts:

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6. **Schapkaitz E**, Libhaber E, Rhemtula H, Gerber A, Jacobson BF, Chauke L, Buller HR: Pregnancy-related venous thromboembolism and HIV infection. *Int J Gynaecol Obstet* 2021 Oct;155(1):110-118.

The declaration of the contribution of the candidate and co-author agreements are provided in Appendix 8.2.

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LIST OF CONGRESS PRESENTATIONS

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2. **E Schapkaitz**. Pregnancy and Anticoagulation in the management and prevention of VTE. Women in Thrombosis Congress 14-16 May 2022, Pretoria, South Africa
3. **E Schapkaitz**, S Middeldorp, K Dheda. Women who have been diagnosed with TB, HIV or COVID -19: What are the risks of coagulation? Women in Thrombosis Congress 15-16 May 2021, Johannesburg, South Africa
4. **E Schapkaitz**, BF Jacobson, E Libhaber, M Meiring and H R Büller. von Willebrand Factor Propeptide to Antigen Ratio: Evidence of Endothelial Activation in HIV Infected Pregnancy. International Society of Thrombosis and Haemostasis Congress (ISTH) 17-21 July 2021, Philadelphia, USA
5. **E Schapkaitz**. Diagnostics and Management of HIV and Vascular Complications During Gestation. Women's Health Issues in Thrombosis and Haemostasis (WHITH). 9th International Symposium. 1 October 2021
6. **E Schapkaitz**. Pregnancy and Anticoagulation –Practical Considerations. South African Society of Thrombosis and Haemostasis (SASTH) Congress 7 November 2021, Johannesburg, South Africa
7. **E Schapkaitz**. Thromboprophylaxis in HIV infected pregnant women. Women in Thrombosis Congress 22-23 February 2020, Johannesburg, South Africa
8. **E Schapkaitz**. Pregnancy, HIV infection and ART. Women in Thrombosis Congress 22-24 February 2019, Johannesburg, South Africa

ABSTRACT

The human immunodeficiency virus (HIV) epidemic affects an estimated 30% of pregnant women living in South Africa. Increasing evidence suggests that women living with HIV are at a heightened risk for venous thrombo-embolism (VTE), which is a significant contributor to maternal mortality. In addition to a higher prevalence of obstetric and venous risk factors, this increased risk of VTE has been attributed to the effects of HIV and/or its treatment. HIV is characterized by immune activation and inflammation, which promote endothelial dysfunction and activation of coagulation. This is more pronounced with untreated HIV, yet this pro-inflammatory and pro-thrombotic balance may persist with long-term suppressive antiretroviral therapy (ART). However, the extent to which ongoing inflammation disrupts maternal haemostasis and predisposes pregnant women living with HIV to its prothrombotic consequences, is currently unknown.

The aims of the work presented in this thesis in women living with HIV with access to ART were firstly to identify antepartum and postpartum risk factors for VTE; secondly to assess procoagulant changes in maternal haemostasis; and thirdly to determine risks of thrombosis and bleeding associated with thromboprophylaxis for VTE prevention.

An epidemiological case-control study was performed in 128 cases with pregnancy related VTE and 640 matched controls. This study found at least a two-fold increased risk for VTE among pregnant and postpartum women living with HIV. In addition, antepartum risk factors, that may explain the disproportion of VTE risk in HIV, included medical co-morbidities and chronic hypertension, while postpartum risk factors included a personal history of VTE, medical co-morbidities, systemic infection, prolonged hospital admission and postpartum haemorrhage. Opportunistic infections, ART and the degree of immunosuppression were not associated with VTE risk.

A sub-study followed and investigated antiphospholipid antibodies (aPL) in 215 women with thrombosis and/or obstetric complications. In this study, 15 (13.2%) of the women with HIV were positive at baseline for one of the five criteria aPL. The prevalence of aPL was not significantly increased among women with HIV, as compared to HIV negative women.

Furthermore, the aPL profiles were not significantly different between the two groups. Lupus anticoagulant (LAC) positivity, on a single occasion, was associated with thrombosis ($p < 0.003$).

Subsequently two prospective cross-sectional studies were conducted which assessed endothelial activation as well as fibrinolysis, coagulation and platelet activation in pregnant women with HIV, in each trimester. The studies included three groups: HIV negative, HIV with virological suppression (< 50 copies/mL) and HIV with viral load (VL) of >50 copies/mL. Endothelial activation was evaluated by measuring von Willebrand factor (VWF) antigen, VWF propeptide, multimer patterns and ADAMTS-13 antigen, activity, and antibody levels. The results showed an increase in the ratio of VWF propeptide to VWF antigen in the first, second and third trimester, in the HIV virologically suppressed group (1.7 ± 0.7 , 1.7 ± 0.4 , 1.6 ± 0.5) and the HIV 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 , $p < 0.05$). Virological suppression was not associated with a significant reduction in this ratio, in each trimester. In addition, increased high molecular weight multimers were observed in the HIV groups, despite only a mild reduction in ADAMTS-13 activity compared to the HIV negative group ($p < 0.001$).

Thereafter, fibrinolytic activity was evaluated by measuring d-dimer and plasminogen activator inhibitor-1 (PAI-1). Coagulation activity was determined by measuring thrombin-antithrombin (TAT) complex concentrations, and platelet factor-4 and platelet indices, namely mean platelet volume (MPV) and platelet distribution width as a measure of platelet activation. The results showed increased log d-dimer levels in the first, second and third trimester, in the HIV virologically suppressed group (-1.2 ± 0.5 , -0.9 ± 0.4 , -0.5 ± 0.3) and the HIV group with VL > 50 copies/mL (-1.1 ± 0.4 , -0.7 ± 0.4 , -0.5 ± 0.5) compared to the HIV negative group (-1.4 ± 0.2 , -1.1 ± 0.3 , -0.8 ± 0.3 , $p < 0.05$). Additionally, log PAI-1 levels were increased in the first, second, and third trimester, in the HIV virologically suppressed group (1.0 ± 0.4 , 1.3 ± 0.4 , 1.5 ± 0.4) and the HIV with VL > 50 copies/mL (0.8 ± 0.5 , 1.2 ± 0.4 , 1.5 ± 0.3) compared to the HIV negative group (0.4 ± 0.5 , 0.8 ± 0.3 , 1.3 ± 0.3 , $p < 0.05$). Virological suppression was not associated with a significant reduction in first and third trimester d-dimer and PAI-1 levels. Thrombin-antithrombin complex levels were not increased, in the HIV virologically suppressed group as compared to the HIV negative group, beyond the first trimester. With regard to platelet parameters, only log MPV measured in the third trimester was decreased in the HIV

virologically suppressed group (2.3 ± 0.1) and the HIV group with VL > 50 copies/mL (2.3 ± 0.1) compared to the HIV negative group (2.5 ± 0.2) ($p < 0.001$).

The last study was a longitudinal study of 129 pregnant women at intermediate or high risk of VTE, who received thromboprophylaxis. Venous thrombo-embolism occurred antepartum in 1.4%, 95% confidence interval (CI) 0.04-7.7 of intermediate and 3.4%, 95% CI 0.4-11.7 of high risk pregnancies. Major, clinically relevant non-major and minor bleeding events occurred in 7.1%, 95% CI 2.4-15.9 of intermediate and 8.5%, 95% CI 2.8-18.7 of high risk pregnancies. Owing to the small number of events, this study could not assess for HIV as a predictor of thrombosis and bleeding.

Thus, in conclusion, the findings described in the studies in this thesis contribute to our knowledge in pregnant women living with HIV in the following ways. Firstly, HIV emerged as a significant antepartum and postpartum risk factor for VTE. Traditional obstetric and venous risk factors were also linked to the risk of thrombosis and could be useful for identifying women with HIV, who may benefit from postpartum and/or antepartum thromboprophylaxis. Secondly, this thesis identified heightened markers of endothelial activation and impaired fibrinolysis. Markers such as the ratio of VWF propeptide to VWF antigen, d-dimer and PAI-1 may provide a biological mechanism for the increased risk of pregnancy-related VTE in HIV. Finally, this thesis provided rates of thrombosis and bleeding in women who received thromboprophylaxis in pregnancy and the postpartum period which can be used to advise women with HIV of the associated risks.

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LIST OF ABBREVIATIONS

ASH: American Society of Haematology

a β 2GP1: anti-beta2-glycoprotein 1 antibodies

aCL: anticardiolipin antibodies

aPL: antiphospholipid antibodies

APS: Antiphospholipid syndrome

aPS/PT: antiphosphatidylserine-prothrombin antibodies

ART: antiretroviral therapy

BMI: body mass index

CNS: central nervous system

CI: confidence interval

DVT: deep vein thrombosis

DOAC: direct oral anticoagulant

HIV: human immunodeficiency virus

LMWH: low molecular weight heparin

LAC: lupus anticoagulant

MPV: mean platelet volume

OR: odds ratio

PAI-1: plasminogen activator inhibitor-1

PDW: platelet distribution width

PF4: platelet factor-4

PPH: postpartum haemorrhage

PE: pulmonary embolism

RCOG: Royal College of Obstetrics and Gynaecology

SA: South Africa

SLE: systemic lupus erythematosus

TAT: thrombin-antithrombin

TF: tissue factor

TFPI-2: tissue factor pathway inhibitor -2

VTE: venous thrombo-embolism

UFH: unfractionated heparin

UK: United Kingdom

VL: viral load

VKA: Vitamin K antagonists

VWF: von Willebrand factor

WHO: world health organisation

CHAPTER 1: Introduction and background to thesis

1.1 Introduction to the thesis

Globally, the World Health Organization (WHO) estimates that 1.3 million pregnant women are living with human immunodeficiency virus (HIV) infection (1). The prevalence of HIV varies markedly by geographic distribution, with the largest burden of disease in Eastern and Southern Africa. South Africa (SA) is at the epicentre of the HIV epidemic with the largest number of infections in the world. Among pregnant women attending antenatal clinics in SA, the estimated prevalence is 30%, 95% confidence interval (CI) 29.4–30.6 (2). Access to antiretroviral therapy (ART) for pregnant women in SA has significantly improved, with greater than 95% ART coverage in 2019, following the implementation in 2015 of revised eligibility criteria (2).

Despite widespread access to ART, pregnancy in women living with HIV is associated with an increased morbidity as compared with the general population. Recent epidemiologic studies have described venous thrombo-embolism (VTE) as well as placental vascular complications and preterm delivery with HIV and/or its treatment (3-6). Venous thrombo-embolism, which comprises deep vein thrombosis (DVT) and its complication pulmonary embolism (PE), remains a significant contributor to morbidity in pregnant and postpartum women (7, 8).

Early in the HIV epidemic, the risks of VTE in pregnant and postpartum women with HIV were attributed to an increased prevalence of classical venous and/or obstetric risk factors for VTE (9, 10). Subsequently, research has focused on the role of HIV associated inflammation and immune activation (11-13). In non-pregnant populations, HIV proteins and pro-inflammatory cytokines have been shown to promote endothelial dysfunction and systemic activation of coagulation (12, 14). In these populations, a mechanistic link has been demonstrated between endothelial activation, coagulation and VTE (15, 16). Moreover, increasing evidence suggests that this pro-thrombotic and pro-inflammatory state does not steadily improve with suppressive,

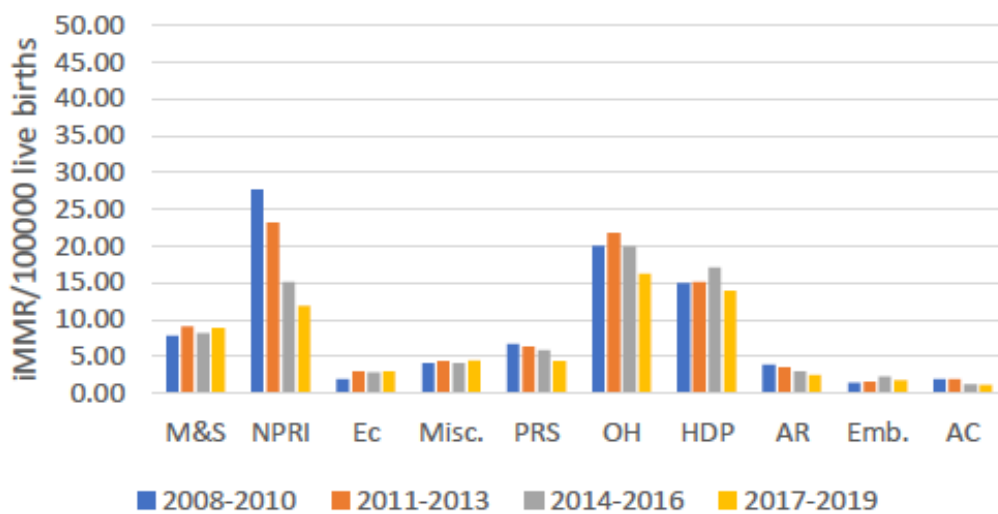
long-term ART (17-20). Little research, however, has been conducted in pregnant women, in the era of suppressive ART, that addresses the degree to which persistent inflammation predisposes pregnant women with HIV to its prothrombotic consequences.

1.2 Background to pregnancy related venous thrombo-embolism

1.2.1 Epidemiology of venous thrombo-embolism in pregnancy and the puerperium

The prevalence of pregnancy related VTE is estimated to be 0.5-2 per 1000 deliveries (21 , 22-25). Pregnancy increases the risk of VTE by four to five-fold (21). Pregnancy is characterised by hypercoagulability which continues for several weeks after delivery and the risk for VTE is greatest in the first six weeks postpartum (26). The risk of VTE peaks at three weeks postpartum with estimates of up to 84-fold increase in the risk of VTE in this period, have been reported (27, 28). Of the VTE cases, DVT accounts for approximately 70 to 80% and 20 to 30% present as PE (22, 29). Deep vein thrombosis is more prevalent during the antepartum period, while PE is more frequent during the postpartum period (21). Deep vein thrombosis in pregnancy frequently presents in the left leg and is more proximal often involving the iliofemoral veins, as compared to non-pregnant women (29, 30). Symptomatic pelvic vein thrombosis is a rare complication associated with caesarean deliveries and peripartum infections (31).

Venous thrombo-embolism is an important cause of maternal mortality and long-term morbidity. In high-income countries, PE has been identified as a major cause of direct maternal mortality which accounts for approximately 20% of pregnancy-related deaths (8, 24). In SA, a resource limited country, the major cause of death continues to be infection related. Nonetheless, maternal mortality reports (1999 to 2019) have not shown a significant reduction in the mortality rate from PE (Fig 1.1) (32, 33). Rates of 3 to 4.5 per 100 000 live births for maternal mortality secondary to PE have been reported during this period. A maternal death is a devastating event with far-reaching effects for the woman's family and community. Importantly, VTE can also have significant morbidities, including post-thrombotic syndrome, recurrent thrombosis, functional impairments, and reduced quality of life.



Key: iMMR, institutional maternal mortality ratio; M&S, pre-existing medical and surgical conditions; NPRI, non-pregnancy related infection; Ec, ectopic pregnancy; Miscar, miscarriage; PRS, pregnancy related sepsis; OH, obstetric haemorrhage; HDP, hypertensive disorders of pregnancy; AR, anaesthetic-related deaths; Emb, pulmonary embolism; AC, acute collapse, cause unknown

Figure 1.1 Trends in South African institutional maternal mortality rate per underlying cause, 2008-2019

Reproduced with permission from S N Cebekhulu (32, 33)

1.2.2 Haemostatic changes of pregnancy

Many factors such as physiological changes in coagulation and fibrinolysis contribute to this prothrombotic state of pregnancy and the puerperium. Pregnant women may also experience damage to the vascular endothelium during delivery. Further progesterone-induced vasodilatation and inferior vena cava and pelvic vein compression by the uterus cause stasis of the lower extremities. Changes in haemostasis begin early in pregnancy with the migration and invasion of the trophoblast cells. The trophoblast cells, which express procoagulant and anticoagulant surface receptors, maintain the local haemostatic balance. As pregnancy progresses, increasing concentrations of coagulation factors, maternal platelet and endothelial cell microparticles, platelet activation, reduced levels of specific anticoagulants and decreased fibrinolysis are responsible for the acquired hypercoagulability of pregnancy (Fig 1.2) (34). These changes in the haemostatic system are designed to ensure the integrity of the expanding maternal and fetal circulations at the placental interface and to control bleeding at the time of

placental separation.

Coagulation is initiated by tissue factor (TF) which is constitutively expressed at low levels by the trophoblast cell layer of the intervillous space (35). Tissue factor bearing microparticles, which are shed upon activation or apoptosis during trophoblast differentiation, also contribute to activation of coagulation (36, 37). Normal pregnancy is characterised by an increase in the concentrations of coagulation factors VII, VIII, IX, X, XII, prothrombin fragments 1 and 2 and thrombin-antithrombin (TAT) complexes as well as a rise in von Willebrand factor (VWF) and fibrinogen (38). Platelet counts decline during pregnancy, in particular in the third trimester. In addition to haemodilution, sequestration and consumption of platelets in the placental circulation are responsible for lower platelet counts in pregnancy. Platelets which release vasoactive substances, in particular from the alpha granules, are active mediators of primary haemostasis. However, the mechanisms and degree of platelet activation in normal pregnancy have not been properly characterised (39-41).

Physiological anticoagulants decrease with advancing gestational age, with a significant reduction in protein S activity as well as resistance to activated protein C (42, 43). There is a reduction in free as well as total protein S activity. This can be attributed in part to the increased circulating complement 4B-binding protein during pregnancy, which complexes with 60% of circulating protein S. Moreover, the transcription of protein S in pregnancy is upregulated by oestrogen response elements in the *PROS1* gene promoter (44).

Fibrinolytic activity is impaired from the second trimester and returns to normal after delivery. This is largely as a result of the decreased activity of tissue plasminogen activator as well as increased physiological synthesis of plasminogen activator inhibitor type 1 (PAI-1) and placental derived plasminogen activator inhibitor type 2 (34). Fibrinolysis is also inhibited by a progressive increase in maternal thrombin-activatable fibrinolysis inhibitor levels (45). Levels of fibrin degradation products increase with advancing gestational age which reflect an overall increase in fibrin production during pregnancy.

The hypercoagulability of pregnancy is balanced by upregulation of a variety of physiological anticoagulants on the trophoblast cells from the tenth week of gestation (46, 47). Tissue factor pathway inhibitor-1 in addition to placental tissue factor pathway inhibitor-2 (TFPI-2) progressively increase during pregnancy and prevent excessive thrombin formation (48). Another anticoagulant is annexin A5, which is abundant on the apical surfaces of the trophoblast cells (49). Annexin A5 binds phosphatidylserine and thereby modulates the fluidity of the maternal circulation (50). In addition, thrombomodulin, which is expressed on endothelial and trophoblast cells, ameliorates the procoagulant activity of thrombin and inactivates essential co-factors, V and VIII (47).

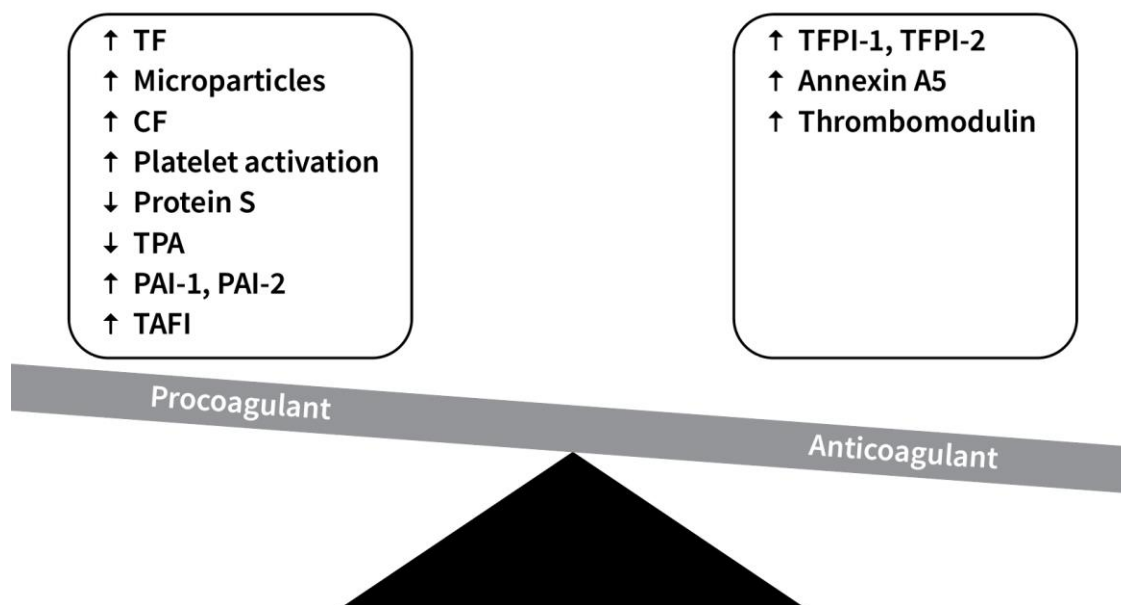


Figure 1.2 Haemostatic changes during pregnancy

Key: TF, tissue factor; CF, coagulation factors; TAT, thrombin antithrombin; APCR, acquired resistance to activated protein C; TPA, tissue plasminogen activator; PAI, plasminogen activator inhibitor; TAFI, thrombin-activatable fibrinolysis inhibitor; TFPI, tissue factor pathway inhibitor

1.2.3 Risk factors for venous thrombo-embolism in pregnancy and the puerperium

Whilst pregnancy and the puerperium are associated with a background risk for VTE, multiple risk factors which further increase this risk have been identified (51-53). Risk factors for VTE are prevalent among pregnant and postpartum populations. In a recent study of 21,000 Irish women, at least one postpartum risk factor for VTE was present in 75% of women, while at least two risk factors were present in approximately 40% of women (54). These individual risk factors have been shown to differ according to the antepartum and postpartum periods. In particular, a personal history of thrombosis and inherited thrombophilia have been shown to be strong risk factors for VTE (22, 55-57).

Personal history of thrombosis. In women with a personal history of thrombosis, the conditions of the first thrombotic event influence the recurrence risk in pregnancy and the puerperium. In particular, a prior unprovoked or pregnancy or oestrogen related VTE are associated with a higher risk of recurrence as compared to a previous provoked VTE, associated with transient risk factors (56-59). In a study of 1,104 women, among women with a personal history of unprovoked and pregnancy or oestrogen related VTE, the rate of VTE recurrence was 7.5%, 95% CI 4.0–13.7 in pregnancy. During the puerperium, the reported risks of recurrent VTE were greatest for women with a prior pregnancy related VTE (15.5%, 95% CI 7.7–28.7) (57).

Thrombophilia. An estimated 20 to 50% of women with a personal history of thrombosis have an associated thrombophilia, according to studies from high income countries (60). The extent of the increase in risk varies according to the thrombophilia and the presence of a first degree relative with a VTE history (61, 62) (Table 1.1). A positive family history is associated with a two to four-fold risk for VTE (63). Factor V Leiden and prothrombin gene G20120A mutations, which are prevalent among Caucasian populations (61, 62), have not been associated with thrombosis among populations of African ethnicity (64).

Table 1.1 The risk of pregnancy related venous thrombo-embolism in women with thrombophilia mutations and a family history (55)

Thrombophilia	Estimated relative risk of VTE OR (95% CI)	Estimated absolute risk of VTE % of pregnancies (95% CI) Studies with a positive family history	Estimated absolute risk of VTE % of pregnancies (95% CI) Non-family studies
Antithrombin deficiency	9.5 (1.6-31.9)	16.6 (0.0-45.1)	*
Protein C deficiency	9.3 (2.1-43.1)	7.8 (0.0-33.8)	*
Protein S deficiency	7.0 (1.3-21.9)	4.8 (0.0-20.0)	*
Factor V Leiden, homozygous	35.8 (0.4-137.8)	8.3 (0.0-29.6)	5.6 (0.0-34.3)
Factor V Leiden, heterozygous	6.4 (4.0-9.7)	2.4 (0.9-4.4)	0.4 (0.0-0.9)
Prothrombin G20210A mutation, homozygous	21.1 (0.0 -727.4)	*	*
Prothrombin G20210A mutation, heterozygous	5.1 (2.6- 9.8)	1.0 (0.0-2.5)	0.8 (0.1-2.0)
Compound heterozygous factor V Leiden and prothrombin G20210A mutation	21.2 (1.6- 89.0)	2.5 (0.0-9.5)	*
Key: OR, odds ratio; CI, confidence interval; VTE, venous thrombo-embolism			
*Insufficient data			

Antiphospholipid syndrome. Antiphospholipid syndrome (APS) is another important risk factor for thrombosis (65). The estimated prevalence of APS is 51 (95% CI 31.0- 72.0) per 100,000 women (66). Antiphospholipid syndrome is characterised by persistent antiphospholipid antibodies (aPL) in conjunction with clinical criteria (67, 68). The clinical criteria include small-vessel, arterial or venous thrombosis and/or pregnancy-related morbidity¹. The most

¹ ≥ three unexplained consecutive pregnancy losses < 10 weeks, foetal death ≥ 10 weeks, placental insufficiency or severe preeclampsia-eclampsia necessitating delivery < 34 weeks

frequently affected arterial and venous sites are the cerebral arteries and deep veins of the legs respectively. Thrombosis also occurs at unusual sites such as the hepatic, visceral or cerebral veins. The aPL profile as well as concomitant clinical risk factors influence the thrombotic risk. The risks of thrombosis are associated with the type of aPL, the combination of more than one positive aPL, medium to high antibody titres, and the persistence of aPL. The presence of combined positivity for lupus anticoagulant (LAC), anticardiolipin (aCL) and anti-beta2-glycoprotein 1 (a β 2GP1) antibodies is particularly associated with an increased risk of thrombosis as well as thrombosis recurrence (69). The cumulative incidence of thrombosis is 12%, 95% CI 10.0–15.0 at one year, 26%, 95% CI 22.0–30.0 at five years, and 44%, 95% CI 39.0–50.0 at ten years (70). Lupus anticoagulant specifically, is a recognised risk factor for thrombosis. Nonetheless, the predictive value of a single positive LAC is unclear and inconsistent results have been reported (71-73). Further studies are indicated to identify target epitopes for autoantibodies responsible for isolated LAC. In recent years, clinical studies have also illustrated the role of “non-criteria aPL” in thrombotic events as well as pregnancy morbidity (74). In particular, antibodies against the domain I of beta2-glycoprotein and antiphosphatidylserine-prothrombin antibodies (aPS/PT) may be useful to confirm or exclude high risk aPL profiles in patients with incomplete profiles (75, 76).

The risk of thrombosis in women with APS is also influenced by other clinical factors for venous and arterial thrombosis. Studies have identified chronic hypertension, hypercholesterolemia and smoking, which are known risks for arterial thrombosis (77, 78). In addition, underlying systemic lupus erythematosus (SLE) is associated with a 20-fold increased risk of VTE as compared to primary APS (79). The adjusted Global APS Score, which incorporates cardiovascular risk factors and aPL profile, is predictive of thrombotic recurrence risk (80, 81). Additional parameters continue to be evaluated for their value in APS diagnosis and thrombosis prediction.

Clinical risk factors for VTE. In addition, there are several clinical antepartum and postpartum risk factors which are associated with a modest effect on VTE (Tables 1.2 and 1.3). These include advanced age and parity, medical co-morbidities, significant pregnancy complications

and caesarean delivery (22, 53, 82). According to a United Kingdom (UK) population study, maternal obesity and multiparity were associated with an approximately three-fold and four-fold increased risk of PE respectively (82). Maternal obesity is an increasingly relevant risk factor worldwide. Obesity (83) and other risk factors, in combination, have also been shown to interact and further increase the risk of VTE, namely immobilisation and body mass index (BMI) ≥ 25 kg/m²; as well as postpartum infection following normal vaginal delivery (52).

Ethnicity also influences clinical VTE risk factors. Only a few studies have examined the associations in African pregnant women, despite the higher prevalence of thrombosis (21, 22-25). The increased risk of thrombosis among African-American populations has been attributed to factors such as chronic hypertension, diabetes mellitus, chronic renal disease, obesity, sickle cell disease and HIV infection (64, 84). A recent review has been dedicated to understanding the prothrombotic mechanisms of VTE in women with HIV in pregnancy and the postpartum period (see Chapter one, Publication one).

Several risk stratification tools which consider clinical VTE risk factors have been recommended in order to guide VTE prevention (85-89). For example, the Maternity Clot Risk prediction model is a risk score for postpartum VTE, which was developed using clinical databases in the UK and validated with Swedish and UK data (88, 90). The model included 15 statistically significant risk factors, of which, emergency caesarean delivery, stillbirth, varicose veins, preeclampsia, systemic infection, and medical co-morbidities emerged as the most relevant predictors of postpartum VTE. This model has not been prospectively validated. More recently, the performance of a risk-stratification protocol was evaluated retrospectively in 24 229 women (91). Thromboprophylaxis was associated with an increased risk of wound hematomas (adjusted odds ratio [OR] 2.3, 95% CI 1.5–3.6), unplanned surgical procedures (adjusted OR 1.3, 95% CI 1.1–1.6), and blood transfusions (adjusted OR 1.3, 95% CI 1.2–1.6). Randomized studies are necessary to determine the optimal threshold for thromboprophylaxis for clinical VTE risk factors. (90, 92, 93). One such trial, the international PARTUM (Postpartum Aspirin to Reduce Thromboembolism Undue Morbidity) randomized trial,

comparing postpartum low-dose acetylsalicylic acid with placebo in women with intermediate VTE risk factors, is currently underway (94).

Table 1.2 Antepartum risk factors for venous thrombo-embolism

Risk factors for VTE	aOR/IRR (95% CI)
Maternal Characteristics	
Age > 35 years	1.4 (1.0–2.0)
Parity ≥ 3	0.9 (0.5–1.8)
Obesity (BMI ≥30 kg/m ²)	1.4 (0.9–2.2) (53)
Smoking (10-30 cigarettes/day)	1.2 (0.8–1.6)
Gross varicose veins	2.2 (1.6–4.8)
Medical co-morbidities	
Heart disease *	7.1 (6.2–8.3) (22)
Sickle cell disease*	6.7 (4.4–10.1) (22)
Systemic lupus erythematosus*	8.7 (5.8–13.0) (22)
Pre-existing diabetes mellitus	3.5 (1.1–11.0) (53)
Cancer	2.0 (0.9-4.4) (53)
Inflammatory bowel disease	3.5 (1.1-10.9) (53)
Pregnancy characteristics	
Multiple pregnancy	0.8 (0.3–2.6) (53)
ART (singleton)	4.3 (2.0-9.4) (52)
Dehydration/hyperemesis	2.5 (1.4–4.5) (95)
Pre-eclampsia with intra-uterine growth restriction	1.0 (0.3–4.0) (52)
Antepartum immobilization with BMI < 25 kg/m ²	7.7 (3.2–19.0) (52)
Antepartum immobilization with BMI ≥25 kg/m ²	62.3 (11.5–337.6) (52)
Acute respiratory tract infection	1.7 (0.9-2.9) (53)
Urinary tract infection	1.8 (1.2-2.7) (53)
Key: aOR, adjusted odds ratio; IRR, incidence rate ratio; CI, confidence interval; BMI, body mass index; ART, assisted reproductive technology *as a predictor of antepartum and postpartum VTE	

Table 1.3 Postpartum risk factors for venous thrombo-embolism

Risk factors for VTE	aOR/IRR (95% CI)
Maternal Characteristics	
Age > 35 years	1.4 (1.2–3.0) (53)
Parity ≥ 3	1.9 (1.2–3.0) (53)
Obesity (BMI ≥30 kg/m ²)	3.5 (2.5–4.7) (53)
Smoking (10-30 cigarettes/day)	1.3 (1.0–6.9) (53)
Gross varicose veins	3.9 (2.6–5.9) (53)
Medical co-morbidities	
Heart disease	5.3 (1.3–21.5) (53)
Sickle cell disease*	6.7 (4.4–10.1) (22)
Systemic lupus erythematosus	5.4 (0.8-38.3) (53)
Pre-existing diabetes mellitus	0.7 (0.3–1.9) (53)
Cancer	1.1 (0.5-2.8) (53)
Inflammatory bowel disease	4.1 (1.7-9.6) (53)
Delivery characteristics	
Multiple pregnancy	0.9 (0.4-2.1) (53)
Stillbirth	4.1 (1.7–9.6) (53)
Preterm delivery (< 37 weeks)	2.3 (1.7–3.1) (53)
Pre-eclampsia with intra-uterine growth restriction	5.8 (2.1-16.0) (52)
Mid-cavity or rotational operative delivery	1.2 (0.7-2.1) (53)
Elective caesarean delivery	1.3 (0.7-2.2) (52)
Emergency caesarean delivery	2.7 (1.8–4.1) (52)
Vaginal delivery and postpartum infection	20.2 (6.4-63.5) (52)
Caesarean delivery and postpartum infection	6.2 (2.4-16.2) (52)
Postpartum infection	4.1 (2.9–5.7) (22)
Postpartum haemorrhage (> 1000 mL and surgery)	12.0 (3.9–36.9) (52)
Key: aOR, adjusted odds ratio; IRR, incidence rate ratio; CI, confidence interval; BMI, body mass index *as a predictor of antepartum and postpartum VTE	

1.2.4 Risk assessment for venous thrombo-embolism in pregnancy and the puerperium

The presence of risk factors as well as their level of risk provides guidance on the indications for antepartum and postpartum thromboprophylaxis. The decision to use thromboprophylaxis should also take into account patient preferences (96). Current guidelines vary considerably in their recommendations for risk stratification and the use of thromboprophylaxis (97). For example, the Royal College of Obstetrics and Gynaecology (RCOG) guideline considers a number of antepartum and postpartum risk factors and employs a weighted risk score that categorises women into low, intermediate and high risk groups for VTE (97, 98, 100). In contrast, the American College of Chest Physicians considers postpartum thromboprophylaxis for risk factors with an OR >10 after a normal vaginal delivery and an OR >6 after emergency caesarean delivery (98). More recently, the American Society of Haematology (ASH) guideline advises thromboprophylaxis in women who are considered at high risk for VTE. This guideline employed the Grading of Recommendations Assessment, Development and Evaluation approach to evaluate evidence from systematic reviews (99). Owing to the low level of evidence, the ASH guideline suggests against thromboprophylaxis in women with only one clinical risk factor (excluding high risk factors namely thrombophilia or a prior VTE). (85, 87). The optimal approach to VTE prevention in pregnancy and the puerperium is an important research need (99).

1.2.5 Thromboprophylaxis in pregnancy and the puerperium

Low molecular weight heparin (LMWH) is the preferred anticoagulant in pregnancy based on available safety and efficacy data. A systematic review of LMWH thromboprophylaxis published in 2005 reported data from 30 studies and 1348 pregnancies (100). The rates of VTE and postpartum haemorrhage (PPH) were 0.84%, 95% CI 0.5-1.3 and 2%, 95% CI 1.5–2.6 respectively. Of the total pregnancies, wound hematomas accounted for 0.65%, 95% CI 0.4-1.0 of PPH. Subsequently, Sirico *et al.* reviewed data from eight studies and 22,162 pregnant women, of whom 1320 (6%) received LMWH thromboprophylaxis (101). Low molecular weight heparin was associated with a higher risk of PPH (relative risk 1.45, 95% CI 1.0–2.1) as compared to controls. In this study, in contrast to the former study, rates of blood transfusion

were assessed. However, there was no significant increase in blood transfusion at delivery as compared to controls.

Previously unfractionated heparin (UFH) was the standard anticoagulant in pregnancy. A concern with the use of UFH in pregnancy was the associated risk of reduced bone density and osteoporotic fracture (102). In contrast, LMWH is associated with lower risks of maternal complications. Further advantages of LMWH include; predictable pharmacokinetics with an increased half-life, subcutaneous administration and substantially reduced risk of heparin-induced thrombocytopenia (100).

There is currently limited data on fetal safety with the direct oral anticoagulants (DOACs) and as such the International Society of Thrombosis and Haemostasis advise against the use of DOACs in pregnant and breastfeeding women (103). Nevertheless, exposure to DOACs in early pregnancy has not been associated with a significant risk for embryopathy according to a recent review of registries, case reports and pharmacovigilance data (104). Accordingly, major birth defects were documented in 12 of 336 (4%, 95% CI 2.0-6.0) pregnancies with available outcomes. The observed major birth defects were highly variable and not typical of harmful drug exposures in pregnancy. The rate of miscarriage, however, was 22%, which is double that which is expected in the overall population.

Vitamin K antagonists (VKA) such as warfarin are also not recommended during pregnancy for thromboprophylaxis (99). These drugs are not recommended in the first trimester owing to the highly variable rates of warfarin embryopathy (105-108). Lower doses e.g. < 5mg/day have been associated with a reduced rate of complications. However, a dose dependent relationship has not been consistently found (109, 110). Furthermore, VKA exposure in any trimester has been associated with central nervous system (CNS) complications, miscarriages and stillbirths (105, 109, 111-113). Central nervous system abnormalities have been rarely described, (114, 115). However, most studies do not include histological assessments, which may lead to

underestimation of foetal CNS abnormalities. Significantly increased rates of miscarriage ranging from 14 to 40% have been reported as compared to the overall population.

There is limited evidence for graduated compression stockings or use of mechanical prophylaxis such as intermittent pneumatic compression, postpartum (116).

Antepartum prophylaxis is recommended early in pregnancy (59). With regards to postpartum prophylaxis, the risk of VTE is greatest within the first three weeks postpartum. In women considered as high risk, thromboprophylaxis should be extended for up to six weeks' postpartum (26). The optimal LMWH dose in high risk pregnancies is inconclusive with current guidelines recommending a fixed low or an intermediate dose of LMWH (98, 117, 118). Recent guidance has stemmed from the multi-centre, multi-national Highlow randomized controlled trial which found no statistically significant difference in efficacy and safety outcomes between fixed low dose and intermediate dose LMWH in women with prior VTE (119). In high risk pregnancies, monitoring of thromboprophylaxis with anti-Xa activity has not been recommended. During pregnancy, the pharmacodynamics of LMWH are altered as a result of the increased volume of distribution and enhanced renal clearance. However, studies to date have not demonstrated an improvement in efficacy and safety outcomes with regular monitoring (120-123).

Delivery requires a multidisciplinary approach to reduce the associated risks of maternal PPH, and VTE. In women at high risk, a scheduled delivery is preferable to minimize the period without anticoagulation (99). However, there are increasing safety reports of unscheduled deliveries, whereby women are instructed to discontinue LMWH upon the signs of labour (124). An unscheduled delivery may be associated with an increased risk of bleeding and limited accessibility to neuraxial analgesia and anaesthesia (125). A time interval of 12-24 hours is indicated between the last prophylactic LMWH dose and a scheduled induction of labour or elective caesarean section, provided haemostasis is adequate. These time intervals are also

applicable to neuraxial analgesia and anaesthesia in view of the risk of spinal or epidural hematomas. After delivery, it is recommended that LMWH be reintroduced at least six hours after removal of the catheter (126, 127). Neurological monitoring is mandatory for at least 12 hours and ideally for 72 hours following neuraxial analgesia and anaesthesia. In the postpartum period, LMWH, fondaparinux and VKA are safe. However, the DOACs should be avoided in women who plan to breastfeed (128).

1.2.6 Summary

Thrombosis is a significant cause of maternal morbidity and mortality and its prevention should be a priority for health care services. Implementation of safe and effective risk-reduction measures in pregnancy and the puerperium requires accurate identification of women at increased risk for thrombosis. For women receiving thromboprophylaxis, multidisciplinary collaboration is important in order to plan the management of pregnancy, delivery and postpartum.

1.3 Research aims and objectives

The aims and objectives of the work presented in this thesis women living with HIV with access to ART in South Africa were:

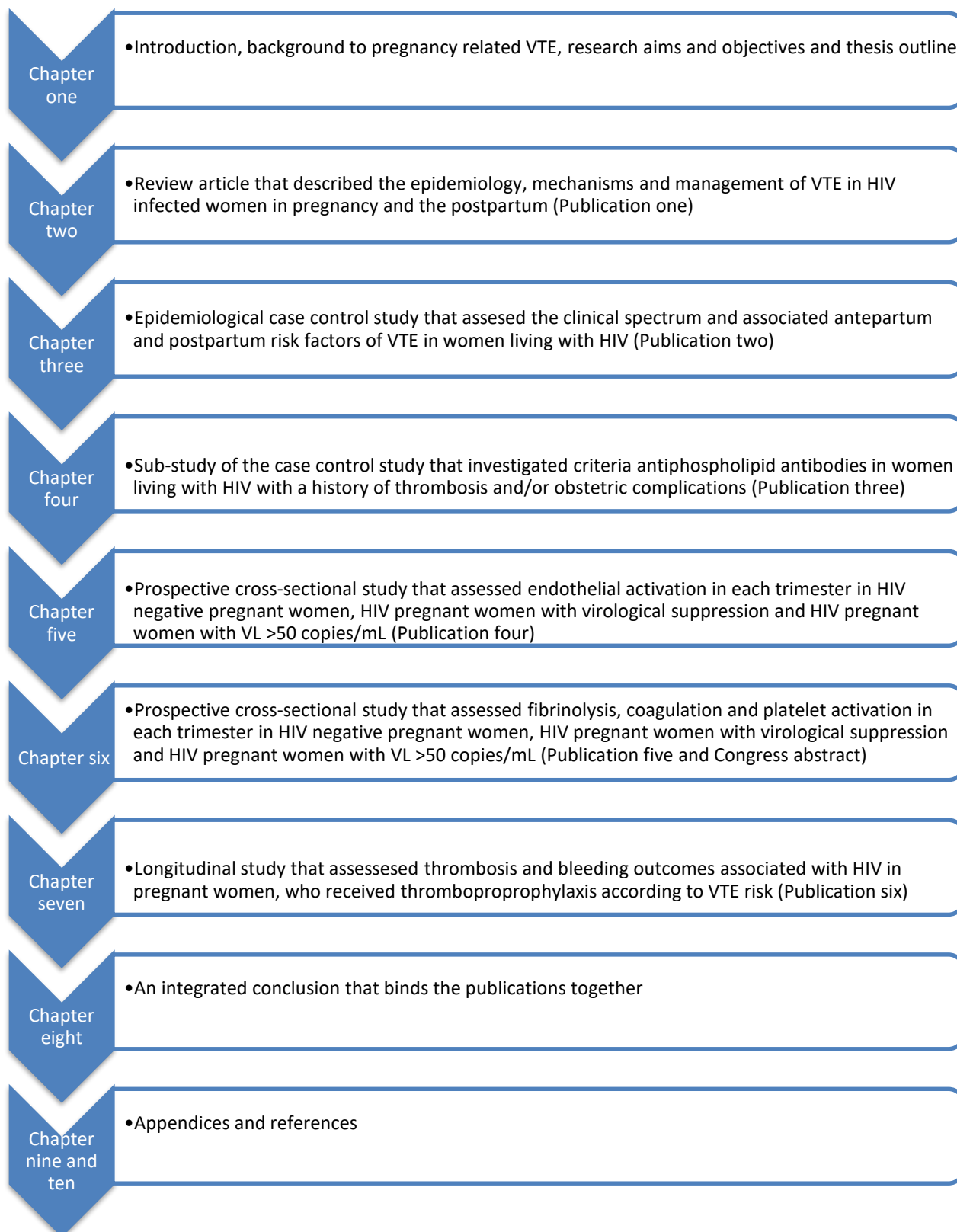
- 1. To identify risk factors for pregnancy related VTE**
 - a. To identify antepartum and postpartum risk factors for VTE
 - b. To determine the prevalence, profile and significance of criteria aPL in women with a history of thrombosis and/or obstetric complications

- 2. To assess procoagulant changes in maternal haemostasis and its relationship with HIV viral load (VL), in each trimester**
 - a. To assess endothelial activation by measuring VWF antigen, VWF propeptide, multimer patterns and ADAMTS-13 antigen, activity, and antibody levels in HIV negative pregnant women, HIV pregnant women with virological suppression and HIV pregnant women with VL >50 copies/mL, in each trimester
 - b. To assess fibrinolytic activity by measuring d-dimer and PAI-1, coagulation by measuring TAT complex concentration and platelet activation by measuring platelet factor-4 (PF4) and platelet indices in HIV negative pregnant women, HIV pregnant women with virological suppression and HIV pregnant women with VL >50 copies/mL, in each trimester

- 3. To assess thrombosis and bleeding outcomes associated with thromboprophylaxis for the prevention of pregnancy related VTE**
 - a. To assess thrombosis and bleeding outcomes associated with antepartum and postpartum thromboprophylaxis in women classified as intermediate or high risk of VTE

1.4 Thesis outline

This is a thesis by publication that consists of nine chapters. Each publication addresses a specific aim, however, there is overlap and progression of the arguments (Fig 1.3).



Key: VTE, venous thrombo-embolism; HIV, human immunodeficiency virus; VL, viral load

Figure 1.3 Thesis outline

CHAPTER 2: Pregnancy related venous thromboembolism associated with HIV infection and antiretroviral therapy

Summary: This invited peer-reviewed review article described the epidemiology, mechanisms and management of VTE in HIV infected women in pregnancy and the postpartum period. This review highlighted the interactions between HIV, ART, inflammation and coagulation as well as the relevance for pregnancy associated thrombosis.

Statement of originality document: Please see Appendix 8.2

Pregnancy Related Venous Thromboembolism-Associated with HIV Infection and Antiretroviral Therapy

Elise Schapkaitz, MMed (Hematology), FCPATH¹ Barry F. Jacobson, PhD¹ Elena Libhaber, PhD²

¹Department of Molecular Medicine and Hematology, University of Witwatersrand, Johannesburg, South Africa

²Department of Research Methodology and Statistics, University of Witwatersrand, Johannesburg, South Africa

Address for correspondence Elise Schapkaitz, MMed (Hematology), FCPATH, PO Box 28985, Sandringham 2131, South Africa (e-mail: elise.schapkaitz@nhls.ac.za).

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Abstract

Human immunodeficiency virus (HIV) infection in pregnancy is associated with substantial morbidity and mortality. Improved access to effective antiretroviral therapy (ART) has shifted the spectrum of pregnancy-related complications among HIV-infected pregnant women. In addition to placental vascular complications and preterm delivery, increased rates of venous thromboembolism (VTE) have been described. HIV infection is characterized by immune activation, inflammation, and endothelial dysfunction, which contribute to the activation of coagulation and its prothrombotic consequences. Indeed, activated coagulation factors have been reported to be increased and natural anticoagulants reduced in HIV. Several mechanisms for this persistent prothrombotic balance on ART have been identified. These may include: co-infections, immune recovery, and loss of the gastrointestinal mucosal integrity with microbial translocation. In addition to the direct effects of HIV and ART, traditional venous and obstetric risk factors also contribute to the risk of VTE. A research priority has been to understand the mechanisms of VTE in HIV-infected pregnant women receiving suppressive ART and to translate this into HIV-specific thromboprophylaxis recommendations. Management requires a multidisciplinary approach and further studies are indicated to guide the prevention and management of pregnancy-associated VTE in this population. The current review describes the epidemiology, mechanisms, and management of VTE in HIV-infected women in pregnancy and the postpartum period.

Keywords

- ▶ human immunodeficiency virus
- ▶ pregnancy
- ▶ venous thromboembolism

Globally, an estimated 1.3 million pregnant women are living with human immunodeficiency virus (HIV) infection since its discovery in 1983.¹ The HIV epidemic has evolved and the focus of maternal care has shifted. In the early stages of the epidemic, the prevention of mother-to-child transmission of HIV was the primary focus. An extensive body of research and clinical practice guidelines were established around this complication.^{2–5} Significant progress has been made in the prevention of mother-to-child transmission of HIV with 85%

of all pregnant and breastfeeding women currently accessing antiretroviral therapy (ART).¹ In the current age of suppressive ART, HIV-infected pregnant women confront a new set of chronic, non-infectious complications. Several epidemiological studies have suggested that HIV-infected patients have increased rates of venous thromboembolism (VTE) in pregnancy.^{6–8}

In recent years, there has been a rapid increase in investigations regarding the mechanisms of thrombotic

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complications in treated HIV infection. Traditional venous and obstetric risk factors for VTE, including medical comorbidities and prolonged hospital admission, have been shown to be heightened during the early phase of the epidemic in HIV-infected pregnant and postpartum women.^{8–10} Subsequently investigations have focused on the roles of immune activation and inflammation in the pathogenesis of VTE.^{11–13} Considerably less, however, is known about the epidemiology, prevention, and management of thrombotic complications in pregnancy. The focus of this review is therefore to examine the current evidence on the interactions between HIV, inflammation, and coagulation as well as to discuss the relevance for thrombotic manifestations in pregnancy and puerperium.

Epidemiology of VTE in Pregnant Women with HIV

VTE, which comprises deep vein thrombosis (DVT) and its complication, pulmonary embolism (PE) is one of the largest contributors to morbidity and mortality in pregnant and postpartum women.¹⁴ Epidemiologic studies performed over the last decade have described an increased incidence of VTE in addition to placental vascular complications and preterm delivery in association with HIV and/or its treatment.^{2,6,7,15,16} Similar to other infectious and inflammatory conditions,¹⁷ HIV infection has been associated with a two to threefold increased risk of VTE in pregnancy and the postpartum period.⁸ In the AIDS Therapy Evaluation in the Netherlands project (ATHENA) prospective cohort of 14,389 HIV-infected participants, the reported incidence of a first pregnancy-related VTE was 9.4 per 1,000 person years, with an upper limit of the 95% confidence interval (CI) of 17.3 per person years.⁶ Likewise, in various other clinical settings, such as malignancy and hospitalization, studies have demonstrated a twofold to 10-fold increased risk of VTE in HIV as compared with matched HIV negative controls.^{18,19} HIV viral load and disease characteristics are also strong predictors of VTE risk,²⁰ yet evidence suggests that the risk of VTE persists in pregnant women on suppressive ART.^{6,8}

VTE in HIV-infected pregnant women is associated with increased morbidity relative to uninfected populations, which is likely to influence this population's long-term health significantly. Post-thrombotic syndrome and recurrent thrombosis are significant morbidities which have been reported to be increased in HIV-infected pregnant women with DVT.^{8,21} Likewise, HIV-infected pregnant women with PE are at a higher risk of developing functional impairments, such as chronic pulmonary hypertension and cor pulmonale.⁸

Prothrombotic Mechanisms in HIV

The Strategies for Management of Antiretroviral Therapy (SMART) study provided some of the earliest and most robust evidence for the more important role of HIV and its associated inflammatory effects beyond ART or traditional risk factors.^{22,23} Several reviews have been dedicated to understanding the pathogenesis of HIV.^{24–29} It has been

Table 1 Coagulation abnormalities which contribute to the hypercoagulable state of HIV

Platelet activation	IL- β , Thromboxane A ₂ , soluble CD40 ligand, soluble P-selectin, platelet factor-4, soluble platelet glycoprotein IV
Thrombin generation	Endogenous thrombin potential, thrombin antithrombin complex
Anticoagulant deficiencies	Antithrombin deficiency, protein S deficiency, protein C deficiency, acquired activated protein C resistance
Increased coagulation factors	Factor VIII activity, antiphospholipid antibodies, lupus anticoagulant, microparticles
Endothelial cellular adhesion molecule activation	P-selectin, E-selectin, VCAM-1, ICAM-1, soluble thrombomodulin, von Willebrand factor
Hypofibrinolysis	Tissue plasminogen activator, plasminogen activator inhibitor-1, fibrinogen, D-dimer

Abbreviations: ICAM-1, intercellular adhesion molecule-1; IL, interleukin; VCAM-1, vascular cell adhesion molecule-1.

proposed that HIV may modulate coagulation activity via the tissue factor (TF) pathway. In brief, HIV viral proteins Tat and Nef and envelope glycoproteins gp120/41, activate pro-inflammatory cytokines including tumor necrosis factor- α , interleukin (IL)-6, and IL-1 β . In response to these pro-inflammatory cytokines, TF is upregulated on the surface of monocytes, platelets, and circulating microparticles. This contributes to systemic activation of coagulation via the extrinsic pathway.^{12,30} Indeed, several parameters which have important regulatory functions in hemostasis are disturbed in HIV (**Table 1**). This is evidenced by a progressive increase in factor VIII activity and decreasing levels of antithrombin, functional protein S, and protein C activity as well as acquired activated protein C resistance.^{31–35} The most frequent coagulation abnormality associated with HIV infection, according to several clinical studies, is a low functional protein S.^{31–33,36} In these reports, the decrease in both total and free protein S has been attributed to decreased synthesis as well as an abnormal distribution between bound and free forms associated with increased C4b-binding protein.^{31–33,36} These abnormal coagulation parameters, which are associated with an increased risk for VTE, provide a biologic mechanism for the increased incidence of VTE among HIV-infected individuals.³⁷

HIV viral and envelope proteins and/or pro-inflammatory cytokines also cause endothelial activation and dysfunction. Upregulation of monocyte chemoattractant protein-1 and cell adhesion molecules; E-selectin, vascular cell adhesion molecule-1 and intercellular adhesion molecule-1 further promote leukocyte migration from the systemic circulation.^{38,39} Endothelial signaling pathways are also activated with downstream reduction in nitric oxide synthesis. Other biochemical markers of endothelial injury in HIV, which have

important regulatory functions in hemostasis, include von Willebrand factor (VWF) and soluble thrombomodulin.^{20,40,41} In particular, the release of increased ultra-large VWF multimers, which results in the formation of platelet-VWF thrombi in the microcirculation, has been proposed as one of the possible mechanisms for the increased risk of thrombotic thrombocytopenic purpura like syndrome in HIV.^{42,43} The increased large multimers, nonetheless, have not been associated with a significant deficiency in, and/or antibodies directed against, ADAMTS-13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) to cause overt pathology.^{42,43} Endothelial activation is favored to be the predominant mechanism for these increased large multimers in HIV.³¹

Platelets have also been shown to circulate in an activated state in HIV infection.^{44–46} Along with their role in vessel wall adhesion and aggregation, activated platelets mediate inflammatory responses through the release of α -granules and neutrophil-platelet interactions. Neutrophil-platelet interactions involve the binding of P-selectin (CD62P) and CD40 on platelets to P-selectin glycoprotein ligand and CD40 ligand on the neutrophil surface.⁴⁷ Neutrophil activation results in the production of cytotoxic neutrophils extravasation traps which may promote platelet activation and thrombosis.⁴⁸ Studies of platelet activation in the era of ART, however, have reported discordant results.^{44–46,49–51} The different treatment regimens have been proposed as an explanation for this finding. According to a recent systematic meta-analysis, a twofold increase in P-selectin was observed in patients receiving suppressive ART containing protease inhibitors as compared with treatment naïve patients.⁵² This suggests that long-term protease inhibitors in HIV-infected individuals, which are associated with sustained platelet activation, may lead to increased comorbidities such as cardiovascular disease.

The presence of circulating antiphospholipid antibodies (aPL) is another important cause of thrombosis and pregnancy morbidity.⁵³ In HIV-infected women, the presence of a lupus anticoagulant has been associated with an increased risk for thrombosis and/or obstetric complications.⁵⁴ Earlier studies proposed a link between HIV and anticardiolipin antibodies (aCL), anti- β 2-glycoprotein 1 (a β 2GPI) and anti-prothrombin/phosphatidylserine.^{55–61} In particular, an increased frequency of aCL IgG of 17 to 93% was found in HIV-infected individuals.^{55,56,62,63} The wide variation in aCL levels has been attributed to factors such as: ART exposure, and the degree of virological suppression and immune dysregulation.⁶⁴ In the current era of ART, a decline in the incidence of aPL has been reported.^{54,60} According to a recent study of women with a history of thrombosis and/or obstetric complications, aCL was not significantly increased among HIV-infected women, of whom 71.9% were virologically suppressed with a median CD4 count of $540 [352] \times 10^6/L$ on long-term ART.⁵⁴ It is thus likely that circulating antibodies in HIV infection against phospholipids, phospholipid-protein complexes, or phospholipid-binding proteins, similar to other chronic viral infections, may act as a trigger for infection-induced aPL production. Infection-related aPL are

often transient.⁶⁵ Nonetheless, in HIV studies, repeat confirmatory aPL testing has been infrequently performed and future studies are indicated.^{54,60}

Another well-described mechanism for the prothrombotic state in HIV infection is decreased fibrinolysis. In HIV, the balance between tissue-type plasminogen activator (t-PA) and plasminogen activator inhibitor-1 (PAI-1) is disrupted.^{32,66,67} This results in impaired fibrinolysis, which is an important driver of morbidity and mortality.^{22,23,32} Increased PAI-1 levels in the 16-year follow-up of the Women's Interagency HIV Study, were significantly associated with mortality among women, of whom more than half were receiving long-term ART.³² Similarly, D-dimer, a degradation product of crosslinked fibrin, has been independently associated with morbidity (cardiovascular disease, chronic kidney disease, liver failure, and malignancies), all-cause mortality, and non-AIDS deaths in individuals receiving suppressive ART.^{22,68}

While inflammation is more pronounced with virological and immune suppression, evidence suggests that inflammation does not consistently improve with long-term suppressive ART.^{69–72} This may explain elevated VTE rates despite virological suppression. Subsequently, investigations have focused on biomarkers of inflammation, coagulation, and endothelial activation.^{11,73–76} Studies conducted over the past decade, in non-pregnant HIV-infected populations on ART, have suggested a mechanistic link between markers of coagulation and endothelial activation and VTE.^{20,77} In a retrospective study, levels of P-selectin, D-dimer, and hyaluronic acid, measured prior to VTE, were significantly higher in HIV-infected cases with VTE as compared with matched HIV-infected controls.²⁰ Importantly, the majority of the HIV-infected cases and controls were on long-term ART and the risk of VTE was independent of the HIV viral load as well as ART status. In another retrospective review of the Dutch National HIV registry database, levels of VWF antigen, were significantly higher in HIV-infected cases with first and recurrent VTE as compared with matched HIV-infected controls.⁷⁷ Similarly, the majority of the HIV-infected cases and controls were on long-term ART and the risk of VTE was independent of virological and immune suppression.⁷⁷ Several mechanisms for this persistent pro-inflammatory and pro-thrombotic balance on ART have been identified^{78,79} (► Fig. 1).

Co-infections in HIV

Co-infections, predominantly in resource-limited settings, play an important role in further modulating VTE risk by contributing to immune activation and inflammation. HIV is associated with reactivation of latent infections normally controlled by the immune system.⁸⁰ Viruses such as cytomegalovirus, Epstein-Barr virus, Hepatitis C and B virus, Human Papillomavirus and other Herpes viruses serve as a source of antigen which activate CD8+ T cells and other microbial products, which further activate the innate immune system.⁸¹ In particular, cytomegalovirus co-infection has been found to be a significant independent risk factor for inflammation-related morbidity and mortality despite

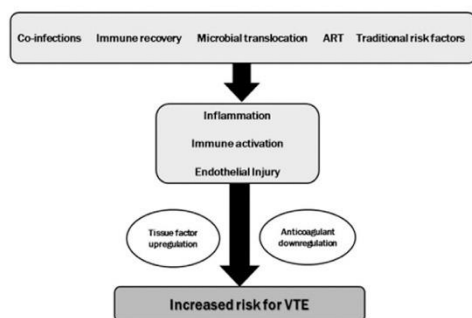


Fig. 1 Mechanisms for the persistent pro-inflammatory and pro-thrombotic balance on antiretroviral therapy. Presented is a conceptual model of the factors which contribute to the pro-inflammatory and pro-thrombotic state on suppressive antiretroviral therapy. Persistent immune activation and inflammation as well as endothelial activation and dysfunction contribute to systemic activation of coagulation. Tissue factor is upregulated on the surface of monocytes, platelets, and circulating microparticles and anticoagulant levels, e.g., antithrombin, functional protein S, and protein C activity decline. These abnormal coagulation parameters provide a biological mechanism for the increased risk of thrombosis. Abbreviations: ART, antiretroviral therapy; VTE, venous thrombo-embolism.

suppressive ART.⁸² More recently, coronavirus disease 2019 (COVID-19), has been associated with higher rates of mortality in specific HIV cohorts.^{83,84} The increased risk of severe outcomes has been related to the presence of multiple comorbidities, including; unsuppressed viral load, chronic hypertension, diabetes mellitus, cardiovascular disease, tuberculosis, and smoking.⁸⁵

Concomitant opportunistic infections are an additional risk factor for thrombosis. According to a recent meta-analysis, a significant proportion of HIV-infected individuals from resource-limited settings continue to present in the late stages of disease with opportunistic infections, despite widespread access to HIV testing and ART.⁸⁶ *Mycobacterium tuberculosis* co-infection is the most common opportunistic infection associated with HIV.⁸⁷ Indeed, HIV and/or concomitant tuberculosis are the most prevalent risk factors for VTE in hospitalized patients in South Africa.^{88,89} The hypercoagulable state of HIV and concomitant tuberculosis contribute to this increased risk and have been associated further with mortality.⁹⁰

Microbial Translocation in HIV

Importantly, monocyte activation and gut epithelial dysfunction have been shown to play a potentially important role in several studies. HIV facilitates translocation of microbial products into the lamina propria of the gastrointestinal tract and the systemic circulation. Factors responsible for disruption of the gastrointestinal barrier include: damage and decreased repair of the epithelium, depletion of intestinal lymphoid tissue CD4⁺ T cells, and dysfunction of IgA-secreting immune cells. These microbial products stimulate toll-like receptors, innate pattern recognition molecules, which activate pro-inflammatory cytokines and TF-bearing mono-

cytes.^{12,91} A large prospective study of individuals diagnosed with early HIV infection on long-term ART showed significantly higher levels of soluble markers associated with monocyte activation (sCD14 and sCD163), inflammation (soluble tumor necrosis factor receptor type II), and mucosal integrity (intestinal fatty-acid binding protein) when compared with HIV-uninfected groups.⁹² Thus, while ART may effectively suppress viral replication, homeostasis of the gastrointestinal tract mucosa is not completely restored. This contributes to ongoing inflammation and activation of coagulation. Recent studies, which have linked monocyte activation and microbial translocation to adverse perinatal outcomes, have supported this mechanism for persistent inflammation on ART.^{69,70}

Immune Recovery in HIV

HIV-related immunological and virological parameters are also important risk factors for persistent inflammation and immune activation. Pregnancy, however, is not a contributing factor to delayed immune recovery in HIV. The multicenter, longitudinal Women and Infants Transmission Study assessed lymphocyte subsets of HIV-infected pregnant women as compared with non-pregnant HIV-infected controls. In this study, the CD4⁺/CD8⁺ T cell ratio remained stable during pregnancy.⁹³ Current data suggest that at initiation of ART, more advanced disease is a risk factor for VTE.²⁰ In the Options Project, significantly higher levels of immune activation were found in patients who were initiated on ART 2 years or more after infection as compared with controls who started ART within 6 months of infection.⁹⁴ Subsequently, early initiation of ART has been associated with decreased activation of CD8⁺ T cells and monocytes.⁹⁵ Nonetheless, early initiation of ART remains a challenge for resource-limited settings.⁹⁶ In addition to the degree of pre-ART immunodeficiency, more recent data support a role for CD4⁺ T cell recovery during ART in conferring a risk for VTE recurrence.²¹ This data supports current guideline recommendations for early ART initiation.

Adverse Metabolic Effects of ART in HIV

Since the introduction of universal ART for pregnant and breastfeeding women in 2014, ART has dramatically improved the survival of patients with HIV infection.^{97,98} First-generation nucleoside analogue reverse transcriptase inhibitors and protease inhibitors were associated with marked abnormalities of lipid and glucose metabolism and lipodystrophy.^{99,100} Newer ART regimens, within the same classes, have shown significantly reduced metabolic side-effects.¹⁰¹ For example, protease inhibitors, ritonavir and lopinavir, have been associated with lipid abnormalities such as increased low-density lipoprotein (LDL) and triglycerides, insulin resistance, diabetes and metabolic syndrome. Increased LDL and triglycerides have also been observed with efavirenz. Additional studies of the class of integrase inhibitors are required to clarify the risks of unexplained weight gain and impaired glucose metabolism.^{102,103} Specific ART regimens may also independently affect the risk of specific comorbidities. The Data Collection on Adverse

Events of Anti-HIV Drugs (D.A.D.) study identified protease inhibitors in particular, ritonavir-boosted darunavir, as a risk factor for cardiovascular disease.^{99,104} Likewise, metabolic abnormalities are established risk factors for adverse pregnancy outcomes such as gestational hypertension, pre-eclampsia, and preterm delivery, which too are predictors of VTE events. It is therefore important to consider the metabolic profile of each drug to individualize the treatment according to personal risk factors.

Management of VTE in Pregnant Women with HIV

While our knowledge of the interactions between inflammation and coagulation has vastly increased, data on the prevention and management of VTE tailored to HIV-infected pregnant women remains limited.^{6–8,105} Recommendations for the management of VTE in the setting of HIV are largely reliant on guidelines and data from the general population.

Antithrombotic Management of VTE in HIV-Infected Pregnancy

The preferred management of pregnancy-related VTE consists of anticoagulation with low-molecular weight heparin adjusted according to the peak anti-factor Xa level.¹⁰⁶ Limited duration anticoagulation is appropriate for most women with a pregnancy-related VTE. This is evidenced by data from the Computerized Registry of Patients with VTE (RIETE) which reported a 2-year VTE recurrence rate of 3.3%, with an upper limit of 95% CI of 5.0% for women whose initial event was pregnancy related.¹⁰⁷ Among HIV-infected individuals, significantly higher rates of VTE recurrence as compared with HIV-uninfected individuals have been reported.^{21,77} In the ATHENA Dutch National HIV prospective cohort (2003–2015), the incidence rates for VTE recurrence were 5.2 per 100 person-years of follow-up for the HIV group as compared with 3.1 per 100 person-years of follow-up for the HIV-uninfected group (hazard ratio: 1.70, 95% CI 1.23–2.36, $p = 0.003$).²¹ This risk of VTE recurrence, after adjusting for age, gender, and provoked versus unprovoked initial VTE, was highest in the first year after discontinuing anticoagulation (hazards ratio 1.67, 95% CI 1.04–2.70). In contrast, CD4+ T cell recovery with suppressive ART, was associated with a lower risk of VTE recurrence among the HIV-infected individuals.²¹ Accordingly, this suggests that immune status is a reversible risk factor for VTE in the modern era of widespread access to ART. The duration of anticoagulant treatment for pregnancy-related VTE in HIV-infected women should therefore be guided by a personalized assessment of risk factors.¹⁰⁸ Studies evaluating prolonged anticoagulation and the associated risk of major bleeding in HIV-infected women are essential.

VTE Thromboprophylaxis in HIV-Infected Pregnancy

Risk assessment in the antepartum and postpartum periods is important to identify women at risk for VTE, who may benefit from thromboprophylaxis. HIV and ART are not considered as risk factors for VTE according to current

thromboprophylaxis guidelines. In the modern treatment era, the risk of VTE in pregnancy has been found to be independent of ART status.⁸ Regarding HIV, in the largest retrospective cohort study, the rates of VTE observed did not support the use of thromboprophylaxis in HIV-infected pregnancy.⁶ In HIV-infected pregnant women, the upper limit of the 95% CI of the VTE rate was 17.3 per 1,000 person years. This limit is below the American College of Chest Physicians threshold of 20 per 1,000 person years for VTE in pregnancy.¹⁰⁹ However, studies are lacking from Eastern and Southern Africa, which are associated with the highest burden of maternal HIV infection. The current recommendations, can be attributed to the paucity of high quality studies to guide management in HIV-infected pregnant women.^{6–8,21} There is an urgent need to prioritize research in this population group.

A high prevalence of traditional venous and obstetric risk factors has been reported among HIV-infected women of African ethnicity. A recent record review (2017–2020) identified antepartum risk factors, including medical comorbidities and postpartum risk factors, including a personal history of VTE, systemic infection, prolonged hospital admission, postpartum hemorrhage, and medical comorbidities. Medical comorbidities included: heart disease, chronic hypertension, malignancy, and chronic kidney disease.⁸ Guidelines broadly recommend antepartum and postpartum thromboprophylaxis for women with high-risk factors which include a personal history of unprovoked or pregnancy-related VTE, whereas postpartum thromboprophylaxis is suggested for women with intermediate risk factors such as medical comorbidities or prolonged hospital admission.^{109,110} However, in women with low-risk factors for VTE, such as systemic infection, immobility, or postpartum hemorrhage, there is no clear guidance on the role of thromboprophylaxis. As such, the recommended approach is therefore to risk assess each HIV-infected pregnant patient individually and to engage patients in a shared decision regarding thromboprophylaxis in the antepartum and postpartum periods until further evidence becomes available.

Conclusion

The maternal care of HIV-infected women has undergone a significant transition. The improved understanding of the relationship between persistent inflammation and coagulation suggests that chronic non-infectious complications, in particular, VTE may disproportionately impact this population's long-term well-being. While knowledge of the pathophysiology and mechanisms of HIV-related VTE have vastly increased, salient gaps exist with regards to the epidemiology, prevention, and management of HIV and VTE. A coordinated approach with collaboration between the fields of HIV, obstetrics and hematology is required to develop strategies to prevent pregnancy-associated VTE. This represents an opportunity to shape the long-term trajectory of maternal care in HIV-infected pregnant women.

Authors' Contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication. E.S. wrote the original draft. E.S., B.F.J., and E.L. reviewed and edited the manuscript.

Conflict of Interest

None declared.

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THIEME

CHAPTER 3: Pregnancy related venous thromboembolism and HIV infection

Summary: This case control study was designed to assess risk factors of pregnancy related VTE in order to guide local thromboprophylaxis recommendations. The study identified HIV as an antepartum and postpartum risk factor of VTE. This risk of thrombosis was independent of opportunistic infections, virological suppression and ART. In addition, a higher prevalence of traditional obstetric and venous risk factors was found in pregnant women with HIV.

Statement of originality document: Please see Appendix 8.2

Pregnancy-related venous thromboembolism and HIV infection

Elise Schapkaitz¹  | Elena Libhaber² | Haroun Rhemtula³ | Annika Gerber³ | Barry F. Jacobson¹ | Lawrence Chauke³ | Harry R. Büller⁴

¹Department of Molecular Medicine and Hematology, Faculty of Health Sciences, University of Witwatersrand Medical School, Johannesburg, South Africa

²HS Research Office and School of Clinical Medicine, Faculty of Health Sciences, University of Witwatersrand Medical School, Johannesburg, South Africa

³Department of Obstetrics, Faculty of Health Sciences, University of Witwatersrand Medical School, Johannesburg, South Africa

⁴Department of Vascular Medicine, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

Correspondence

Elise Schapkaitz, PO Box 28985, Sandringham 2131, South Africa.
Email: elise.schapkaitz@nhls.ac.za

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Abstract

Objective: To assess risk factors for venous thromboembolism (VTE) in African women in order to guide thromboprophylaxis.

Methods: A case-control study was performed at a specialist obstetric unit in South Africa from July 1, 2017 to June 30, 2020. We identified 128 cases with VTE and 640 controls, matched for gestation.

Results: Prepartum risk factors associated with VTE included; medical comorbidities (odds ratios [OR] 5.32, 95% confidence intervals [CI] 1.82–15.56), human immunodeficiency virus (HIV) (OR 2.84, 95% CI 1.50–5.41), and hospital admission or immobility (OR 5.33, 95% CI 1.17–24.22). Postpartum, the following were identified as significant risk factors; medical comorbidities (OR 23.72, 95% CI 8.75–64.27), hospital admission or immobility (OR 13.18, 95% CI 5.04–34.49), systemic infection (OR 4.48, 95% CI 1.28–15.68), HIV (OR 3.20, 95% CI 1.49–6.87), pre-eclampsia and fetal growth restriction (OR 2.74, 95% CI 1.18–6.36), and postpartum hemorrhage (OR 4.38, 95% CI 1.75–10.97). Antiretroviral therapy, opportunistic infections, and viral load >50 copies/ml, however, were not associated with VTE risk among HIV-infected participants.

Conclusion: HIV was a significant risk factor for pregnancy-related thrombosis. This was independent of traditional HIV risk factors. As such, future studies are recommended to explore the mechanisms of thrombosis associated with HIV infection.

KEYWORDS

epidemiology, human immunodeficiency virus, postpartum risk factors, prepartum risk factors, venous thromboembolism

1 | INTRODUCTION

Pregnancy is recognized as an acquired hypercoagulable state. Increased concentrations of coagulation factors, platelet activation, decreased fibrinolysis, and a reduction in free and total protein S activity are responsible for the hypercoagulability of pregnancy. The resultant increase in thrombin generation, however, predisposes to venous thromboembolism (VTE), which comprises deep vein thrombosis and its complication, pulmonary embolism. Over the past decade, risk factors that further increase this risk of VTE have been

identified by a number of high-quality cohort and case-control studies from the developed world.^{1–6} In particular, a personal history of thrombosis and inherited thrombophilia are the most important risk factors for pregnancy-associated VTE.³ In addition, established risk factors include; age >35 years, medical comorbidities, significant pregnancy complications, and cesarean delivery.^{3,4,6} Accordingly, several evidence-based guidelines have been developed that recommend the use of pharmacological thromboprophylaxis during pregnancy and the postpartum period in women with an increased risk of thrombosis.^{7–11}

In South Africa, a resource-limited setting, these guidelines have been increasingly adopted. Of particular concern, however, is the fact that human immunodeficiency virus (HIV) and its treatment, which are known risk factors for VTE, have received limited consideration in the current guidelines. In South Africa, particularly, this risk factor is of relevance, considering a HIV-adjusted maternal prevalence of 20.4%. Studies of non-pregnant HIV-infected individuals have described a two- to ten-fold increased risk of VTE.¹² Epidemiologic studies have also evaluated the significance of HIV as a risk factor for VTE among pregnant and postpartum women.¹³ Unfortunately, the interpretation of these studies is limited by the small numbers (fewer than 10) of HIV-infected cases. Various risk factors predisposing to a pro-thrombotic and pro-inflammatory state have been described in association with HIV. These include opportunistic infections, malignancy, and immunodeficiency.¹⁴ In addition, several thrombophilias have also been observed in HIV; namely, the presence of a circulating lupus anticoagulant, activated protein C resistance, increased soluble plasminogen activator inhibitor-1, and decreased synthesis as well as an abnormal distribution between bound and free forms of protein S.¹⁵ Moreover, HIV-infected women are increasingly conceiving on antiretroviral therapy (ART). ART has been associated with an increased risk of stillbirths and preterm delivery, which are also predictors of thrombosis.¹⁶ Nevertheless, very little is currently known about the impact of HIV on the incidence of VTE in pregnant and postpartum women.

We therefore conducted a retrospective case-control study to evaluate the clinical spectrum as well as prepartum and postpartum risk factors for pregnancy-related VTE in African women to guide thromboprophylaxis in a resource-limited setting.

2 | MATERIALS AND METHODS

A single-center study was conducted at the obstetric unit at Charlotte Maxeke Johannesburg Academic Hospital in Johannesburg, South Africa from July 1, 2017 to June 30, 2020. This is a specialist referral academic center for high-risk pregnant women including those with VTE. All cases of VTE during pregnancy or within 42 days postpartum were identified. Controls (without a history of arterial thrombosis or VTE) were selected from the population of patients presenting to the obstetric unit during the study period. The cases and selected controls were of African ethnicity, matched for gestation in a 1:5 ratio. During the study period, 98.9% of maternity admissions were of African ethnicity. Written informed consent was obtained from the cases, and the study protocol was approved by the Institutional Review Board (M 191038).

Data including demographics, detailed clinical information relating to diagnosis and associated prepartum and/or postpartum risk factors were collected from clinical records. The diagnoses of thrombosis were independently confirmed by three of the study investigators (ES, AG, and HR). VTE was diagnosed in patients with clinical, laboratory, and radiologic findings (compression ultrasound;

ventilation-perfusion lung-scan; computed tomographic pulmonary angiography, adapted and standardized for pregnancy). All women with VTE in pregnancy were treated with dose-adjusted twice-daily low-molecular-weight heparin (enoxaparin) (anti-Xa level of 0.5–1.0 units/ml 3–4 h after the last dose) followed by warfarin in the postpartum period (International Normalized Ratio of 2–3). The median durations of treatment for deep vein thrombosis and pulmonary embolism were 6 months (interquartile range [IQR] 3 months) and 9 months (IQR 6 months), respectively.

In the subgroup of HIV-infected women, data on opportunistic infections and ART were collected. Associated prepartum risk factors were obtained. These included; age >35 years, personal history of VTE, self-reported family history of unprovoked or estrogen-related VTE in a first-degree relative, inherited thrombophilia, antiphospholipid syndrome (APS), medical comorbidities (including at least one of the following: malignancy, heart disease, active systemic lupus erythematosus, inflammatory polyarthropathy, inflammatory bowel disease, chronic kidney disease, sickle cell disease, current intravenous drug user), chronic hypertension (systolic blood pressure >140 mm Hg and/or diastolic blood pressure >90 mm Hg), systemic infection (perioperative and opportunistic infection), HIV, parity three or more, multiple pregnancy, pre-eclampsia with fetal growth restriction (FGR), non-obstetric surgery, hospital admission or immobility (at least 3 days bed rest), gross varicose veins, hyperemesis gravidarum, in vitro fertilization, smoking and obesity (body mass index [BMI, calculated as weight in kilograms divided by the square of height in meters] ≥ 30). The following postpartum risk factors were also recorded; non-obstetric surgery, obesity (BMI ≥ 40), preterm delivery (<37 weeks), stillbirth (intrauterine fetal death >28 weeks), mode of delivery, prolonged labor (>24 h) and postpartum hemorrhage requiring transfusion.

During pregnancy, all women were screened for HIV when first presenting to prenatal care, with retesting at follow-up prenatal visits. A positive HIV test was further characterized with a CD4 count and HIV viral load. This was determined using the Cytomics FC 500 MPL Flow Cytometry System (Beckman Coulter, Brea, CA, USA) and the Cobas 6800/8800 system (Roche Diagnostics, Basel, Switzerland), respectively. The lower limit of detection for HIV RNA was 50 copies/ml.

In 82 cases (64.1%), inherited thrombophilia testing was performed for fibrinogen (STA-Liquid Fib; Diagnostica Stago), antithrombin (STA Stachrom kits; Diagnostica Stago), protein C (STA Stachrom kits; Diagnostica Stago), functional protein S (Staclo protein S; Diagnostica Stago), and Factor V Leiden and prothrombin G20210A mutations (LightCycler 480; Roche Diagnostics and DNA sequence analysis; Applied Biosystems). Only blood sampling 1-month after completion of anticoagulation treatment was analyzed, to exclude any pregnancy-related, acute thrombosis-related and anticoagulant-related alterations in coagulation and fibrinolysis.

Statistical analysis was performed using STATISTICA 13.2 software and SAS 9.1 software (SAS Inc., Cary, NC, USA). Normally distributed continuous data were presented as mean $\pm$ standard deviation and variables with non-Gaussian distribution as median (IQR).

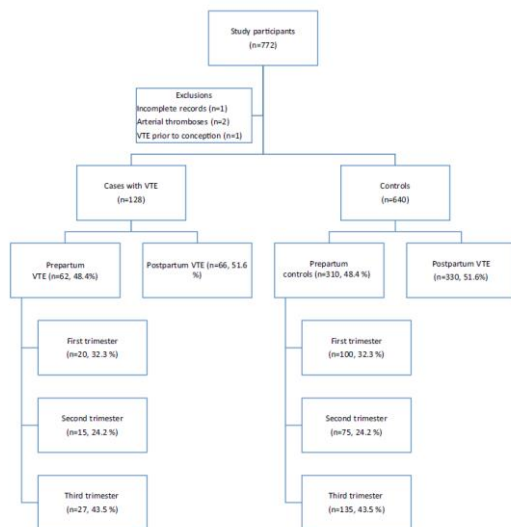
Categorical data were presented as frequencies and percentages. Comparisons of prepartum and postpartum risk factors in the cases and controls were performed using χ^2 test or two-tailed Fisher's exact test. Subgroup analysis with multiple pairwise comparisons was subsequently performed applying the Bonferroni correction with a *P* value less than 0.0083 considered statistically significant. Univariate logistic regression analysis was conducted to predict thrombosis with the correspondent adjusted odds ratios (aOR) and 95% confidence intervals (CI). Multiple logistic regression analysis was then applied to build the models by including risk factors with a

P value of less than 0.05, which were identified by univariate analysis. As a result of the limited number of cases, the explanatory variables were restricted to a maximum number of six per model. The resultant model was selected by exhibiting the highest -2 log likelihood value and area under the curve on receiver operating characteristic analysis. Two-tailed *P* value of less than 0.05 was considered statistically significant.

3 | RESULTS

During the study period, 26 323 deliveries were registered. VTE was diagnosed in 128 women, of which 67 were deep vein thromboses, 58 were pulmonary embolisms, and three were both. The majority of thromboses were diagnosed during the postpartum period ($n = 66$, 51.6%) at a median of 6 days (IQR 12 days) and in the third trimester ($n = 27$, 21.1%) at 32 weeks (IQR 4 weeks) (Figure 1). Table 1 shows the characteristics of the study population. Inherited thrombophilia was identified in 17 (20.7%) cases, protein S deficiency was confirmed in 12 (14.6%), protein C deficiency in four (4.9%), and a rare prothrombin gene C20209T mutation was recorded in a single patient (1.2%) with a massive pulmonary embolism. All other screening results were negative. Forty-four (34.4%) women were HIV-infected; 16 (36.3%) with a median viral load of 3287 copies/ml (IQR 26 062 copies/ml) and 28 (63.6%) with virologic suppression. Complications of thrombosis included cor pulmonale ($n = 2$, 1.6%), post-thrombotic syndrome ($n = 2$, 1.6%), and maternal death as a result of right ventricular infarction and decompensated cardiac failure ($n = 1$, 0.8%). Patients who developed complications all tested positive for HIV. Additionally, four patients (3.1%) presented with a recurrent thrombosis after completion of anticoagulation during the study period. Of these, three (2.3%) were HIV infected; two with a deficiency of protein S despite virologic suppression and another not on ART with comorbid tuberculosis.

On univariate analysis, the following prepartum risk factors were independently associated with thrombosis; personal history of VTE,



VTE, venous thrombo-embolism

FIGURE 1 Flow diagram of study selection of patients with venous thromboembolism and controls

TABLE 1 Baseline characteristics of cases of venous thromboembolism and corresponding matched controls^a

Characteristic	Controls (n = 640)	Cases with VTE (n = 128)	P value
Clinical			
Age, years	29 ± 7	30 ± 6	0.146
Parity	2 ± 1	2 ± 1	0.795
BMI	27.4 ± 5.1	27.9 ± 6.1	0.297
HIV-infected	114 (17.8)	44 (34.3)	<0.001
Laboratory			
White cell count, ×10 ⁹ /L (ref: 3.9–12.6 × 10 ⁹ /L)	8.0 ± 2.6	10.2 ± 4.2	<0.001
Hemoglobin, g/L (ref: 116–164 g/L)	117.6 ± 16.3	106.7 ± 18.8	0.001
Platelet count, ×10 ⁹ /L (ref: 186–454 × 10 ⁹ /L)	249.4 ± 63.2	293.9 ± 107.0	0.003
Mean platelet volume, fL (ref: 7.3–11.3 fL)	10.4 ± 1.7	10.6 ± 1.2	0.547

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by the square of height in meters); VTE, venous thromboembolism.

^aValues are given as mean ± SD or as number (percentage).

TABLE 2 Prepartum and postpartum risk factors in the study patients^a

Variable	Controls	Cases with VTE	P value
Prepartum risk factors	(n = 310)	(n = 62)	
Age > 35 years	63 (20.3)	16 (25.8)	0.336
Personal history of VTE	0 (0)	5 (8.1)	<0.001
Positive family history for VTE	2 (0.6)	2 (3.2)	0.105
Inherited thrombophilia ^b	2 (14.3)	11 (25.6)	0.479
APS ^c	0 (0)	2 (6.7)	0.067
Medical comorbidities ^d	8 (2.6)	8 (12.9)	<0.001
Chronic hypertension	20 (6.5)	7 (11.3)	0.186
Systemic infection	3 (1.0)	3 (4.8)	0.046
HIV	56 (18.1)	24 (38.7)	<0.001
ART	52 (16.8)	21 (33.9)	0.443
Parity ≥3	74 (23.8)	15 (24.2)	0.957
Multiple pregnancy	6 (1.9)	2 (3.2)	0.527
Pre-eclampsia with FGR ^e	10 (5.3)	1 (3.1)	0.603
Hospital admission or immobility	4 (1.3)	4 (6.5)	0.021
Gross varicose veins	12 (3.9)	3 (4.8)	0.724
BMI ≥ 30	96 (31.0)	23 (37.1)	0.346
Postpartum risk factors	(n = 330)	(n = 66)	
Age > 35 years	69 (20.9)	14 (21.2)	0.956
Personal history of VTE	0 (0)	2 (3.0)	0.002
Positive family history for VTE	4 (1.2)	1 (1.5)	0.841
Inherited thrombophilia ^b	4 (13.3)	6 (15.4)	0.811
APS ^c	0 (0)	1 (3.2)	0.045
Medical comorbidities ^d	9 (2.7)	18 (27.3)	<0.001
Chronic hypertension	42 (12.7)	14 (21.2)	0.074
Systemic infection	7 (2.1)	11 (16.7)	<0.001
HIV	58 (17.6)	20 (30.3)	0.019
ART	48 (14.5)	17 (25.8)	0.817
Parity ≥3	90 (27.3)	23 (34.8)	0.215
Multiple pregnancy	16 (4.8)	5 (7.6)	0.370
Pre-eclampsia with FGR	34 (10.3)	19 (28.8)	<0.001
Non-obstetric surgery	1 (0.3)	5 (7.6)	0.003
Hospital admission or immobility	11 (3.3)	22 (33.3)	<0.001
Gross varicose veins	10 (3.0)	1 (1.5)	0.503
BMI ≥40	12 (3.6)	5 (7.6)	0.159
Preterm delivery	77 (23.3)	29 (43.9)	<0.001
Stillbirth	23 (7.0)	8 (12.1)	0.160
Elective cesarean delivery	83 (25.2)	12 (18.2)	0.229
Emergency cesarean delivery	130 (39.4)	27 (40.9)	0.818

(Continues)

medical comorbidities, systemic infection, HIV, and hospital admission or immobility (Table 2). Postpartum risk factors associated with thrombosis included personal history of VTE, APS, medical

TABLE 2 (Continued)

Variable	Controls	Cases with VTE	P value
Prolonged labor	8 (2.4)	5 (7.6)	0.042
Postpartum hemorrhage	21 (6.4)	14 (21.2)	<0.001

Abbreviations: APS, antiphospholipid syndrome; ART, antiretroviral therapy; BMI, body mass index (calculated as weight in kilograms divided by the square of height in meters); FGR, fetal growth restriction; VTE, venous thromboembolism.

^aValues are given as number (percentage).

^b43 cases and 14 controls prepartum; 39 cases and 30 controls postpartum.

^c39 cases and 59 controls prepartum; 31 cases and 116 controls postpartum.

^dMedical comorbidities refer to having at least one of the following: heart disease, active connective tissue disease, malignancy, diabetes mellitus with nephropathy or chronic kidney disease.

^e32 cases and 188 controls prepartum.

comorbidities, systemic infection, HIV, pre-eclampsia with FGR, non-obstetric surgery, hospital admission or immobility, preterm delivery, prolonged labor, and postpartum hemorrhage. The most frequent medical comorbidity among the prepartum and postpartum cases was heart disease, which included peripartum cardiomyopathy ($n = 10$, 7.8%), dilated cardiomyopathy ($n = 2$, 1.6%), and pulmonary hypertension ($n = 5$, 3.9%).

Explanatory variables from the univariate analyses were selected to construct the multiple models. In the prepartum model, medical comorbidities, HIV, and hospital admission or immobility were identified as risk factors (Table 3). For the postpartum risk factors, two models were developed. Model one included the following variables: medical comorbidities, systemic infection, HIV, hospital admission or immobility, postpartum hemorrhage, and pre-eclampsia with FGR. Model two included medical comorbidities, systemic infection, HIV, hospital admission or immobility, postpartum hemorrhage, and preterm delivery. Collinearity was detected between significant postpartum variables, preterm delivery, and pre-eclampsia and FGR. Pre-eclampsia and FGR (OR 2.74, 95% CI 1.18–6.36, $P > 0.019$) showed a stronger association with postpartum VTE compared with preterm delivery (OR 1.62, 95% CI 0.81–3.25, $P = 0.080$) and was retained for further analysis.

Subsequently, prepartum and postpartum risk factors for VTE were analyzed according to HIV infection. Table 4 shows the characteristics of HIV-infected cases and controls compared with corresponding HIV-negative patients. Antepartum risk factors independently associated with HIV and thrombosis included medical comorbidities and chronic hypertension. The following postpartum risk factors were also associated with HIV and thrombosis; namely, a personal history of VTE, medical comorbidities, systemic infection, hospital admission or immobility, and postpartum hemorrhage. However, there was no difference in the rates of stillbirth between the groups ($P = 0.154$). Furthermore, the risk of thrombosis was not significantly associated with immune suppression, as evidenced by viral load and CD4 count, opportunistic infections, and ART (Table 5).

TABLE 3 Univariate and multiple logistic regression analysis of venous thromboembolism

Variable	Univariate logistic regression			Multiple logistic regression		
	OR	95% CI	P value	Adjusted OR ^a	95% CI	P value
Prepartum (AUC 0.66, P = 0.372)						
Medical comorbidities ^b	5.59	2.01–15.54	<0.001	5.32	1.82–15.56	0.002
HIV infection	2.86	1.59–5.15	<0.001	2.84	1.50–5.41	0.001
Hospital admission or immobility	5.28	1.28–21.70	0.021	5.33	1.17–24.22	0.030
Postpartum (AUC 0.86, P = 0.172)						
Medical comorbidities ^b	13.38	5.68–31.47	<0.001	23.72	8.75–64.27	<0.001
HIV infection	2.04	1.12–3.70	0.019	3.20	1.49–6.87	0.003
Systemic infection	9.23	3.42–24.83	<0.001	4.48	1.28–15.68	0.019
Hospital admission or immobility	14.50	6.58–31.93	<0.001	13.18	5.04–34.49	<0.001
Postpartum hemorrhage	3.96	1.89–8.28	<0.001	4.38	1.75–10.97	0.003
Pre-eclampsia with FGR	3.52	1.86–6.68	<0.001	2.74	1.18–6.36	0.019

Abbreviations: AUC, area under the curve; CI, confidence interval; FGR, fetal growth restriction; OR, odds ratio.

^aAdjusted for age.

^bMedical comorbidities refer to having at least one of the following: heart disease, active connective tissue disease, malignancy, diabetes mellitus with nephropathy or chronic kidney disease.

4 | DISCUSSION

The changing demographic of pregnant women in resource-limited settings with regards to the HIV epidemic has raised concerns, particularly in view of the fact that HIV is an important cause of thrombosis in its own right. This case-control study describes an approximately three-fold increased risk for thrombosis among African pregnant women with HIV infection. Importantly, in this single-academic center, HIV-infected pregnant women with thrombosis were characterized by a higher morbidity compared with HIV-negative cases. This included VTE recurrence ($n = 3$, 2.3%) and long-term disability secondary to post-thrombotic syndrome, chronic pulmonary hypertension, and cor pulmonale ($n = 5$, 3.9%).

This increased risk of thrombosis among HIV-infected women can be attributed to classical prepartum and postpartum risk factors. Significantly, this study revealed a higher prevalence of prepartum risk factors among HIV-infected cases namely; medical co-morbidities and chronic hypertension and postpartum risk factors namely, a personal history of VTE, medical comorbidities, systemic infection, hospital admission or immobility, and postpartum hemorrhage. In contrast to earlier studies, opportunistic infections were not associated with thrombosis risk.¹⁴ Only a single HIV-infected patient was diagnosed with tuberculosis, which can be attributed to widespread access of pregnant women to suppressive ART during the study period.

Interestingly, previous HIV studies have demonstrated a strong association between the risk of thrombosis and thrombophilic abnormalities.¹⁵ We observed a protein S deficiency in seven (25%) of the 28 HIV-infected pregnant and postpartum cases in whom testing was performed. It is significant to note that these cases were not associated with a family history of thrombosis. An earlier study of HIV-infected pregnant women before ART also demonstrated a further downregulation of functional protein S, relative to uninfected

pregnant controls matched for gestational age.¹⁷ Increasing evidence suggests that HIV-related factors contribute to this observed pro-thrombotic state, independent of traditional venous risk factors.¹⁴ HIV is characterized by increased release of pro-inflammatory cytokines that recruit tissue-factor-bearing monocytes to the chronically activated endothelium.¹⁸ This acts as a surface for activation of coagulation and fibrinolysis. Accordingly, the introduction of ART has been associated with an improvement in makers of inflammation and coagulation. Normalization is not often achieved, however.¹⁹ This persistent pro-inflammatory and pro-thrombotic balance on suppressive ART has been strongly linked with thrombosis in non-pregnant populations.¹⁴ Likewise, in this pregnant population, the risk of thrombosis was independent of viral load, CD4 count, and ART status. Moreover, recent studies of pregnant women on ART have demonstrated increased levels of surrogate markers of intestinal mucosal integrity and microbial translocation.²⁰ This suggests a possible mechanism for persistent inflammation in HIV-infected pregnant women. However, the extent to which HIV, in the era of ART, disrupts the maternal hemostatic balance and as such warrants thromboprophylaxis is unknown and merits evaluation. Furthermore, considerable differences exist between current thromboprophylaxis guidelines with regards to the management of thrombophilias such as protein S deficiency. A recent systematic review and meta-analysis, however, concluded that thromboprophylaxis should be considered in protein S deficiency as it has been associated with a high-risk estimate for pregnancy-associated VTE.²¹

Traditionally, African ethnicity has been associated with a higher prevalence of VTE of 4.48 per 1000 hospital deliveries.²² In contrast, the prevalence of VTE ranges from 1.7 to 2 per 1000 deliveries in pregnant women of white and Asian ethnicity.³ Nonetheless, only a few studies have examined the associations in African populations. In African-American women, this increased

TABLE 4 Prepartum and postpartum risk factors in the study participants according to HIV infection^a

Variable	HIV-infected controls (n = 56)	HIV-negative controls (n = 254)	HIV-infected with VTE (n = 24)	HIV-negative with VTE (n = 38)	P value
Prepartum risk factors					
Age >35 years	28 (50.0)*	35 (13.8)	8 (33.3)	8 (21.1)	<0.001
Personal history of VTE	0 (0)**	0 (0)	1 (4.2)	4 (10.5)	<0.001
Positive family history for VTE	1 (1.8)	1 (0.4)	1 (4.2)	1 (2.6)	0.222
Inherited thrombophilia ^b	2 (100.0)*	0 (0)	6 (33.3)	5 (20.0)	0.009
Protein S	2 (100.0)*	0 (0)	5 (27.8)	4 (16.0)	0.007
Protein C	0 (0)	0 (0)	1 (5.6)	0 (0)	0.531
Prothrombin gene mutation	0 (0)	0 (0)	0 (0)	1 (4.0)	0.729
APS ^c	0 (0)	0 (0)	0 (0)	2 (7.1)	0.084
Medical comorbidities	1 (1.8)	7 (2.8)***	4 (16.7)	4 (10.5)	0.002
Heart disease	0 (0)***	2 (0.8)***	3 (12.5)	2 (5.3)	<0.001
Active connective tissue disease	0 (0)	3 (1.2)	1 (4.2)	2 (5.3)	0.156
Diabetes mellitus with nephropathy	1 (1.8)	2 (0.8)	0 (0)	0 (0)	0.764
Chronic hypertension	5 (8.9)	15 (5.9)***	6 (25.0)	1 (2.6)	0.004
Systemic infection	2 (3.6)	1 (0.4)	1 (4.2)	2 (5.3)	0.048
Parity ≥3	21 (37.5)	53 (20.9)	9 (37.5)	6 (15.8)	0.013
Multiple pregnancy	1 (1.8)	5 (2.0)	1 (4.2)	1 (2.6)	0.901
Pre-eclampsia with FGR ^d	1 (2.5)	9 (6.1)	0 (0)	1 (5.3)	0.665
Hospital admission or immobility	0 (0)	4 (1.6)	2 (8.3)	2 (5.3)	0.052
Gross varicose veins	2 (3.6)*	10 (3.9)	3 (12.5)	0 (0)	0.108
BMI ≥30	19 (33.9)	77 (30.3)	9 (37.5)	14 (36.8)	0.761
Postpartum risk factors					
Age >35 years	21 (36.2)	48 (17.6)	4 (20.0)	10 (21.7)	0.019
Personal history of VTE	0 (0)	0 (0)***	1 (5.0)	1 (2.2)	0.007
Positive family history for VTE	2 (3.4)	2 (0.7)	0 (0)	1 (2.2)	0.335
Inherited thrombophilia ^b	3 (75.0)*	1 (3.8)	2 (20.0)	4 (13.8)*****	0.002
Protein S	1 (25.0)	0 (0)	2 (20.0)	1 (3.4)	0.040
Protein C	2 (50.0)	1 (3.8)	0 (0)	3 (10.3)	0.015
APS ^c	0 (0)	0 (0)	0 (0)	1 (4.3)	0.143
Medical comorbidities	0 (0)****	9 (3.3)**	5 (25.0)***	13 (28.2)*****	<0.001
Heart disease	0 (0)****	1 (0.4)**	3 (15.0)***	9 (19.6)*****	<0.001
Active connective tissue disease	0 (0)	4 (1.5)	0 (0)	2 (4.3)	0.303
Diabetes mellitus with nephropathy	0 (0)	3 (1.1)	0 (0)	1 (2.2)	0.697
Malignancy	0 (0)	1 (0.4)	1 (5.0)	0 (0)	0.030
Chronic kidney disease	0 (0)	0 (0)**	1 (5.0)***	1 (2.2)	0.007
Chronic hypertension	8 (13.8)	34 (12.5)	4 (20.0)	10 (21.7)	0.339
Systemic infection	2 (3.4)	5 (1.8)**	4 (20.0)**	7 (15.2)	<0.001
Parity ≥3	21 (36.2)	69 (25.4)	11 (55.0)	12 (26.1)	0.018
Multiple pregnancy	3 (5.2)	13 (4.8)	1 (5.0)	4 (8.7)	0.751
Pre-eclampsia with FGR	5 (8.6)	29 (10.7)**	5 (25.0)	14 (30.4)*****	<0.001
Non-obstetric surgery	0 (0)	1 (0.4)**	1 (5.0)	4 (8.7)	<0.001
Hospital admission or immobility	0 (0)****	11 (4.0)**	5 (25.0)***	17 (37.0)*****	<0.001
Gross varicose veins	1 (1.7)	9 (3.3)	1 (5.0)	0 (0)	0.532
BMI ≥40	4 (6.9)	8 (2.9)	3 (15.0)	2 (4.3)	0.052
Preterm delivery	20 (34.5)	57 (21.0)**	8 (40.0)	21 (45.7)	<0.001

(Continues)

TABLE 4 (Continued)

Variable	HIV-infected controls	HIV-negative controls	HIV-infected with VTE	HIV-negative with VTE	P value
Stillbirth	2 (3.4)	21 (7.7)	1 (5.0)	7 (15.2)	0.154
Elective cesarean delivery	19 (32.8)	64 (23.5)	4 (20.0)	8 (17.4)	0.290
Emergency cesarean delivery	25 (43.1)	105 (38.6)	5 (25.0)	22 (47.8)	0.322
Prolonged labor	0 (0)	8 (2.9)	2 (10.0)	3 (6.5)	0.092
Postpartum hemorrhage	3 (5.2)	18 (6.6)**	5 (25.0)***	9 (19.6)	0.001

Abbreviations: APS, antiphospholipid syndrome; BMI, body mass index (calculated as weight in kilograms divided by the square of height in meters); FGR, fetal growth restriction VTE, venous thromboembolism.

^aValues are given as number (percentage).

^b2 HIV-infected controls, 12 HIV-negative controls, 18 HIV-infected cases and 25 HIV-negative cases, prepartum; 4 HIV-infected controls, 26 HIV-negative controls, 10 HIV-infected cases and 29 HIV-negative cases, postpartum.

^c14 HIV-infected controls, 26 HIV-negative controls, 9 HIV-infected cases and 28 HIV-negative cases, prepartum; 34 HIV-infected controls, 82 HIV-negative controls, 8 HIV-infected cases and 23 HIV-negative cases, postpartum.

^d40 HIV-infected controls, 148 HIV-negative controls 13 HIV-infected cases and 19 HIV-negative cases, prepartum.

* $P < 0.0083$ in HIV-infected controls versus HIV negative controls.

** $P < 0.0083$ in HIV-negative controls versus HIV negative cases.

*** $P < 0.0083$ in HIV-negative controls versus HIV infected cases.

**** $P < 0.0083$ in HIV-infected controls versus HIV infected cases.

***** $P < 0.0083$ in HIV-infected controls versus HIV negative cases.

TABLE 5 HIV risk factors in the HIV-infected study participants^a

Variable	HIV-infected controls (n = 114)	HIV infected with VTE (n = 44)	P value
Years from diagnosis	3 ± 4	5 ± 6	0.058
CD4 cell count (<200 × 10 ⁶ /L)	14 (12.3)	6 (13.6)	0.674
HIV viral load (>50 copies/ml)	38 (33.3)	16 (36.4)	0.746
ART, n (%)			
First-line therapy ^b	96 (84.2)	34 (77.3)	0.354
Second-line therapy ^c	4 (3.5)	4 (9.1)	0.220
No therapy	14 (12.3)	6 (13.6)	0.794
Opportunistic infection	0 (0)	1 (2.3)	0.106

Abbreviations: ART, antiretroviral therapy; VTE, venous thromboembolism.

^aValues are given as number (percentage).

^bFirst-line consisted of tenofovir, emtricitabine and efavirenz.

^cSecond-line consisted of zidovudine, lamivudine (3TC) and Aluvia (lopinavir/ritonavir).

risk has been attributed to factors such as chronic hypertension, peripartum cardiomyopathy, obesity, sickle cell disease, and other inherited thrombophilias, namely protein S and antithrombin deficiency.³ Likewise, in this population of African women, medical comorbidities, in particular peripartum cardiomyopathy, were identified as a significant risk factor for prepartum and postpartum VTE. Maternal obesity, owing to an increasing prevalence, has also emerged as an important risk factor for thrombosis in high-income countries.^{2,5,6} However, this risk factor was not associated with an increased risk of VTE in this study population. In South

Africa, there is a bimodal distribution, with a rise in obesity in particular, among HIV-infected patients on long-term ART.²³ In keeping with this trend, 15% (n = 3) of the postpartum HIV-infected cases had a BMI of 40 or more. However, this may differ in a larger population study.

Overall, the findings of this study support many of the existing guideline recommendations for identifying pregnant and postpartum women requiring thromboprophylaxis. Current guidelines recommend thromboprophylaxis for risk factors such as a personal history of VTE and the presence of high-risk thrombophilias.⁷⁻¹¹ The majority of thrombotic episodes in the present study, however, occurred in the absence of such risk factors, in accordance with previous studies of African-American populations.²⁴ Nonetheless, this study has identified at least a two-fold increased risk associated with medical comorbidities; a two-fold increased risk associated with HIV, and a one-fold increased risk associated with hospital admission or immobility in the prepartum period. Additionally, in the postpartum period, medical comorbidities were associated with at least a nine-fold increased risk; systemic infection, a one-fold increased risk; HIV, a two-fold increased risk; hospital admission or immobility, a five-fold increased risk; postpartum hemorrhage, a two-fold increased risk; and pre-eclampsia and FGR, a one-fold increased risk. This may be useful for identifying African women for whom thromboprophylaxis is indicated.

To the best of our knowledge, this is one of the first hospital-based studies to examine the significance of HIV and ART as prepartum and postpartum risk factors. Nonetheless, our results must be interpreted in light of certain limitations. First, this was a single-center study in a referral center for patients at risk for obstetric complications. As a result, the participants represent a selective group of HIV cases from the total pool of HIV-infected women in the country. This may limit the interpretation of the associated risk factors.

However, the associations with HIV and thrombosis obtained in this study are as expected, based on reports in non-pregnant HIV-infected populations. A second limitation of this study is that the possible association of risk factors, such as non-obstetric surgery, prolonged labor, personal history of VTE and APS could not be included in the multiple regression model owing to the low incidence in cases and controls. Patients attending the unit with a personal history of thrombosis or APS were considered high risk and received thromboprophylaxis. Third, testing for inherited thrombophilia was only performed in a subgroup of cases. This may in part explain the low inherited thrombophilia incidence (17, 20.7%). This is in contrast to reports in the literature of up to 50% with thrombosis.²⁵ In addition, FV Leiden and the classical prothrombin gene G20120A mutations, which are not associated with African ethnicity, were not evident in this study population.³ The clinical characteristics of this study population may not accurately reflect other ethnic groups. Fourth, a limited number of independent variables could be included in the statistical models in the multiple logistic regression analysis as a result of the limited number of cases, which limits the precision of the risk estimates. This also contributed to the wide confidence intervals. Finally, the study was conducted as a case-control study (referral/selection bias for cases, recall bias on patient interview) which could result in an overestimation of individual risk factors.

In conclusion, we have identified prepartum and postpartum risk factors associated with thrombosis in African women attending a specialist obstetric unit. Importantly, HIV emerged as a significant prepartum and postpartum risk factor. The increased VTE risk in the subgroup of HIV-infected pregnant women can be attributed to a higher prevalence of traditional obstetric and venous risk factors. Nevertheless, this risk was independent of opportunistic infections, virologic suppression, and ART. In view of the disproportionate burden of HIV, in particular in young women aged 15–24 years in South Africa, it is important to investigate further HIV as a risk factor after taking into account all confounding factors. A multicenter study with sufficient statistical power is indicated before a formal recommendation can be made.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest.

AUTHOR CONTRIBUTIONS

All authors performed the research. ES contributed to the study design, wrote the manuscript, and performed data collection, entry, and analysis. EL was responsible for the study design, data analysis and critical review. HR contributed to data collection and critical review. AG was responsible for data collection and critical review. BFJ contributed to the study design and critical review. LC was responsible for the critical review of the manuscript. HRB contributed to the study design and critical review of the manuscript.

ORCID

Elise Schapkaitz  <https://orcid.org/0000-0002-1534-2930>

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CHAPTER 4: Profile of antiphospholipid antibodies in HIV infected and HIV uninfected women with a history of thrombosis

Summary: This study, expanded on Chapter two on risk factors of pregnancy related VTE by assessing the role of aPL in the pathogenesis of thrombosis. In this study population, the prevalence of aPL was not significantly increased among women living with HIV in the modern era of ART, as compared to HIV negative women. The study identified baseline positivity for LAC as a risk factor for thrombosis and/or obstetric complications.

Statement of originality document: Please see Appendix 8.2

Profile of antiphospholipid antibodies in HIV-infected and HIV-uninfected women with a history of thrombosis

Elise Schapkaitz¹  | Elena Libhaber² | Barry F. Jacobson¹ | Annika Gerber³ | Haroun Rhemtula³ | Harry R. Büller⁴

¹Department of Molecular Medicine and Haematology, Faculty of Health Sciences, University of Witwatersrand Medical School, Johannesburg, South Africa

²HS Research Office and School of Clinical Medicine, Faculty of Health Sciences, University of Witwatersrand Medical School, Johannesburg, South Africa

³Department of Obstetrics, Faculty of Health Sciences, University of Witwatersrand Medical School, Johannesburg, South Africa

⁴Department of Vascular Medicine, Academic Medical Centre, University of Amsterdam, Amsterdam, The Netherlands

Correspondence

Elise Schapkaitz, PO Box 28985
Sandringham 2131, South Africa.
Email: elise.schapkaitz@nhls.ac.za

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Abstract

Introduction: Increased antiphospholipid antibodies (aPL) have been described in human immunodeficiency virus (HIV) infection. However, the association between aPL and the increased risk of thrombosis in HIV requires further clarification.

Methods: We reviewed the medical records of 215 consecutive women with a history of thrombosis and/or obstetric complications (158 HIV-uninfected and 57 HIV-infected) between July 2017 and March 2021. Participants ($n = 215$) without clinical criteria manifestations for antiphospholipid syndrome were included as matched controls. Testing for lupus anticoagulant (LAC), anticardiolipin (aCL) and anti-beta2-glycoprotein1 (aβ2GP1) IgM and IgG was performed.

Results: Thirty-two (10.1%) HIV-uninfected and 15 (13.2%) HIV-infected participants were positive at baseline for one of the five criteria aPL, with no statistically significant difference. The profile of the HIV-infected participants with thrombosis ($n = 11$) included LAC in 15.8%, aCL IgG in 3.5% and aβ2GP1 IgG in 1.8%. In contrast, the HIV-infected controls ($n = 4$), included aCL IgM in 1.8% and aβ2GP1 IgM in 5.3%. Only LAC was significantly associated with thrombosis ($p < 0.003$). On repeat testing, in a HIV-infected sub-population, 2/7 with thrombosis were positive, while 3/3 controls tested negative.

Conclusion: In contrast to earlier reports, the prevalence and expression of aPL in HIV-infected women with a history of thrombosis in the present study, in the era of antiretroviral therapy, were similar to HIV-uninfected women. Baseline LAC positivity was associated with a significantly increased risk for thrombosis in HIV. Future studies are recommended to explore additional coagulation abnormalities in HIV.

KEYWORDS

antiphospholipid antibodies, human immunodeficiency virus, lupus anticoagulant, South Africa, thrombosis

1 | INTRODUCTION

Antiphospholipid syndrome (APS) is an important cause of thrombosis and pregnancy morbidity in women with an estimated prevalence of 51 per 100 000 (95% CI: 31–72).¹ APS is diagnosed by the presence of criteria antiphospholipid antibodies (aPL) which are

lupus anticoagulant (LAC), anticardiolipin (aCL) and anti-beta2-glycoprotein 1 (aβ2GP1) IgM and IgG antibodies.^{2,3} Persistent aPL positivity together with thrombosis and/or obstetric complications are indications for the combination of prophylactic low-molecular-weight heparin and low-dose aspirin in pregnant women.⁴

International registries and specific disease cohorts have described the characteristics and associated risk factors of aPL-positive patients.⁵ Earlier studies proposed an association between human immunodeficiency virus (HIV) infection and aPL. Prior to the widespread use of antiretroviral therapy (ART), an increasing frequency of aPL primarily, aCL IgG was reported in HIV infection.^{6–12} This increased prevalence of aPL correlated with the HIV viral load (VL) and the degree of immune dysregulation and has subsequently diminished in the current era of ART.¹³

Increased aPL have been widely reported in various other infections such as syphilis, malaria, leprosy, hepatitis C virus and parvovirus B19.¹⁴ These infections related to aPL, however, are often transient. Similarly, aPL triggered by HIV have not usually been described in association with thrombosis and/or obstetric morbidity.^{6,15,16} Only a small number of isolated cases have been reported.¹⁷

HIV-infected women in the reproductive age group are associated with an increased morbidity as compared to the general population.^{18–22} We and other investigators have observed that HIV infection is associated with a pro-inflammatory and hypercoagulable state with clinical consequences such as venous thromboembolism (VTE).^{23–25} In HIV infection, several haemostatic parameters are disturbed. In particular, aPL might well play a role in thrombotic complications in HIV-infected women.¹⁴ Further venous and cardiovascular risk factors may also contribute to the pathogenesis of thrombosis in HIV infection.²⁵ Nonetheless, in HIV-infected women, the association between aPL, thrombosis and obstetric complications and clinical characteristics has not been well described and warrant further investigation.

It is within this context, that we investigated the prevalence, profile and significance of criteria aPL as well as related clinical risk factors of HIV-infected women with a history of thrombosis and/or obstetric complications, in order to advance research that is aimed at identifying risks of thrombosis in the current era of HIV treatment.

2 | METHODS

2.1 | Study design and population

The design and population of the study have been previously described.²⁵ This sub-study identified 215 consecutive participants from the medical records with a history of thrombosis and/or obstetric complications who were referred for aPL testing, as part of the standard investigative work-up between 1 July 2017 and 31 March 2021. Participants with a history of pregnancy-related, provoked and unprovoked VTE as well as arterial thrombosis were included. Additionally, controls (without APS clinical criteria of thrombosis or obstetric complications) were recruited from the population of patients presenting to the Charlotte Maxeke Johannesburg Academic Hospital Antenatal Clinic during the study period. The cases and selected controls were matched for age, ethnicity and HIV infection in a 1:1 ratio. The study protocol was approved by the Institutional Review Board (M-191038).

2.2 | Study protocol

2.2.1 | Data collection

Data were captured on pre-designed data collection sheets which included demographics, a personal history of thrombosis and/or obstetric complications as well as related venous and/or cardiovascular risk factors. Related venous risk factors included thrombophilia, cancer, autoimmune inflammatory rheumatic diseases (AIIRD), heart disease, immobilization or prolonged admission, systemic infection, major surgery, gross varicose veins and age >35 years. AIIRD included cases of systemic lupus erythematosus (SLE), dermatomyositis, rheumatoid arthritis, antineutrophil cytoplasmic antibody-associated vasculitis and mixed connective tissue disease. Cardiovascular risk factors included chronic hypertension (systolic blood pressure >140 mm Hg and/or diastolic blood pressure >90 mm Hg), diabetes mellitus, hyperlipidaemia and body mass index (BMI) ≥ 30 kg/m². In addition, data on ART (regimen, date of commencement and adherence) of the HIV-infected participants were also collected.

2.2.2 | Blood sampling and laboratory methods

Full blood count (FBC) parameters and an aPL profile (consisting of LAC, aCL and $\alpha 2$ GP1 antibodies IgG and IgM), performed pre-conception or during pregnancy, were collected. In addition to baseline testing, follow-up aPL testing performed in a sub-population was recorded after at least 12 weeks. Blood samples for LAC were collected in 3.2% sodium citrate (ratio of one volume trisodium citrate to nine volumes of blood). Platelet poor plasma was separated by centrifugation (twice at 3500 $\times$ g for 15 min) within 30 min of collection. Serum for aCL and $\alpha 2$ GP1 IgG and IgM antibodies was separated by centrifugation at 3500 $\times$ g for 15 min. Aliquots were stored at -80°C for analysis at a single dedicated laboratory at the National Health Laboratory Service, South Africa. Daily quality control was performed prior to analysis. The laboratories are compliant with local and international proficiency testing.

2.2.3 | Haematological testing

FBC parameters were analysed on the Sysmex XN 9000 haematology analysers (Sysmex Corporation).

2.2.4 | HIV testing

All participants were screened for HIV. A positive HIV test (in 57 HIV-infected participants with thrombosis and 57 HIV-infected controls) was further characterized with a CD4 count and HIV VL. This was determined using the Cytomics FC 500 MPL Flow Cytometry System (Beckman Coulter, Gent, Belgium) and the Cobas

6800/8800 system (Roche Diagnostics) respectively. The lower limit of detection for HIV RNA was 50 copies/ml.

2.2.5 | aPL testing

LAC was detected on the STA-R Max[®] automated coagulation analyser (Diagnostica Stago, Asnières sur Seine, France) using a dilute Russell viper venom time (dRVVT) (STA Staclot dRVV screen and confirm, Diagnostica Stago, Asnières, France) and activated partial thromboplastin time (aPTT) (PTT-LA, Diagnostica Stago, Asnières, France) assays. The dRVVT was performed according to the guidelines of the International Society on Thrombosis and Haemostasis (ISTH) using a three-step procedure including screening, mixing and confirmation.²⁶ A screen mix / confirm mix normalized ratio was applied for dRVVT results which exceeded locally validated cut-off values. The aPTT was performed using a screening and mixing procedure. A screen mix normalized ratio was applied for aPTT results, which exceeded locally validated cut-off values. Normal plasma was obtained by pooling platelet-poor plasma from healthy South African donors without any deficit in coagulation factors. The final LAC result of positive or negative was based on screening, mixing and/or confirmatory results. The aCL and a β 2GP1 antibodies IgG and IgM were performed in duplicate using the fluorescent enzyme immunoassay ImmunoCap[®] ELiA (Phadia, Thermo Scientific) according to the manufacturer's instructions. Positive results were defined by the manufacturer's recommended cut-off values which were derived from the 99th percentile, following confirmation in 20 healthy volunteers, in accordance with ISTH guidelines.²⁷

2.2.6 | Definitions

APS was defined by at least one clinical criterion (thrombosis or obstetric complications) and one laboratory criterion (LAC, aCL, a β 2GP1 antibodies confirmed 12 weeks after the initial testing). This was formulated during the consensus conference in Sapporo and revised in Sydney.^{2,3}

Thrombosis was defined as one or more clinical events of arterial, venous or small vessel thrombosis, diagnosed by objective criteria. VTE was classified as pregnancy-related, unprovoked or provoked. VTE was considered to be provoked if the event occurred within three months of a reversible risk factor.

Obstetric complications were defined as one or more unexplained intra-uterine foetal death (IUFD) at or beyond the tenth week of gestation and more than three consecutive early pregnancy losses and severe preeclampsia or foetal growth restriction (FGR) necessitating delivery before 34 weeks. These complications were considered in the absence of an identifiable other causes (i.e. thrombophilia, anatomical, chromosomal and endocrine abnormalities or infection). FGR was defined as birth weight below the tenth percentile for gestational age.

2.3 | Statistical methods

This study was conducted as an exploratory study as a result of the low reported incidence of APS. Clinical and laboratory data were analysed using Statistica 13.2 software and SAS 9.1 software. Normally distributed continuous data are presented as mean $\pm$ SD and variables with non-Gaussian distribution as median [interquartile range (IQR)]. Categorical data are presented as frequencies and percentages. Comparisons for categorical measurements were performed using chi-squared test or Fisher's exact test when necessary. Comparisons for continuous measurements were performed using a parametric independent t-test or non-parametric Mann-Whitney U-test depending upon normality. Unadjusted odds ratios (OR) and 95% confidence intervals (CI) were calculated to determine the predictors of aPL. Significance was set at a *p* value of <0.05.

3 | RESULTS

Distribution of the participants is presented in Figure 1. The participants with a history of thrombosis included ten (4.7%) participants with ischaemic stroke, 203 (94.4%) participants with VTE and two (0.9%) participants with both venous and arterial thrombosis. The VTE events included 113 deep vein thromboses (DVT), 74 pulmonary emboli (PE) and 16 DVTs and PEs. The mean $\pm$ SD age of the study population was 32 $\pm$ 6 years. On univariate analysis, thrombosis was independently associated with the following risk factors: a personal history of VTE, baseline aPL positivity, AIIRD and major surgery (Table 1). On thrombophilia screening, in 159 participants with thrombosis in whom testing was performed, a protein S deficiency was identified in 20 (12.6%), a protein C deficiency in two (1.3%) and a prothrombin gene mutation in one (0.6%).

Thirty-nine (18.1%) participants with a history of thrombosis and eight (3.7%) controls were positive at baseline for one of the five criteria aPL. This was significantly associated with thrombosis (odds ratio [OR] 5.7, 95% confidence intervals [CI] 2.6–12.6; *p* < 0.001). The characteristics of the aPL-positive and aPL-negative study participants with thrombosis are presented in Table 2. A history of thrombosis and obstetric complications were reported in 11 of the 39 aPL-positive (28.2%) and 48 of the 176 aPL-negative participants (27.3%). There were nine (23.1%) participants with AIIRD in the aPL-positive group as compared to 17 (9.7%) in the aPL-negative group (*p* < 0.024). With regard to laboratory characteristics, haemoglobin levels were significantly lower, albeit within the reference interval, in the aPL-positive group as compared to the aPL-negative group (*p* < 0.006).

On subgroup analysis of the HIV-infected study participants, 82 (71.9%) were virologically suppressed with a median CD4 count of 540 [352] $\times$ 10⁶/L on ART for 7 [5] years. Fifty-seven (26.5%) participants with thrombosis were HIV infected. A history of ischaemic stroke was increased among HIV-infected participants as compared to HIV-uninfected participants (*n* = 7, 12.3% vs. *n* = 3, 1.4%, *p* < 0.004). Arterial thrombosis in the HIV-infected participants was

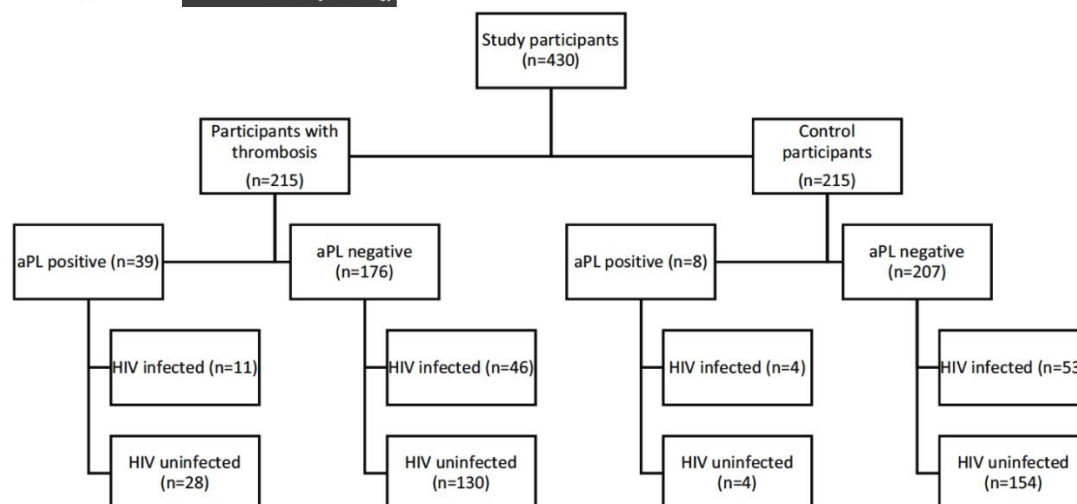


FIGURE 1 Flow diagram of patient selection

TABLE 1 Risk factors associated with thrombosis in the study population

Parameter	Participants with thrombosis n = 215	Controls n = 215	OR (95% CI)	p value
Venous risk factors				
Personal history of VTE (n, %)	205 (95.3)	0 (0)	–	<0.001
Thrombophilia (n, %) ^a	24 (15.1)	–	–	–
Baseline aPL positivity (n, %) ^b	39 (18.1)	8 (3.7)	5.7 (2.6–12.6)	<0.001
AIIRD (n, %) ^c	26 (12.1)	11 (5.1)	2.6 (1.2–5.3)	0.012
Heart disease (n, %)	12 (5.6)	4 (1.9)	3.1 (1.0–9.8)	0.052
Hospital admission/immobility (n, %)	9 (4.2)	3 (1.9)	3.1 (0.8–11.6)	0.094
Major surgery (n, %)	8 (3.7)	0 (0)	–	0.049
Gross varicose veins (n, %)	10 (4.7)	3 (1.9)	3.4 (0.9–12.7)	0.063
Systemic infection (n, %)	6 (2.8)	1 (0.5)	–	0.094
Malignancy (n, %)	1 (0.5)	0 (0)	–	0.500
Age >35 years (n, %)	74 (34.4)	69 (32.1)	1.1 (0.7–1.7)	0.609
Cardiovascular risk factors				
Chronic hypertension (n, %)	30 (14.0)	40 (18.6)	0.7 (0.4–1.2)	0.193
Diabetes mellitus (n, %)	3 (1.4)	0 (0)	–	0.196
Hyperlipidaemia (n, %) ^d	5 (2.9)	0 (0)	–	0.852
BMI ≥30 kg/m ² (n, %)	60 (27.9)	57 (26.5)	1.1 (0.7–1.6)	0.745

Abbreviations: AIIRD, autoimmune inflammatory rheumatic diseases; aPL, antiphospholipid antibodies; BMI, body mass index; CI, confidence interval; OR, odds ratio; VTE, venous thromboembolism.

^aThrombophilia screening was performed in 159 participants with thrombosis. Thrombophilia's included protein S deficiency (n = 20), protein C deficiency (n = 2) and prothrombin gene mutation (n = 1).

^bBaseline aPL positivity refers to positivity on a single occasion for LAC, aCL or aβ2GP1 antibodies IgG and IgM.

^cAIIRD included systemic lupus erythematosus (n = 20), dermatomyositis (n = 1), rheumatoid arthritis (n = 1), antineutrophil cytoplasmic antibody-associated vasculitis (n=1) and mixed connective tissue disease (n = 3).

^dTesting for hyperlipidemia was performed in 175 participants with thrombosis and 20 controls.

not associated with an increased odds of chronic hypertension, diabetes mellitus, hyperlipidaemia or BMI ≥30 kg/m² as compared to the HIV-infected controls. On thrombophilia screening, in 40

HIV-infected participants with thrombosis in whom testing was performed, a protein S deficiency was identified in seven (17.5%) and a protein C deficiency in one (2.5%), which were not significantly

TABLE 2 Characteristics of aPL-positive and aPL-negative participants with thrombosis

Parameter	aPL-positive n = 39 (18.1%)	aPL-negative n = 176 (81.9%)	p value
Demographics			
Age at conception (years), mean $\pm$ SD	31 $\pm$ 5	32 $\pm$ 6	0.257
Parity, mean $\pm$ SD	2 $\pm$ 1	2 $\pm$ 1	0.710
BMI (kg/m ²), mean $\pm$ SD ^a	25.8 $\pm$ 5.0	29.8 $\pm$ 6.9	0.010
Clinical characteristics			
HIV infected (n, %)	11 (28.2)	46 (26.1)	0.791
AIIRD (n, %) ^a	9 (23.1)	17 (9.7)	0.024
Personal history of thrombosis			
Arterial Thrombosis (n, %)	0 (0)	10 (5.7)	0.214
VTE (n, %)	38 (97.4)	165 (93.8)	0.699
Provoked VTE (n, %)	2 (5.3)	14 (8.5)	0.741
Unprovoked VTE (n, %)	13 (34.2)	44 (26.7)	0.351
Pregnancy/oestrogen related VTE (n, %)	23 (60.5)	107 (64.8)	0.617
Venous and arterial thrombosis (n, %)	1 (2.6)	1 (0.6)	0.331
Recurrent VTE	9 (23.1)	23 (13.1)	0.117
Personal history of obstetric complications			
Early pregnancy losses $\geq$ 3 (n, %)	5 (12.8)	22 (12.5)	0.956
IUFD (n, %)	8 (20.5)	22 (12.5)	0.191
Severe preeclampsia or FGR (n, %)	3 (7.7)	9 (5.1)	0.458
Laboratory characteristics			
White cell count ($\times 10^9$ /L), mean $\pm$ SD (ref: 3.9–12.6)	6.8 $\pm$ 3.1	7.8 $\pm$ 2.9	0.076
Haemoglobin (g/L), mean $\pm$ SD (ref: 116–164)	116.3 $\pm$ 22.7	123.3 $\pm$ 18.0	0.037
Platelet count ($\times 10^9$ /L), mean $\pm$ SD (ref: 186–454)	270.9 $\pm$ 129.7	284.9 $\pm$ 99.7	0.455
Mean platelet volume (fL), mean $\pm$ SD (ref: 7.3–11.3)	9.9 $\pm$ 2.3	10.1 $\pm$ 1.6	0.608

Abbreviations: AIIRD, autoimmune inflammatory rheumatic diseases; aPL, antiphospholipid antibodies; BMI, body mass index; FGR, foetal growth restriction; HIV, human immunodeficiency virus; IUFD, intra-uterine foetal death; SD, standard deviation; VTE, venous thromboembolism.

^aAIIRD included systemic lupus erythematosus (n = 20), dermatomyositis (n = 1), rheumatoid arthritis (n = 1), antineutrophil cytoplasmic antibody-associated vasculitis (n = 1) and mixed connective tissue disease (n = 3).

TABLE 3 Characteristics of aPL-positive and aPL-negative HIV-infected participants with thrombosis

Parameter	aPL-positive n = 11 (19.3%)	aPL-negative n = 46 (80.7%)	p value
HIV characteristics			
Years from diagnosis, median (IQR)	5 [8]	4 [8]	0.980
CD4 cell count $>200 \times 10^6$ /L, (n, %)	7 (63.6)	39 (84.8)	0.195
HIV viral load <50 copies/ml, (n, %)	7 (63.6)	34 (73.9)	0.482
ART	8 (72.7)	42 (91.3)	0.092

Abbreviations: aPL, antiphospholipid antibodies; ART, antiretroviral therapy; HIV, human immunodeficiency virus.

different as compared to HIV-uninfected participants ($p = 0.299$). Eleven (19.3%) HIV-infected participants were aPL positive (Table 3). The majority of the aPL-positive women (n=8, 72.7%) were on ART for a median [IQR] of 5 [8] years.

The profiles of the aPL-positive study participants are illustrated in Table S1 and S2. There were 37 (78.7%) participants with single aPL positivity. In addition, nine (19.1%) participants (eight with thrombosis and one control) were double positive and one (2.1%)

participant with thrombosis was triple positive. The most common aPL profiles were LAC and aCL IgG as well as aCL IgM and a β 2GPI IgM, observed in six (12.8%). LAC positivity was significantly associated with thrombosis (24/39, 61.5% with thrombosis vs 1/8, 12.5% controls; $p < 0.009$).

The aPL profiles did not present statistically significant differences between the HIV-uninfected and HIV-infected participants. Among the HIV-infected participants with thrombosis ($n = 57$), nine (15.8%) had a LAC, two (3.5%) aCL IgG antibodies and one (1.8%) a β 2GPI IgG antibodies. In the HIV-infected control participants ($n = 57$), one (1.8%) had aCL IgM antibodies and three (5.3%) had a β 2GPI IgM antibodies. LAC positivity was significantly associated with thrombosis ($p < 0.003$). In contrast, aCL and a β 2GPI IgM antibodies were not associated with thrombosis ($p = 0.118$).

There were 28 (59.6%) of the aPL-positive participants with aPL results from two separate occasions, at least 12 weeks apart as per diagnostic guidelines. The diagnostic criteria for APS were met in eight out of twenty-two participants (with thrombosis): 2/7 (28.6%) HIV-infected and 6/15 (40.0%) HIV-uninfected. The presence of APS was not statistically significantly different between the HIV-uninfected and HIV-infected participants ($p = 1.000$). Repeat testing was positive in one of the six controls tested, who was HIV-uninfected with systemic lupus erythematosus.

4 | DISCUSSION

aPL have been proposed as one of the causes of a hypercoagulable state in HIV infection, that can lead to thrombosis and/or adverse pregnancy outcomes. In this study, baseline aPL positivity was associated with an approximately sixfold increased odds for thrombosis among South African women attending a specialist obstetric unit. Among the HIV-infected participants, baseline LAC positivity was significantly associated with thrombosis. Nonetheless, in the present study, in the era of suppressive ART, the prevalence of aPL was not significantly increased among the subgroup of HIV-infected women, as compared to HIV-uninfected women (13.2% vs. 10.1%).

Earlier studies reported an increased aPL prevalence, which varied according to the aPL isotype and the clinical characteristics of the population studied. Among ART naïve participants, increased rates of aCL IgG of 17%–93% were reported. This was in addition to LAC of 0%–2.9%, aCL IgM of 1%–13%, a β 2GPI IgG of 0%–37% and a β 2GPI IgM of 0%–10%.^{6,7,28,29} Despite these findings of an increased aPL prevalence, these were not associated with an increased risk of thrombotic complications (OR 0.8, 95% CI 0.1–2.1).¹⁴ The findings of these earlier studies suggested that circulating antibodies in HIV infection against phospholipids, phospholipid-protein complexes and phospholipid-binding proteins, similar to other chronic viral infections, could act as a trigger for infection-induced aPL production. Alternatively, aPL could be induced by phospholipid exposure as a consequence of inflammation, coagulation and platelet activation in HIV.

Contrary to previous reports, in the present study, we observed a lower frequency of aCL IgG of 3.5%. The majority ($n = 82$, 71.9%) of the HIV-infected study participants were virologically suppressed on ART for 7 [5] years. In keeping with this trend, Sedlacek et al. described a decline in aPL levels in a longitudinal study of 30 HIV-infected patients on suppressive ART for 5 years.¹¹ The authors reported a decrease, albeit non-significant, at five years in L-alpha-phosphatidylethanolamine IgG, aCL IgG, L-alpha-phosphatidylserine IgG and L-alpha-phosphatidylinositol IgG levels as compared to presentation. Similarly, Martinez et al., described an association between aCL antibodies and ART, immunosuppression and immune dysregulation in HIV infection.¹³ So too, the lower aCL prevalence in the current study can be, in part, attributed to the lesser degree of immune suppression. The lower rates of aCL IgG, in addition to aCL IgM, a β 2GPI IgG and a β 2GPI IgM, in the present study also may be as a result of the type of solid-phase assays as well as the use of higher recommended cut-off values for positivity, as compared to earlier studies.^{7,8,11,28}

It is interesting to note that the aPL profiles of the HIV-infected women without thrombosis, in this study, were consistent with a low-risk profile for thrombosis. We observed aCL and a β 2GPI IgM antibodies at a combined rate of 7%. These were not associated with LAC or IgG antibodies. The presence of single aCL and a β 2GPI IgM in large studies of thrombotic APS has been rarely described, at rates of 3.5%–5.4%, and has not been shown to be significant risk factors for thrombosis.³⁰ In contrast, among the HIV-infected women with thrombosis, LAC was a significant predictor of thrombosis. The single-positive LAC, observed among HIV-infected women with thrombosis, may be explained by additional co-factors, such as complement C4 or factor H.³¹ Another possible explanation is the significant variation among aCL and a β 2GPI solid-phase assays as well as among laboratories.^{32,33} A strong association between LAC and non-criteria aPL such as anti-prothrombin/phosphatidylserine (aPS/PT) has also been described in patients with APS.^{34,35} An increasing frequency aPS/PT IgG ranging from 34% to 87% has been previously reported in South African HIV-infected groups.^{7,9} Nonetheless, studies in HIV-infected women in the era of ART are lacking. Non-criteria aPL warrant further validation studies in HIV-infected women with previous thrombosis and/or obstetric complications.

This increased risk of VTE can also be attributed to classical venous risk factors. Significantly, this study revealed a higher prevalence of personal history of VTE, AIIRD and major surgery on univariate analysis. Several coagulation abnormalities have also been described in association with HIV and thrombosis,^{36,37} which have been attributed to increased systemic inflammation and immune activation.³⁸ In the present study, deficiencies of protein S and C were observed in 17.5% and 2.5% of HIV-infected participants, respectively, which is comparable to findings of similar South African cohorts with HIV and thrombosis.^{39,40} We, however, did not find a significant relationship between protein S and C deficiencies and HIV infection. These results should be interpreted with caution as testing was only performed in a subgroup of participants. Furthermore, the reference ranges were locally validated using healthy South African

donors; however, specific reference ranges for HIV-infected females have not been clearly defined. Among the HIV-infected study participants, an increased frequency of arterial thromboses was observed, as compared to HIV-uninfected participants. However, consistent with other local studies, this was not associated with chronic hypertension or obesity.^{41,42} In a study of acute coronary syndrome in HIV-infected patients, Becker et al. identified few conventional cardiovascular risk factors, namely lower HDL cholesterol levels and smoking.⁷ This suggests a potentially more important role for HIV related factors in contributing to arterial thrombosis—independent of cardiovascular risk factors.

This study represents one of the largest analyses of HIV-infected women with thrombosis in the era of ART. Participants were investigated for APS at a core laboratory. Nonetheless, the results of the study should be interpreted critically owing to insufficient statistical power. As such, the possible association of risk factors could not be assessed by multiple regression analysis owing to the low incidence. A second limitation of this study is that LAC testing was not performed according to ISTH recommendations: Interpretation of the aPTT relied on a mixing test. As a confirmatory aPTT step was not performed, weak aPL may have yielded false-negative results. There were 19 (40.4%) aPL-positive participants, who did not have repeat testing at 12 weeks. aPL testing was also performed during pregnancy in a sub-population. During pregnancy, shortened clotting times, as a result of raised factor VIII levels, can lead to false-negative LAC results. A final limitation is that the patients were enrolled at a single tertiary centre, which could have selected for a high-risk population compared with the total pool of HIV-infected women in South Africa.

5 | CONCLUSION

In conclusion, this study investigated criteria aPL in South African women with a history of thrombosis and/or obstetric complications. In the era of suppressive ART, the prevalence of aPL was not significantly increased among the HIV-infected women. Positivity for LAC, in addition to traditional venous risk factors, was associated with a significantly increased risk for thrombosis in HIV-infected women. Nonetheless, this study population is small and aPL registries are recommended to improve our understanding of the role of aPL in HIV-infected women with a history of thrombosis.

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CONFLICT OF INTEREST

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

AUTHOR CONTRIBUTIONS

All authors performed the research. E Schapkaitz (Dept of Molecular Medicine and Haematology, University of Witwatersrand) involved in study design, manuscript author, data collection, entry and analysis. E Libhaber (HS Research and School of Clinical Medicine, University of Witwatersrand) involved in study design, data analysis and critical review. BF Jacobson (Dept of Molecular Medicine and Haematology, University of Witwatersrand) involved in study design and critical review. A Gerber (Dept of Obstetrics, University of Witwatersrand) involved in data collection and critical review. H Rhemtula (Dept of Obstetrics, University of Witwatersrand) involved in data collection and critical review. H R Büller (Dept of Vascular Medicine, University of Amsterdam) involved in study design and critical review.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

ORCID

Elise Schapkaitz  <https://orcid.org/0000-0002-1534-2930>

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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Supplementary Tables

Table 4.1 The profile of the baseline aPL positive study participants

N	Thrombotic complication	HIV	LAC aPTT	LAC dRVVT	LAC concl	aCL IgM (U/ml)	aCL IgG (U/ml)	aβ2GP1 IgM (U/ml)	aβ2GP1 IgG (U/ml)	Repeat LAC aPTT	Repeat LAC dRVVT	Repeat LAC concl	Repeat aCL IgM (U/ml)	Repeat aCL IgG (U/ml)	Repeat aβ2GP1 IgM (U/ml)	Repeat aβ2GP1 IgG (U/ml)
1	DVT	+	-	-	-	<29.9	28.0	<5.7	<17.7							
2	DVT	+	+	+	+	<29.9	<23.8	<5.7	<17.7	-	-	-				
3	DVT	+	+	+	+	<29.9	<23.8	<5.7	<17.7	-	-	-				
4	DVT	+	+	-	+	<29.9	<23.8	<5.7	<17.7	-		-				
5	PE	+	-	+	+	<29.9	<23.8	<5.7	<17.7		-	-				
6	PE	+	+	-	+	<29.9	<23.8	<5.7	<17.7	+		+				
7	PE	+	+	-	+	<29.9	<23.8	<5.7	<17.7	-		-				
8	DVT	+	-	-	-	<29.9	<23.8	<5.7	19							18
9	PE	+	+	-	+	<29.9	43.0	<5.7	<17.7							
10	PE	+	+	-	+	<29.9	<23.8	<5.7	<17.7	*						
11	DVT and PE	+	-	+	+	<29.9	<23.8	<5.7	<17.7		*					
12	-	+	-	-	-	<29.9	<23.8	12	<17.7						<5.7	
13	-	+	-	-	-	<29.9	<23.8	11	<17.7						<5.7	
14	-	+	-	-	-	30	<23.8	<5.7	<17.7							
15	-	+	-	-	-	<29.9	<23.8	12	<17.7						<5.7	
16	DVT	-	-	-	-	<29.9	28	<5.7	108							
17	DVT	-	+	+	+	<29.9	<23.8	<5.7	<17.7	-	-	-				
18	DVT	-	+	-	+	<29.9	<23.8	<5.7	<17.7	*						
19	DVT	-	+	-	+	<29.9	<23.8	<5.7	<17.7		*					
20	DVT	-	-	+	+	<29.9	<23.8	<5.7	<17.7		*					
21	PE	-	-	-	-	<29.9	60	<5.7	<17.7					49		
22	DVT	-	+	-	+	<29.9	<23.8	<5.7	<17.7	*						
23	PE	-	-	+	+	<29.9	<23.8	<5.7	<17.7		*					
24	PE	-	-	-	-	<29.9	26	<5.7	<17.7					<23.8		
25	PE	-	*	*		30	24	<5.7	<17.7				<29.9	<23.8		

26	DVT and PE	-	-	-	-	<29.9	<23.8	34	<17.7						22	
27	DVT	-	+	-	+	<29.9	<23.8	<5.7	<17.7	-		-				
28	DVT and PE	-	+	-	+	<29.9	<23.8	<5.7	22	-		-				
29	DVT and PE	-	+	+	+	<29.9	114	<5.7	149	*	*				97	
30	DVT	-	-	-	-	33	<23.8	<5.7	<17.7				<29.9			
31	PE	-	-	-	-	71	<23.8	27	<17.7							
32	DVT	-	+	-	+	<29.9	<23.8	<5.7	<17.7	-		-				
33	PE	-	+	-	+	<29.9	<23.8	<5.7	<17.7	-	-					
34	PE	-	-	-	-	50	<23.8	16	<17.7							
35	DVT	-	-	-	-	<29.9	<23.8	10	<17.7							
36	DVT	-	*	*		<29.9	61	<5.7	100							
37	PE	-	-	-	-	<29.9	44	<5.7	<17.7							
38	PE	-	+	-	+	<29.9	<23.8	<5.7	<17.7	**						
39	DVT and PE	+	-	+	+	47	<23.8	<5.7	<17.7		-	-		30		
40	Retinal vein thrombosis and TIA	-	-	-		<29.9	40	<5.7	<17.7							
41	DVT	-	+	+	+	<29.9	64	<5.7	<17.7	+	+	+			49	
42	DVT	-	*	*		<29.9	128	<5.7	<17.7						83	
43	DVT	+	+	-	-	<29.9	<23.8	<5.7	<17.7	-						
44	-	-	-	+	+	<29.9	<23.8	<5.7	<17.7							
45	-	-	-	-		31	<23.8	15	<17.7				<29.9			
46	-	-	-	-		30	<23.8	<5.7	<17.7				<29.9			
47	-	-	-	-		59	<23.8	<5.7	<17.7					39		

Key: DVT, deep vein thrombosis; PE, pulmonary embolus; TIA, transient ischaemic attack; HIV, human immunodeficiency virus; aCL, anticardiolipin; aβ2GP1, anti-beta2-glycoprotein 1; dRVVT, dilute Russell viper venom time; aPTT, activated partial thromboplastin time; LAC, lupus anticoagulant

Bold: All positive results are written in bold

*Anticoagulant interference

** Insufficient sample

Table 4.2 The lupus anticoagulant profile of the baseline aPL positive study participants

N	Thrombotic complication	HIV	aPTT (screen N ratio)	aPTT (screenmix N ratio)	dRVVT (screen N ratio)	dRVVT (screen/confirm N ratio)	LAC concl	Repeat aPTT (screen N ratio)	Repeat aPTT (screen mix N ratio)	Repeat dRVVT (screen N ratio)	Repeat dRVVT (screen/confirm N ratio)	Repeat LAC concl
1	DVT	+	0.98		0.96		-					
2	DVT	+	1.49	1.24	1.33	1.22	+	1.05		0.96		-
3	DVT	+	1.47	1.22	1.42	1.37	+	1.31	1.10	1.18		-
4	DVT	+	1.20	1.21	1.16		+	1.11		0.98		-
5	PE	+	1.24	1.14	1.33	1.22	+	1.17		1.19		-
6	PE	+	1.76	1.37	0.95		+	2.36	1.45			+
7	PE	+	1.56	1.30	1.14		+	1.31	1.12			-
8	DVT	+	0.95		1.06		-					
9	PE	+	1.80	1.28	1.22	0.85	+					
10	PE	+	1.59	1.29	1.20	1.09	+		*			
11	DVT and PE	+	1.29	1.17	1.34	1.24	+				*	
12	-	+	1.00		0.83		-					
13	-	+	0.84		0.91		-					
14	-	+	1.00		0.82		-					
15	-	+	1.11		0.84		-					
16	DVT	-	1.25	1.15	0.91		-					
17	DVT	-	2.06	1.39	1.26	1.28	+	1.08		0.94		-
18	DVT	-	1.46	1.23	1.04		+		*			
19	DVT	-	1.97	1.38	1.17		+				*	
20	DVT	-	1.22	1.13	1.22	1.20	+				*	
21	PE	-	1.17		0.82		-					
22	DVT	-	1.30	1.20	1.05		+		*			
23	PE	-	0.99		1.56	1.46	+				*	
24	PE	-	0.94		0.88		-					
25	PE	-		*		*						
26	DVT and PE	-	0.99		1.11		-					

27	DVT	-	2.30	1.40	1.03		+	1.23	1.16			-
28	DVT and PE	-	1.24	1.21	1.10		+	0.86				-
29	DVT and PE	-	1.88	1.60	2.64	1.63	+		*		*	
30	DVT	-	1.06		1.18		-					
31	PE	-	1.08		0.79		-					
32	DVT	-	1.55	1.28	1.50	1.16	+	0.89				-
33	PE	-	1.25	1.20	1.21	1.12	+	1.12		1.02		
34	PE	-	1.07		1.08		-					
35	DVT	-	0.90		0.83		-					
36	DVT	-		*		*						
37	PE	-	1.18		1.06		-					
38	PE	-	1.70	1.20	1.40	1.04	+	1.3	**			
39	DVT and PE	-	1.51	1.13	1.39	1.25	+			1.04		-
40	Retinal vein thrombosis and TIA	-	0.98		1.03							
41	DVT	-	1.75	1.53	1.75	1.75	+	1.60	1.50	1.62	1.62	+
42	DVT	-		*		*						
43	DVT	-	1.51	1.21	0.82		+	0.98				-
44	-	-	1.18		1.29	1.28	+					
45	-	-	0.97		1.10							
46	-	-	0.94		0.95							
47	-	-	0.94		0.88							

Key: DVT, deep vein thrombosis; PE, pulmonary embolus; TIA, transient ischaemic attack; HIV, human immunodeficiency virus; dRVVT, dilute Russell viper venom time; aPTT, activated partial thromboplastin time; LAC, lupus anticoagulant

Bold: All positive results are written in bold

*Anticoagulant interference

** Insufficient sample

CHAPTER 5: von Willebrand factor propeptide to antigen ratio in HIV infected pregnancy: Evidence of endothelial activation

Summary: This prospective cross-sectional study was designed to assess endothelial activation in pregnant women living with HIV in each trimester. The study found an increase in the VWF propeptide to VWF antigen ratio, as an index of acute endothelial activation, in HIV women with VL > 50 copies/mL as well as HIV women with virological suppression, as compared to matched HIV negative women. Virological suppression was not associated with a significant reduction in the VWF propeptide to VWF antigen ratio, which is suggestive of persistent endothelial activation on suppressive ART.

Statement of originality document: Please see Appendix 8.2

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

Elise Schapkaitz¹ | Elena Libhaber² | Barry F. Jacobson¹ | Muriel Meiring³ | Harry R. Büller⁴

¹Department of Molecular Medicine and Hematology, Charlotte Maxeke Johannesburg Academic Hospital National Health Laboratory System Complex and University of Witwatersrand, Johannesburg, South Africa

²Department of Research Methodology and Statistics, Faculty of Health Sciences, University of Witwatersrand Medical School, Johannesburg, South Africa

³Department of Hematology and Cell Biology, University of Free State and National Health Laboratory Service, Bloemfontein, South Africa

⁴Department of Vascular Medicine, Academic Medical Centre, University of Amsterdam, the Netherlands

Correspondence

Elise Schapkaitz, PO Box 28985 Sandringham 2131, South Africa.
Email: elise.schapkaitz@nhls.ac.za

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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

Handled and decision: Claire McLintock

Final decision: Claire McLintock, 16 August 2021

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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

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.

ADAMTS-13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) antigen, activity, and antibody levels.

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 immuno-turbidimetric 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, FRET5-VWF73 by ADAMTS-13 was measured at 37°C using spectrofluorometry (Synergy HT Reader, Biotek).

2.3 | Statistical methods

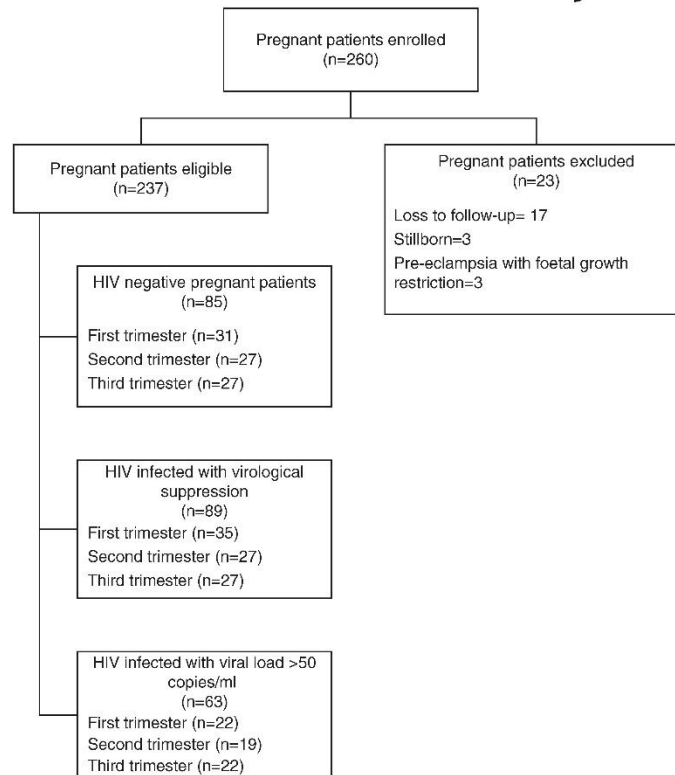
A target sample size of at least 25 participants per study group from each trimester of pregnancy was calculated from the initial pilot study based on an analysis of variance (ANOVA) over all fixed effect size of 0.30 with no interactions with a power of 90% and an $\alpha = .05$. Clinical and laboratory data were analyzed using Statistica 13.2 software and SAS 9.1 software. Normally distributed, continuous data were presented as mean $\pm$ 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 $\pm$ 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 $\pm$ 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] × 10⁶/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] × 10⁶/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 5⁴ 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 $\pm$ SD	31.0 $\pm$ 6.0	34.0 $\pm$ 6.0***	32.0 $\pm$ 6.0	<.001
Parity, median [IQR]	1.0 [1.0]	2.0 [2.0]*	2.0 [2.0]	.008
BMI (kg/m ²), mean $\pm$ SD	28.3 $\pm$ 5.6	27.6 $\pm$ 5.8	27.4 $\pm$ 6.0	.545
Smoking history >10/day (n, %)	0 (0)	0 (0)	0 (0)	-
Laboratory characteristics				
White cell count (x10 ⁹ /L), mean $\pm$ SD (ref: 3.9–12.6)	7.9 $\pm$ 2.0	7.7 $\pm$ 2.4**	6.7 $\pm$ 2.1*	.012
Hemoglobin (g/L), mean $\pm$ SD (ref: 116–164)	124.5 $\pm$ 12.8	119.3 $\pm$ 14.7	114.5 $\pm$ 16.6**	<.001
Platelet count (x 10 ⁹ /L), mean $\pm$ SD (ref: 186–454)	254.7 $\pm$ 74.0	249.2 $\pm$ 66.0	261.3 $\pm$ 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 $\pm$ SD	39.0 $\pm$ 2.0	37.0 $\pm$ 4.0*	38.0 $\pm$ 2.0	.040
Birthweight (g), mean $\pm$ SD	3284.3 $\pm$ 535.3*	2942.2 $\pm$ 585.6***	3027.9 $\pm$ 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 (x10 ⁶ /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.

^aFirst-line consisted of tenofovir, emtricitabine and efavirenz or tenofovir, lamivudine and dolutegravir.

^bSecond-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)	92.8–150.6	First trimester	123.0 [64.9]	141.9 [86.6]	147.7 [77.2]	.131
Log VWF antigen			4.7 ± 0.4	5.0 ± 0.4*	5.0 ± 0.4	.021
	98.7–174.4	Second trimester	167.9 [47.8]	186.8 [62.2]	175.9 [97.1]	.764
			5.1 ± 0.3	5.2 ± 0.3*	5.2 ± 0.3	.042
	134.0–245.6	Third trimester	208.0 [65.1]	225.3 [98.4]	228.7 [82.8]	.283
			5.3 ± 0.3	5.4 ± 0.4	5.4 ± 0.4	.038
VWF propeptide (U/dl)	116.9–169.4	First trimester	162.0 [60.7]	219.0 [100.6]	218.9 [101.3]	<.001
Log VWF propeptide			5.1 ± 0.4	5.4 ± 0.4***	5.4 ± 0.4***	<.001
	116.0–154.9	Second trimester	209.8 [103.6]	301.9 [129.3]	269.3 [204.7]	.009
			5.3 ± 0.4	5.8 ± 0.4***	5.6 ± 0.4	<.001
	120.8–188.0	Third trimester	230.7 [135.5]	289.0 [219.3]	262.3 [277.4]	.019
			5.4 ± 0.6	5.7 ± 0.7*	5.6 ± 0.8*	.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 pregnancy²¹ 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.

ORCID

Elise Schapkaitz  <https://orcid.org/0000-0002-1534-2930>

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SUPPORTING INFORMATION

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

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CHAPTER 6: Evaluation of markers of fibrinolysis and coagulation in pregnant women with HIV

Summary: This prospective cross-sectional study expanded on Chapter five on endothelial activation by further assessing activation of coagulation and fibrinolysis in pregnant women living with HIV in each trimester. The study found an increase in d-dimer and PAI-1 levels, as a measure of fibrinolytic activity, in HIV women with VL > 50 copies/mL as well as HIV women with virological suppression, as compared to matched HIV negative women. Virological suppression was not associated with a significant reduction in first and third trimester d-dimer and PAI-1 levels, which is suggestive of persistently impaired fibrinolytic activity on suppressive ART. The results of a sub-study, presented at the International Society of Laboratory Haematology Congress, further assessed platelet activation in pregnant women living with HIV, in each trimester. No significant differences were found in PF4 in women with virological suppression and VL > 50 copies/ml, as compared to HIV negative women. With regard to platelet parameters, mean platelet volume (MPV) measured in the third trimester was significantly decreased in women with virological suppression as compared to HIV negative women. Mean platelet volume, measured in the third trimester, may be a valuable marker of platelet activation in pregnant women with HIV on suppressive ART.

Statement of originality document: Please see Appendix 8.2



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Full Length Article

Evaluation of markers of fibrinolysis and coagulation in pregnant women with human immunodeficiency virus

Elise Schapkaitz^{a,*}, Elena Libhaber^b, Barry F. Jacobson^a, Marketa Toman^c, Annika Gerber^d, Harry R. Büller^e

^a Dept. of Molecular Medicine and Hematology, Charlotte Maxeke Johannesburg Academic Hospital National Health Laboratory System Complex and University of the Witwatersrand, South Africa

^b Dept. of Research Methodology and Statistics, Faculty of Health Sciences, University of the Witwatersrand Medical School, South Africa

^c Department of Chemical Pathology, National Health Laboratory Service (NHLS) at the Faculty of Health Sciences, University of the Witwatersrand Medical School, South Africa

^d Department of Obstetrics, Charlotte Maxeke Johannesburg Academic Hospital, South Africa

^e Dept. of Vascular Medicine, Academic Medical Centre, University of Amsterdam, the Netherlands

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ABSTRACT

Introduction: Human immunodeficiency virus (HIV) in pregnant women is characterized by immune activation and inflammation despite suppressive antiretroviral therapy (ART). The extent to which ongoing inflammation contributes to activation of coagulation and fibrinolysis is unknown.

Materials and methods: This cross-sectional study included pregnant women in the following three groups: HIV negative ($n = 109$), HIV infected virologically suppressed ($n = 109$) and HIV infected with HIV viral load (VL) of >50 copies/mL ($n = 80$). Fibrinolytic activity was evaluated by measuring d-dimer and plasminogen activator inhibitor-1 (PAI-1) as well as thrombin-antithrombin (TAT) complex concentrations, as an index of coagulation, in the first, second and third trimesters.

Results: In this population, with a mean age of 33 ± 6 years, pregnancy outcomes were recorded for 277 (93.0%) participants with live births. HIV infected participants with virological suppression and VL of >50 copies/mL showed significantly increasing levels of d-dimer and PAI-1 in the first, second and third trimesters, as compared to HIV negative participants. No significant differences were observed between HIV infected participants with virological suppression and HIV infected participants with VL > 50 copies/mL for levels of first and third trimester d-dimer and PAI-1 in each trimester. In addition, TAT complex levels in the first trimester were significantly increased in HIV infected virologically suppressed participants as compared to HIV negative participants.

Conclusion: HIV infected virologically suppressed pregnant women show evidence of persistently impaired markers of fibrinolysis. Future research should explore the risk of adverse pregnancy complications among HIV infected pregnant women in the modern era of ART.

1. Introduction

Globally, an estimated 1.3 million pregnant women are living with human immunodeficiency virus (HIV) infection, with the highest burden of disease observed in Eastern and Southern Africa. Despite significantly improved access to antiretroviral therapy (ART), pregnancy in HIV infected women continues to be associated with increased morbidities including venous thrombo-embolism (VTE), placental vascular complications and preterm delivery [1–5]. Immune activation and

inflammation contribute to the excess risk associated with HIV infection in pregnancy and suppressive ART has not consistently been associated with an improvement in markers of inflammation [6,7]. However, the extent to which ongoing inflammation contributes to activation of coagulation and fibrinolytic activity and its prothrombotic consequences has not been well described in pregnant HIV infected women of African ethnicity in the modern era of suppressive ART.

Studies conducted over the past decade in non-pregnant HIV-infected populations have investigated the association between markers of

* Corresponding author at: PO Box 28985, Sandringham 2131, South Africa.
E-mail address: elise.schapkaitz@nhls.ac.za (E. Schapkaitz).

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coagulation and fibrinolysis and morbidity and mortality [8–13]. The d-dimer, a degradation product of cross-linked fibrin, and plasminogen activator inhibitor-1 (PAI-1), the principal inhibitor of fibrinolysis have previously been examined as markers of impaired fibrinolysis [11,14,15]. D-dimer levels have been independently associated with morbidity (cardiovascular disease, chronic kidney disease, liver failure and malignancies), all-cause mortality and non-AIDS deaths [8,9,14]. Several studies, investigating the effects of suppressive ART, have observed a decline of d-dimer levels [15–19]. Nonetheless, d-dimer levels remained significantly elevated when compared to HIV negative controls [11,20] and pre-HIV levels [21,22] suggesting impaired fibrinolysis may be an important driver of morbidity and mortality in the era of suppressive ART. Increased PAI-1 levels, in the Women's Interagency HIV Study, also independently predicted non-AIDS deaths. Significant differences in PAI-1 levels were observed according to ART use, in this longitudinal cohort of 674 women, of predominantly African ethnicity. Nonetheless, the study population was recruited during the years 1994–1995, prior to widespread ART and the study did not include a HIV negative control group for comparison [15].

Few studies have investigated markers of hemostasis in HIV-infected pregnant women in the era of ART. de Andrade et al., investigated markers of impaired fibrinolysis in pregnant HIV infected Brazilian women receiving ART [23]. D-dimer levels were increased among HIV infected pregnant women, as compared to HIV negative pregnant controls. No evidence of disruption of the balance between tissue-type plasminogen activator (t-PA) and PAI-1 was observed, albeit a small number of HIV infected pregnant women ($n = 11$). Normal pregnancy is characterized by impaired fibrinolysis as a result of an increase in PAI-1 as well as placental derived plasminogen activator inhibitor type 2 (PAI-2), from the second trimester. An early excessive release of PAI-1, however, creates an imbalance of maternal hemostasis, which may predispose to VTE and placental vascular complications [24,25]. In African women, with the highest burden of infection, the association between impaired fibrinolysis and adverse pregnancy complications has not been explored previously.

The present study of HIV infected pregnancy investigated d-dimer and PAI-1, as markers of fibrinolytic activity, in addition to thrombin antithrombin (TAT) complex concentrations, as a measure of coagulation, in each trimester and its relationship with HIV viral load (VL) and adverse pregnancy outcomes. We hypothesized that fibrinolytic and coagulation activity may be activated in HIV infected pregnant women on suppressive ART.

2. Methods

2.1. Study design and population

The design and population of the study were described previously [26]. The study was conducted at the Charlotte Maxeke Johannesburg Academic Hospital Antenatal Clinic, Johannesburg, South Africa between 1 July 2019 and 30 April 2021. The following participants >18 years of age of African ethnicity, with a singleton pregnancy were recruited: HIV negative participants; HIV infected with virological suppression (<50 copies/mL) on ART and HIV infected with VL > 50 copies/mL. The three study groups were matched according to gestation. The gestational age, based on last menstrual period and/or 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. Participants 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. Written informed consent was obtained from all study participants, and the study protocol was approved by the Institutional Review Board (M-181018).

2.2. Study protocol

2.2.1. Data collection

Antenatal visits were scheduled at two to four week intervals from enrolment until delivery, including a visit at six weeks postpartum. Data collected on pre-designed data collection sheets included demographics, obstetric history, delivery characteristics, adverse pregnancy outcomes (namely, preterm delivery and placental vascular complications) and symptomatic VTE during pregnancy and up to six weeks postpartum. 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. Apart from ART, the HIV negative and HIV infected participants received the same level of antenatal care. Study participants enrolled after 2020 were screened for coronavirus disease 2019 (COVID-19) via monitoring of symptoms and/or real-time polymerase chain reaction from the upper respiratory tract.

2.2.2. Blood sampling and laboratory methods

Blood sampling was performed at a single time point in each participant. Blood was collected in 3.2 % sodium citrate (ratio of one volume trisodium citrate to nine volumes of blood; Becton-Dickinson, Oxford, UK), dipotassium (K_2) ethylene-diamine-tetra-acetic acid (EDTA). Platelet-poor plasma was obtained by centrifugation at 3000 $\times$ g for 15 min within 30 min of collection. Aliquots were stored locally at -20°C . The study measurements were performed at the National Health Laboratory Service Research Laboratory, Johannesburg, South Africa. Daily quality control was performed prior to patient sample analysis.

2.2.3. Hematological testing

Full blood count parameters were analyzed on the Sysmex XN 9000 hematology analyzers (Sysmex Corporation, Kobe, Japan) with reagents and protocols from the manufacturer.

2.2.4. Fibrinolysis and coagulation testing

D-dimer was measured with the STA-Liatest immuno-turbidimetric assay (Diagnostica Stago, Asnières, France) on the STA-R Max $\otimes$ automated coagulation analyzer (Diagnostica Stago, Asnières sur Seine, France). The intra-assay and inter-assay coefficients of variation for d-dimer were 4.6 % and 3.3 %, respectively. The reference ranges for women of African ethnicity were 0.34–0.62 mg/L for first trimester; 0.45–0.80 mg/L for second trimester and 0.67–1.26 mg/L for third trimester [27]. The plasma concentrations of PAI-1 (Quantikine, R&D Systems, Inc., Minneapolis, USA) and TAT complex (Abnova, Taipei City, Taiwan) were measured using commercially available sandwich enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions. 50 μL of plasma, standards and quality control were incubated with the specific monoclonal antibody, pre-coated onto a microplate. Unbound antigen and antibody complexes were removed by washing. A polyclonal specific antibody linked directly to a peroxidase (in PAI-1 assay) or in biotinylated form recognized by a streptavidin-peroxidase conjugate (in TAT assay), was added to the wells. Following a wash to remove any unbound material, a peroxidase enzyme substrate was added and the reaction was read at 450 nm. PAI-1 and TAT complex concentrations were quantified by comparing the absorbance with a curve generated from known concentration values of the standard samples. The intra-assay and inter-assay coefficients of variation for PAI-1 were in average 6.8 % and 7.0 %, respectively. The intra-assay and inter-assay coefficients of variation for TAT complex concentrations were 4.9 % and 7.1 %, respectively. The reference range of PAI-1 and TAT complex concentrations were 0.7–14.0 ng/mL and 0.5 to 10 ng/mL, respectively.

2.3. Statistical methods

A target sample size of at least 33 participants per study group from each trimester of pregnancy was calculated from the initial pilot study based on an ANOVA overall fixed effect size of 0.30 with no interactions with a power of 90 % and an $\alpha = 0.05$. Clinical and laboratory data were analyzed using Statistica 13.2 software (California, USA) and SAS 9.1 software (Cary, North Carolina, USA). Normally distributed, continuous data were presented as mean $\pm$ 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 $\pm$ SD. Comparisons for continuous measurements were performed using a parametric one-way ANOVA test with Sheffé's post hoc test or non-parametric Kruskal-Wallis test with multiple independent comparisons of mean ranks 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 19-month study period, 320 pregnant participants, of African ethnicity, were eligible for enrolment in the study. Twenty-two (6.9 %) were lost to follow-up and therefore the final cohort consisted of 298 participants (Fig. 1). The mean $\pm$ SD gestational age was 9 ± 3 weeks for first trimester participants, 23 ± 4 weeks for second trimester participants and 34 ± 4 weeks for 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 older maternal age and subsequently parity as compared to the HIV negative group ($p < 0.001$ and $p < 0.029$

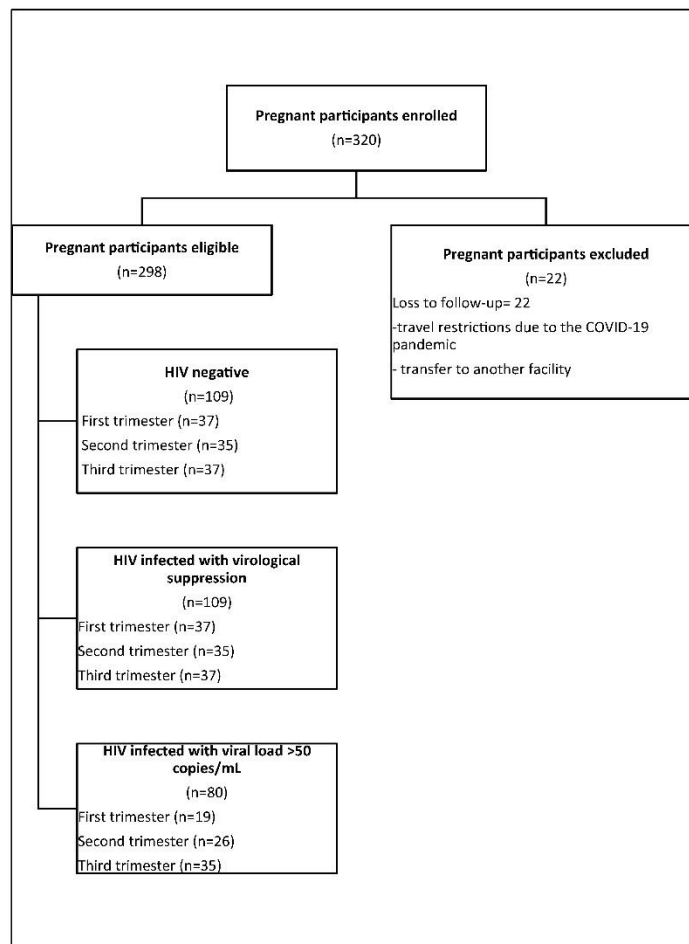


Fig. 1. Flow diagram of study participants enrolled at each trimester.

Table 1

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

Characteristic	HIV negative (n = 109)	HIV and VL <50 copies/mL (n = 109)	HIV and VL >50 copies/mL (n = 80)	p value
Demographics				
Age at study entry (years), mean $\pm$ SD	32.0 $\pm$ 6.0	35.0 $\pm$ 6.0 ^{***}	32.0 $\pm$ 6.0	<0.001
Parity, median [IQR]	2.0 [1.0]	2.0 [2.0] ^{**}	2.0 [2.0]	0.029
BMI (kg/m ²), mean $\pm$ SD	28.3 $\pm$ 5.9	27.3 $\pm$ 6.3	27.1 $\pm$ 5.7	0.212
Smoking history >10/day (n, %)	0 (0)	0 (0)	0 (0)	–
Laboratory characteristics				
White cell count ($\times 10^9$ /L), mean $\pm$ SD (ref: 3.9–12.6)	8.2 $\pm$ 2.0	7.5 $\pm$ 2.3 ^{**}	6.8 $\pm$ 2.2 [*]	0.012
Hemoglobin (g/L), mean $\pm$ SD (ref: 116–164)	125.8 $\pm$ 13.1	117.2 $\pm$ 15.0	114.8 $\pm$ 16.4 ^{**}	<0.001
Platelet count ($\times 10^9$ /L), mean $\pm$ SD (ref: 186–454)	251.0 $\pm$ 66.0	246.0 $\pm$ 68.0	269.0 $\pm$ 69.0	0.719
Obstetric characteristics				
Gestational hypertension (n, %)	2 (1.8)	2 (1.8)	1 (1.3)	1.000
Pre-eclampsia and FGR (n, %)	4 (3.7)	3 (2.8)	1 (1.3)	0.609
Delivery characteristics of live births				
Live births (n, %)	102 (93.6)	99 (90.8)	76 (95.0)	0.514
Gestational age at delivery (weeks), mean $\pm$ SD	38.1 $\pm$ 2.0	37.7 $\pm$ 2.7 ^{**}	38.7 $\pm$ 1.5	0.005
Birthweight (g), mean $\pm$ SD	3268.2 $\pm$ 612.1	2964.5 $\pm$ 596.4 ^{***}	3009.1 $\pm$ 458.8	<0.001
Preterm delivery <37 weeks (n, %)	7 (6.4)	22 (20.2) ^{**}	12 (15.0)	0.012
Low birth weight < 2500 g (n, %)	9 (8.3)	17 (15.6)	7 (8.8)	0.167
Outcomes				
Placental vascular complications (n, %)	11 (10.1)	10 (9.2)	3 (3.8)	0.246
VTE (n, %)	0 (0)	0 (0)	0 (0)	–

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

BMI was calculated from first antenatal booking appointment.

Hypertension was defined as systolic blood pressure > 140 mm Hg and/or diastolic blood pressure > 90 mm Hg.

Pre-eclampsia was defined as hypertension > 20 weeks' gestation and new onset proteinuria (≥ 1 on dipstick) or multi-systemic signs (platelet count < 100×10^9 /L, elevated liver transaminases $\times 2$ baseline, serum creatinine > 1.1 mg/dL doubling of the serum creatinine concentration, pulmonary oedema, cerebral or visual disturbances).

Placental vascular complications included intra-uterine fetal death (n = 11), fetal growth restriction (n = 5), pre-eclampsia (n = 8).

^{*} p < 0.05 versus HIV negative.

^{**} p < 0.01 versus HIV negative.

^{***} p < 0.001 versus HIV negative.

respectively). The HIV infected group with virological suppression was associated with preterm delivery as compared to the HIV negative group (p < 0.012). There was no significant difference in placental vascular complications between the three groups. 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).

Table 2

HIV characteristics of the HIV infected study groups.

Characteristic	HIV and VL <50 copies/mL (n = 109)	HIV and VL >50 copies/mL (n = 80)	p value
HIV characteristics			
Years from diagnosis, median [IQR]	4.0 [7.0]	0.3 [4.0]	<0.001
CD4 cell count ($\times 10^6$ /L), median [IQR]	526 [313]	306 [295]	<0.001
HIV viral load (copies/mL), median [IQR]	<50	360 [8649]	<0.001
ART (n, %)			<0.001
First line therapy ^a	105 (96.3)	26 (32.5)	
Second line therapy ^b	4 (3.7)	15 (18.8)	
No therapy	0 (0)	39 (48.8)	

Key: ART, antiretroviral therapy; HIV, human immunodeficiency syndrome; 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 Abacavir.

The HIV infected study group with virological suppression consisted of 109 participants with a median [IQR] CD4 count of 526 [313] $\times 10^6$ /L on ART for a median of 4.0 [7.0] years. The majority (n = 105, 96.3 %) were on first line treatment. In the HIV infected study group with VL > 50 copies/mL, there were 80 HIV infected pregnant participants with a median VL of 360 [8649] copies/mL and median CD4 count of 306 [295] $\times 10^6$ /L. Of these, 39 (48.8 %) were newly diagnosed with HIV infection. A further 41 (51.3 %) presented with VL > 50 copies/mL on ART for a median of 2 [5] years.

3.2. Laboratory characteristics

3.2.1. D-dimer

Log d-dimer levels, measured in each trimester, showed a significant increase with pregnancy progression (Table 1S). Between each study group in the three trimesters, a significant difference in the overall plasma concentrations of log d-dimer was observed (Table 3). Subgroup analysis with multiple pairwise comparisons showed no statistically significant differences in the first and third trimester between the HIV infected group with virological suppression and the HIV infected group VL > 50 copies/mL. In the second trimester, a significant increase was observed in the HIV infected group VL > 50 copies/mL when compared to the HIV infected group with virological suppression. There was no significant association between d-dimer levels measured in each trimester and adverse pregnancy outcomes (Table 2S).

3.2.2. PAI-1

Log PAI-1 levels, measured in each trimester, showed a significant increase with pregnancy progression (Table 1S). Between each study group in the three trimesters, a significant difference in the overall plasma concentrations of log PAI-1 was observed (Table 3). Subgroup analysis with multiple pairwise comparisons showed no statistically significant differences in each trimester between the HIV infected group with virological suppression and the HIV infected group VL > 50 copies/mL. There was no significant association between PAI-1 levels measured in each trimester and adverse pregnancy outcomes.

3.2.3. TAT complex

Log TAT complex concentrations, measured in each trimester, showed a significant increase with pregnancy progression in the HIV negative group (Table 1S). Between each study group, a significant difference in the overall plasma concentrations of log TAT complex concentrations, in the first trimester, was observed (Table 3). Subgroup analysis with multiple pairwise comparisons showed a statistically significant increase in the first trimester in the HIV infected group with

Table 3

Comparison of parameters of fibrinolysis and coagulation between HIV negative, HIV infected with virological suppression and HIV infected with VL >50 copies/mL study groups.

Parameter	Gestational age	HIV negative	HIV and VL <50 copies/mL	HIV and VL >50 copies/mL	p value
D-dimer (mg/L), median [IQR]	First trimester	0.2 [0.1]–1.4 ±	0.2 [0.1]–1.2 ±	0.3 [0.1]–1.1 ±	0.180
		0.2	0.5	0.4	0.047
	Second trimester	0.3 [0.2]–1.1 ±	0.4 [0.2]–0.9 ±	0.5 [0.3]–0.7 ±	0.020
Log d-dimer, mean ± SD		0.3	0.4	0.4***	<0.001
	Third trimester	0.4 [0.2]–0.8 ±	0.6 [0.3]–0.5 ±	0.6 [0.4]–0.5 ±	0.059
		0.3	0.3***	0.5**	<0.001
PAI-1 (ng/mL), median [IQR]	First trimester	1.5 [1.1]–0.4 ±	2.4 [1.9]–1.0 ±	2.0 [1.7]–0.8 ±	0.005
		0.5	0.4***	0.5**	<0.001
	Second trimester	2.0 [2.3]–0.8 ±	3.5 [2.5]–1.3 ±	3.3 [0.9]–1.2 ±	<0.001
Log PAI-1, mean ± SD		0.3	0.4***	0.4***	<0.001
	Third trimester	3.7 [1.5]–1.3 ±	4.1 [2.8]–1.5 ±	4.1 [2.4]–1.5 ±	0.626
		0.3	0.4*	0.5 ± 0.3	0.012
TAT complex (ng/mL), median [IQR]	First trimester	4.7 [4.0]–1.6 ±	6.2 [5.4]–2.0 ±	4.7 [4.9]–1.6 ±	0.260
		0.4	0.5**	0.7	0.003
	Second trimester	5.1 [3.6]–1.7 ±	6.0 [5.0]–1.9 ±	5.3 [4.3]–1.8 ±	0.693
Log TAT, mean ± SD		0.4	1.9 ± 0.5	1.8 ± 0.5	0.114
	Third trimester	5.8 [2.2]–1.8 ±	6.5 [2.6]–1.9 ±	6.6 [2.9]–1.8 ±	0.663
		0.4	0.4	0.3	0.221
Platelet count (×10 ⁹ /L), median [IQR]	First trimester	256.5 [67.0]–5.6 ±	272.0 [86.0]–5.6 ±	315.0 [255.0]–5.8 ±	0.150
		0.3	5.6 ± 0.2	5.8 ± 0.4	0.122
	Second trimester	235.0 [71.0]–5.5 ±	244.0 [87.0]–5.5 ±	259.0 [109.0]–5.5 ±	0.300
Log Platelet count, mean ± SD		0.2	5.5 ± 0.3	5.5 ± 0.4	0.330
	Third trimester	212.0 [45.0]–5.3 ±	238.0 [82.0]–5.5 ±	245.0 [103.0]–5.4 ±	0.172
		0.2	5.5 ± 0.3	5.4 ± 0.3	0.182
Mean platelet volume (fL), median [IQR]	First trimester	10.1 [1.5]–2.3 ±	9.3 [1.3]–2.3 ±	9.3 [1.4]–2.3 ±	0.189
		0.1	0.1	0.1	0.133
	Second trimester	10.1 [1.2]–2.3 ±	10.1 [1.7]–2.3 ±	10.0 [1.4]–2.3 ±	0.979
Log MPV, mean ± SD		0.1	2.3 ± 0.1	2.3 ± 0.1	0.975
	Third trimester	11.8 [2.5]–2.5 ±	10.2 [1.1]–2.3 ±	10.6 [1.0]–2.3 ±	0.003
		0.2	0.1***	0.1**	<0.001
Platelet distribution width (fL), median [IQR]	First trimester	10.9 [2.4]–2.4 ±	9.5 [2.5]–2.3 ±	10.3 [2.1]–2.3 ±	0.312
		0.2	2.3 ± 0.2	2.3 ± 0.1	0.266
	Second trimester	11.0 [2.9]–2.4 ±	10.7 [3.3]–2.4 ±	11.0 [1.6]–2.4 ±	0.911
Log PDW, mean ± SD		0.2	2.4 ± 0.2	2.4 ± 0.1	0.983
	Third trimester	11.7 [1.5]–2.5 ±	10.4 [2.6]–2.4 ±	10.7 [3.7]–2.4 ±	0.425
		0.1	2.4 ± 0.2	2.4 ± 0.2	0.411

Key: HIV, human immunodeficiency virus; VL, viral load; PAI-1, plasminogen activator inhibitor-1; TAT, thrombin antithrombin; SD, standard deviation; IQR, interquartile range.

D-dimers were performed in 97 HIV negative and 79 HIV and VL >50 copies/mL.

TAT was performed in 101 HIV negative, 102 HIV and VL <50 copies/mL and 69 HIV and VL >50 copies/mL.

* p < 0.05 versus HIV negative;

** p < 0.01 versus HIV negative;

*** p < 0.001 versus HIV negative.

virological suppression when compared to the HIV infected group VL > 50 copies/mL. There was no significant association between TAT levels measured in each trimester and adverse pregnancy outcomes.

3.2.4. Platelet parameters

Log MPV, measured in each trimester, showed a significant increase with pregnancy progression in the HIV negative group (Table 1S). Between each study group, a significant difference in log MPV concentrations, in the third trimester, was observed (Table 3). Subgroup analysis with multiple pairwise comparisons showed no statistically significant differences in each trimester between the HIV infected group with virological suppression and the HIV infected group VL > 50 copies/mL. No significant differences in log PDW concentrations were observed. There was no significant association between MPV and PDW measured in each trimester and adverse pregnancy outcomes.

4. Discussion

Pregnancy is an acquired hypercoagulable state, characterized by changes in the coagulation and fibrinolytic systems [28,29]. We describe d-dimer and PAI-1 concentrations, which gradually increased from the second trimester and reached a maximum in the third trimester. Together with low levels of some anticoagulants and platelet activation, impaired fibrinolytic activity is responsible for the prothrombotic state of pregnancy [30]. Inflammation is a well-recognized cause of impaired fibrinolytic activity with advancing gestational age [31]. Because the resultant increase in fibrin deposition and subsequently reduced uteroplacental blood flow [32] have been associated with an increased risk of placental insufficiency [33] and thrombosis, [34,35] we investigated fibrinolytic activity in HIV infected pregnant women. In this study, HIV infected participants with virological suppression and VL > 50 copies/mL showed increasing d-dimer and PAI-1 levels, as compared to matched HIV negative study participants.

Impaired fibrinolysis plays a significant role in the pathogenesis of HIV infection in non-pregnant women. Earlier studies, in non-pregnant HIV-infected populations, compared the d-dimer to HIV-negative controls [15,18,36,37]. In the Rwanda Women's Inter-association Study and Assessment, log d-dimer levels were significantly increased at baseline in 421 HIV-infected women (0.47 ± 0.36) as compared to a HIV negative control group (n = 185, 0.36 ± 0.42) [18]. The women in this cohort were of African ethnicity with a mean age of 36 ± 7.2 years. Consistent with these earlier studies, we found impaired markers of fibrinolysis in HIV infected pregnant women. Increasing evidence suggests that activation of inflammation, coagulation and fibrinolysis do not consistently improve with long-term suppressive ART [6,7,38,39]. The present study is the first study to compare markers of fibrinolysis between African pregnant women, risk stratified according to HIV VL. In the HIV infected participants on long-term suppressive ART, d-dimer levels, in the third trimester, and PAI-1 levels, in each trimester, were significantly increased, as compared to the HIV negative participants. Moreover, we found no significant difference in PAI-1 (per trimester) and d-dimer levels (in the first and third trimesters) between HIV infected participants on long-term suppressive ART and HIV infected participants with VL > 50 copies/mL, in keeping with some other studies [21,22].

TAT, a well-established surrogate marker of thrombin generation, has not been well investigated in the literature previously. TAT complex levels were increased, in the first trimester, in the HIV infected virologically suppressed group as compared to the HIV negative group. We, however, did not find a significant relationship between TAT complex

levels, and HIV infection, beyond the first trimester. This may be attributed, in part, to the characteristics of our study population, who were at low risk specifically for VTE. There were also no reported cases of VTE in the present study. Moreover, the incidence of pregnancy related complications in particular, gestational hypertension and pre-eclampsia were acceptably low as compared to locally published incidences [40]. Only, older maternal age among the HIV infected virologically suppressed participants, was significant, consistent with earlier studies [15,41]. There is limited data with regards to the effect of maternal HIV infection and related classical venous risk factors, in the current era of ART, on thrombin generation and its relationship with thrombosis in pregnancy [23]. However, among HIV infected non-pregnant patients receiving ART, Jong et al. reported persistently increased plasma concentrations of prothrombin fragment 1 + 2. Increased thrombin generation may have important implications, particularly for those with associated antepartum and/or postpartum VTE risk factors. This highlights the need for longitudinal follow-up to determine the incidence of VTE in HIV infected pregnant women at intermediate to high risk of pregnancy related thrombosis.

Several mechanisms for ongoing impaired fibrinolysis and activation of coagulation on suppressive long term ART have been proposed [42,43]. Recent studies of pregnant women on ART have observed increased intestinal fatty-acid binding protein (I-FABP), a marker of impaired intestinal mucosal integrity [7]. This was accompanied by persistently raised surrogate markers of monocyte activation and microbial translocation, namely; soluble CD163 (sCD163), soluble CD14 (sCD14), and lipopolysaccharide binding protein (LBP) [6,7]. These data suggest that damage and decreased repair of the epithelial barrier; depletion of intestinal CD4+ T-cells in addition to dysfunction of IgA-secreting immune cells play an important role in the translocation of microbial products into the lamina propria of the gastro-intestinal tract and the systemic circulation. Thus, while ART may effectively suppress viral replication, homeostasis of the gastro-intestinal tract mucosa is not completely restored which contributes to ongoing inflammation, activation of coagulation and fibrinolysis and adverse perinatal outcomes [44,45]. In the present study, we observed a significantly higher rate of preterm delivery among HIV infected participants with virological suppression as compared to HIV negative participants, consistent with earlier studies of HIV infected women on ART [46,47]. Nonetheless, we did not find an association between markers of fibrinolysis and coagulation and adverse pregnancy outcomes such as preterm delivery and further statistically powered studies are required.

Our results must be interpreted in light of certain limitations. Firstly, the study was designed as a cross-sectional study. Blood-sampling was performed only at a single time point in each participant, as scheduled follow-up visits were disrupted by travel restrictions due to the COVID-19 pandemic. Furthermore, the study design did not include a non-pregnant HIV infected control group. Secondly, the study investigated markers of fibrinolysis and coagulation, however global assays of thrombin generation and fibrinolytic activity were not performed. Thirdly, platelet activation, which is another contributing factor to the hemostatic changes of pregnancy, was not investigated. Increasingly, placental vascular complications and [48,49] vascular complications associated with HIV infection [50–52] have been attributed to activated circulating platelets. A number of proteins released upon fusion of α -granules with the platelet membrane have been studied as markers of platelet activation. We, however, only studied surrogate markers of platelet activation. We observed a decline in the platelet count which was accompanied by a significant increase in MPV and PDW with pregnancy progression. This phenomenon is most likely due to maternal blood volume expansion. This was associated with a significantly decreased MPV in the third trimester in the HIV infected groups as compared to the HIV negative group. Nonetheless, studies investigating the contribution of ART to platelet activation in non-pregnant populations have revealed variable findings [53,54]. Maternal platelet activation associated with HIV infection and ART, however, has not

been studied extensively and merits further evaluation in the era of ART [55].

In summary, HIV infected pregnant women with VL > 50 copies/ml were significantly associated with markers of impaired fibrinolytic activity, as compared to matched HIV negative women. However, virological suppression was not associated with a significant reduction in d-dimer, PAI-1 and TAT complex levels, in each trimester, which suggests persistently impaired fibrinolytic and coagulation activity on long term ART. Future statistically powered studies should examine whether persistently impaired fibrinolytic and coagulation activity contributes to the excess risk of adverse pregnancy outcomes in HIV infected pregnant and postpartum women.

CRedit authorship contribution statement

Conceptualization: E Schapkaitz
Statistical analysis: E Schapkaitz and E Libhaber
Supervision: E Libhaber, BF Jacobson, HR Buller
Writing – original draft: E Schapkaitz
Review & editing: E Schapkaitz, E Libhaber, BF Jacobson, M Toman, A Gerber, HR Buller.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.thromres.2022.07.001>.

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Supplementary Tables

Table 6.1 Comparison of parameters of fibrinolysis and coagulation between the first, second and third trimesters of pregnancy

Parameter	Gestational age	First trimester	Second trimester	Third trimester	p value
D-dimer (mg/L), median [IQR] Log d-dimer, mean $\pm$ SD	HIV negative	0.2 [0.1]	0.3 [0.2]	0.4 [0.2]	<0.001
		-1.4 $\pm$ 0.2	-1.1 $\pm$ 0.3***	-0.8 $\pm$ 0.3***	<0.001
	HIV and VL <50 copies/mL	0.2 [0.1]	0.4 [0.2]	0.5 [0.3]	<0.001
		-1.2 $\pm$ 0.5	-0.9 $\pm$ 0.4*	-0.7 $\pm$ 0.4***	<0.001
	HIV and VL >50 copies/mL	0.3 [0.1]	0.5 [0.3]	0.6 [0.4]	0.019
		-1.1 $\pm$ 0.4	-0.7 $\pm$ 0.4**	-0.5 $\pm$ 0.5**	<0.001
PAI-1 (ng/mL), median [IQR] Log PAI-1, mean $\pm$ SD	HIV negative	1.5 [1.1]	2.0 [2.3]	3.7 [1.5]	<0.001
		0.4 $\pm$ 0.5	0.8 $\pm$ 0.3***	1.3 $\pm$ 0.3***	<0.001
	HIV and VL <50 copies/mL	2.4 [1.9]	3.5 [2.5]	4.1 [2.8]	0.010
		1.0 $\pm$ 0.4	1.3 $\pm$ 0.4**	1.5 $\pm$ 0.4***	<0.001
	HIV and VL >50 copies/mL	2.0 [1.7]	3.3 [0.9]	4.1 [2.4]	<0.001

		0.8 ± 0.5	1.2 ± 0.4***	1.5 ± 0.3***	<0.001
TAT complex (ng/mL), median [IQR]	HIV negative	4.7 [4.0]	5.1 [3.6]	5.8 [2.2]	0.043
Log TAT, mean ± SD		1.6 ± 0.4	1.7 ± 0.4	1.8 ± 0.4*	0.028
	HIV and VL <50 copies/mL	6.2 [5.4]	6.0 [5.0]	6.5 [2.6]	0.961
		2.0 ± 0.5	1.9 ± 0.5	1.9 ± 0.3	0.986
	HIV and VL >50 copies/mL	4.7 [4.9]	5.3 [4.3]	6.6 [2.8]	0.408
		1.6 ± 0.7	1.8 ± 0.5	1.8 ± 0.3	0.229
Platelet count (x 10 ⁹ /L), median [IQR] Log Platelet count, mean ± SD	HIV negative	256.5 [67.0]	235.0 [71.0]	212.0 [45.0]	0.007
		5.6 ± 0.3	5.5 ± 0.2	5.3 ± 0.2	0.002
	HIV and VL <50 copies/mL	272.0 [86.0]	244.0 [87.0]	238.0 [82.0]	0.455
		5.8 ± 0.4	5.5 ± 0.4	5.4 ± 0.3	0.048
	HIV and VL >50 copies/mL	315.0 [255.0]	259.0 [109.0]	245.0 [103.0]	0.033
		5.6 ± 0.2	5.5 ± 0.3	5.5 ± 0.3	0.460
Mean platelet volume (fL), median [IQR] Log MPV, mean ± SD	HIV negative	10.1 [1.5]	10.1 [1.2]	11.8 [2.5]	<0.001
		2.3 ± 0.1	2.5 ± 0.2	2.3 ± 0.1***	<0.001
	HIV and VL <50 copies/mL	9.3 [1.3]	10.1 [1.7]	10.2 [1.1]	0.084
		2.3 ± 0.1	2.3 ± 0.1	2.3 ± 0.1	0.196

	HIV and VL >50 copies/mL	9.3 [1.4] 2.3 ± 0.1	10.0 [1.4] 2.3 ± 0.1	10.6 [1.0] 2.3 ± 0.1	0.149 0.061
Platelet distribution width (fL), median [IQR] Log PDW, mean ± SD	HIV negative	10.9 [2.4] 2.4 ± 0.2	11.0 [2.9] 2.4 ± 0.2	11.7 [1.5] 2.5 ± 0.1	0.374 0.383
	HIV and VL <50 copies/mL	9.5 [2.5] 2.3 ± 0.1	10.7 [3.3] 2.4 ± 0.1	10.4 [2.6] 2.4 ± 0.2	0.188 0.589
	HIV and VL >50 copies/mL	10.3 [2.1] 2.3 ± 0.2	11.0 [1.6] 2.4 ± 0.2	10.7 [3.7] 2.4 ± 0.2	0.671 0.192
Key: HIV, human immunodeficiency virus; VL, viral load; PAI-1, plasminogen activator inhibitor-1; TAT, thrombin antithrombin; SD, standard deviation; IQR, interquartile range D-dimers were performed in 90 first trimester, 99 second trimester and 103 third trimester participants TAT was performed in 93 first trimester, 97 second trimester and 83 third trimester participants * p<0.05 versus first trimester; ** p <0.01 versus first trimester; *** p <0.001 versus first trimester					

Table 6.2 Analysis of parameters of fibrinolysis and coagulation according to adverse pregnancy complications in the study population

Parameter	Gestational age	Study participants with adverse pregnancy outcomes	Study participants without adverse pregnancy outcomes
D-dimer (mg/L), median [IQR] Log d-dimer, mean $\pm$ SD	First trimester	0.2 [0.1]	0.2 [0.1]
		-1.3 $\pm$ 0.4	-1.2 $\pm$ 0.4
	Second trimester	0.4 [0.2]	0.4 [0.2]
		-0.9 $\pm$ 0.4	-0.9 $\pm$ 0.4
	Third trimester	0.5 [0.4]	0.6 [0.3]
		-0.7 $\pm$ 0.4	-0.6 $\pm$ 0.4
PAI-1 (ng/mL), median [IQR] Log PAI-1, mean $\pm$ SD	First trimester	1.7 [0.8]	2.0 [1.5]
		0.5 $\pm$ 0,4	0.7 $\pm$ 0,5
	Second trimester	2.7 [2.7]	2.8 [1.7]
		1.1 $\pm$ 0.5	1.1 $\pm$ 0.5
	Third trimester	4.0 [1.5]	4.1 [2.2]
		1.3 $\pm$ 0.4	1.4 $\pm$ 0.3

TAT complex (ng/mL), median [IQR] Log TAT, mean $\pm$ SD	First trimester	3.9 [1.2]	5.6 [4.0]
		1.4 $\pm$ 0.3	1.7 $\pm$ 0.5
	Second trimester	5.3 [1.7]	5.3 [4.4]
		1.7 $\pm$ 0.3	1.8 $\pm$ 0.5
	Third trimester	6.3 [2.4]	6.3 [2.6]
		1.8 $\pm$ 0.2	1.9 $\pm$ 0.3
Platelet count ($\times 10^9/L$), median [IQR] Log Platelet count, mean $\pm$ SD	First trimester	265.0 [110.0]	253.0 [68.0]
		5.5 $\pm$ 0.3	5.6 $\pm$ 0.4
	Second trimester	219.0 [53.0]	225.0 [75.0]
		5.4 $\pm$ 0.2	5.4 $\pm$ 0.2
	Third trimester	173.0 [57.0]	209.0 [87.0]
		5.52 $\pm$ 0.2	5.4 $\pm$ 0.3
Mean platelet volume (fL), median [IQR] Log MPV, mean $\pm$ SD	First trimester	9.4 [1.4]	10.1 [1.6]
		2.3 $\pm$ 0.1	2.3 $\pm$ 0.1
	Second trimester	10.0 [1.2]	10.3 [1.3]
		2.3 $\pm$ 0.1	2.3 $\pm$ 0.1
	Third trimester	11.0 [1.9]	10.7 [1.6]

		2.4 ± 0.1	2.4 ± 0.2
Platelet distribution width (fL), median [IQR] Log PDW, mean ± SD	First trimester	9.3 [0.6]	11.1 [2.8]
		2.3 ± 0.2	2.4 ± 0.2
	Second trimester	10.5 [2.6]	11.3 [3.0]
		2.4 ± 0,1	2.4 ± 0,2
	Third trimester	10.5 [0.5]	11.5 [3.2]
		2.3 ± 0.1	2.4 ± 0.2
Key: PAI-1, plasminogen activator inhibitor-1; TAT, thrombin antithrombin; SD, standard deviation; IQR, interquartile range Adverse pregnancy outcomes included preterm delivery (n=41) and placental vascular complications namely; intra-uterine fetal death (n=11), fetal growth restriction (n=5) and pre-eclampsia (n=8) Participants with adverse pregnancy outcomes: first trimester, n=15, second trimester, n=21, third trimester, n=13 Comparisons between the two groups were not statistically significant.			

Abstract Title:

Evaluation of Platelet Function in Pregnant Women with Human Immunodeficiency Virus

Congress:

International Society of Laboratory Hematology Congress (ISLH) 8-10 September 2022,
Bologna, Italy

Presenter:

E. Schapkaitz

Background

Platelets are an important link between inflammation and thrombosis in HIV. Activated platelets mediate inflammation through the release of α -granules and neutrophil-platelet interactions. A number of proteins released upon fusion of the α -granules with the platelet membrane as well as platelet indices have been studied as markers of platelet activation in HIV. Maternal platelet activation, however, has not been studied extensively and merits further evaluation in the era of ART.

Methods

A cross-sectional analysis was performed between July 2019 and October 2021 at the Charlotte Maxeke Johannesburg Academic Hospital Antenatal Clinic, South Africa. The study included the following groups: 97 women living with HIV with virological suppression on ART for 4 [7] years, 54 women living with HIV with VL >50 copies/mL and 103 matched HIV negative women. Platelet factor 4 (Quantikine, R&D Systems, Inc., Minneapolis, USA) and platelet indices, as surrogate markers of platelet activation, were measured in the first (6-12 weeks), second (13-26 weeks) and third trimesters (27-39 weeks) and expressed as median [interquartile range]. Platelet indices were calculated using impedance technology on the Sysmex XN9000 (Sysmex Corporation, Kobe, Japan). These consisted of MPV, which is a measure of platelet size and subsequently the rate of platelet production and platelet distribution width (PDW), which reflects variability in platelet size. Clinical and laboratory data were analysed using Statistica 13.2 software (California, USA). The study was approved by the Ethics committee of the University of the Witwatersrand (M-181018).

Results

In this population, pregnancy outcomes were recorded for 241 (94.9%) participants with live births (Table 6.1). There were no VTE events. Baseline HIV characteristics differed significantly between the HIV groups with virological suppression and VL>50 copies/mL (Table 6.2). Platelets and PF4, measured in each trimester, showed a significant increase with pregnancy progression, while MPV decreased in the HIV negative group (Table 6.3). Between each study group, MPV was significantly decreased in the third trimester in the women living with HIV with virological suppression, as compared to the HIV negative women (Table 6.4).

Conclusion

The study findings suggest that MPV, measured in the third trimester, may be a valuable marker of platelet activation in pregnant women living with HIV with virological suppression. The proposed mechanism for the decrease in MPV levels is as a result of the migration and consumption of large platelets from the circulation to extracellular sites of inflammation. The prognostic value of MPV in the setting of HIV related cardiovascular events has not been established. The significance of this simple and cost-effective surrogate marker in the setting of HIV infected pregnancy is an important future objective.

Acknowledgements

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Enlargement of Poster Tables

Table 6.3 Demographics and clinical, laboratory and delivery characteristics of the three study groups

Characteristic	HIV negative (n=103)	HIV and VL <50 copies/mL (n=97)	HIV and VL >50 copies/mL (n=54)	p value
Demographics				
Age at study entry (years), mean $\pm$ SD	32.0 $\pm$ 6.0	35.0 $\pm$ 6.0 **	33.0 $\pm$ 6.0	0.007
Parity, median [IQR]	2.0 [1.0]	2.0 [2.0] **	2.0 [2.0]	0.029
BMI (kg/m ²), mean $\pm$ SD	28.5 $\pm$ 6.7	27.4 $\pm$ 6.7	27.7 $\pm$ 6.8	0.410
Smoking history >10/day (n, %)	0 (0)	0 (0)	0 (0)	-
Laboratory characteristics				
White cell count ($\times 10^9$ /L), mean $\pm$ SD (ref: 3.9-12.6)	8.0 $\pm$ 2.2	7.6 $\pm$ 2.4	7.0 $\pm$ 2.0**	0.015
Hemoglobin (g/L), mean $\pm$ SD (ref: 116-164)	125.6 $\pm$ 13.4	116.9 $\pm$ 14.5***	114.2 $\pm$ 16.9 ***	<0.001
Platelet count ($\times 10^9$ /L), median [IQR] (ref: 186-454)	238 [64]	248 [86]	259 [110]	0.440
Mean platelet volume (fL), median [IQR] (ref: 7.3-11.3)	10.1 [1.5]	10.1 [1.5]	10.1 [1.4]	0.408
Platelet distribution width (fL), median [IQR]	11.0 [1.9]	10.3 [3.0]	11 [2.4]	0.415
Obstetric characteristics				
Gestational hypertension (n, %)	2 (1.9)	2 (2.1)	1 (1.9)	0.996
Pre-eclampsia and FGR (n, %)	4 (3.9)	3 (3.1)	1 (1.9)	0.786
Delivery characteristics of live births				
Live births (n, %)	99 (96.1)	91 (93.8)	51 (94.4)	0.751
Gestational age delivery (weeks), mean $\pm$ SD	39.0 $\pm$ 2.0	38 $\pm$ 3.0	38.0 $\pm$ 2.0*	0.025
Birthweight (g), mean $\pm$ SD	3255.5 $\pm$ 709.3	2894.7 $\pm$ 668.8 **	2929.0 $\pm$ 529.1*	0.005
Outcome				
VTE (n, %)	0 (0)	0 (0)	0 (0)	-
Key: BMI, body mass index; HIV, human immunodeficiency virus; VL, viral load; VTE, venous thromboembolism; SD, standard deviation, IQR, interquartile range BMI was calculated from first antenatal booking appointment.				

Hypertension was defined as systolic blood pressure > 140 mm Hg and/or diastolic blood pressure > 90 mm Hg. Pre-eclampsia was defined as hypertension > 20 weeks' gestation and new onset proteinuria (+1 on dipstick) or multi-systemic signs (platelet count < $100 \times 10^9/L$, elevated liver transaminases x 2 baseline, serum creatinine >1.1 mg/dL doubling of the serum creatinine concentration, pulmonary oedema, cerebral or visual disturbances).

* p <0.05 versus HIV negative; ** p <0.01 versus HIV negative; *** p <0.001 versus HIV negative

Table 6.4 HIV characteristics

Characteristic	HIV and VL <50 copies/mL	HIV and VL >50 copies/mL	p value
HIV characteristics			
Years from Diagnosis, median [IQR]	4.0 [7.0]	0.2 [3.0]	<0.001
CD4 cell count (x 10 ⁶ /L), median [IQR]	533 [275]	365 [259]	<0.001
HIV viral load (copies/mL), median [IQR]	<50	254 [13608]	<0.001
ART, (n, %)	97(100)	34 (63.0)	<0.001
No therapy	0 (0)	20 (37.0)	
Key: VL, viral load; ART, antiretroviral therapy; HIV, human immunodeficiency syndrome; IQR, interquartile range			

Table 6.5 Comparison of parameters of platelet activation between the first, second and third trimesters of pregnancy

Parameter	Gestational age	First trimester	Second trimester	Third trimester	p value
PF4 (ng/mL), median [IQR] (ref: 3166-13.275)	HIV negative	6920.0 [1720.0]	6320.0 [2340]	5480.0 [1600.0]***	<0.001
	HIV and VL <50 copies/mL	6590.0 [2650.0]	6040.0 [1680.0]	6010.0 [1800.0]	0.559
	HIV and VL >50 copies/mL	4850.0 [2300.0]	5720.0 [2192.0]	5980.0 [1420.0]	0.785
Platelet count (x 10 ⁹ /L), median [IQR] (ref: 186-454)	HIV negative	256.5 [67.0]	235.0 [71.0]	212.0 [45.0]	0.007
	HIV and VL <50 copies/mL	272.0 [86.0]	244.0 [87.0]	238.0 [82.0]	0.455
	HIV and VL >50 copies/mL	315.0 [255.0]	259.0 [109.0]	245.0 [103.0]	0.033
Mean platelet volume (fL), median [IQR] (ref: 7.3-11.3)	HIV negative	10.1 [1.5]	10.1 [1.2]	11.8 [2.5]***	<0.001
	HIV and VL <50 copies/mL	9.3 [1.3]	10.1 [1.7]	10.2 [1.1]	0.084
	HIV and VL >50 copies/mL	9.3 [1.4]	10.0 [1.4]	10.6 [1.0]	0.149
Platelet distribution width (fL), median [IQR]	HIV negative	10.9 [2.4]	11.0 [2.9]	11.7 [1.5]	0.294
	HIV and VL <50 copies/mL	9.5 [2.5]	10.7 [3.3]	10.4 [2.6]	0.188
	HIV and VL >50 copies/mL	10.3 [2.1]	11.0 [1.6]	10.7 [3.7]	0.671
Key: HIV, human immunodeficiency virus; VL, viral load; IQR, interquartile range *** p <0.001 versus first trimester					

Table 6.6 Comparison of parameters of platelet activation between HIV negative, HIV infected with virological suppression and HIV infected with VL >50 copies/mL study groups

Parameter	Gestational age	HIV negative	HIV and VL <50 copies/mL	HIV and VL >50 copies/mL	p value
PF4 (ng/mL), median [IQR] (ref: 3166-13.275)	First trimester	6920.0 [1720.0]	6590.0 [2650.0]	4850.00 [2300.0]	0.075
	Second trimester	6320.0 [2340.0]	6040.0 [1680.0]	5720.0 [2192.0]	0.074
	Third trimester	5480.0 [1600.0]	6010.0 [1800.0]	5980.0 [1420.0]	0.090
Platelet count (x 10 ⁹ /L), median [IQR] (ref: 186-454)	First trimester	256.5 [67.0]	272.0 [86.0]	315.0 [255.0]	0.150
	Second trimester	235.0 [71.0]	244.0 [87.0]	259.0 [109.0]	0.300
	Third trimester	212.0 [45.0]	238.0 [82.0]	245.0 [103.0]	0.172
Mean platelet volume (fL), median [IQR] (ref: 7.3-11.3)	First trimester	10.1 [1.5]	9.3 [1.3]	9.3 [1.4]	0.189
	Second trimester	10.1 [1.2]	10.1 [1.7]	10.0 [1.4]	0.979
	Third trimester	11.8 [2.5]	10.2 [1.1]**	10.6 [1.0]	0.006
Platelet distribution width (%), median [IQR]	First trimester	10.9 [2.4]	9.5 [2.5]	10.3 [2.1]	0.403
	Second trimester	11.0 [2.9]	10.7 [3.3]	11.0 [1.6]	0.911
	Third trimester	11.7 [1.5]	10.4 [2.6]	10.7 [3.7]	0.425
Key: HIV, human immunodeficiency virus; VL, viral load; SD, standard deviation; IQR, interquartile range ** p <0.01 versus HIV negative					

CHAPTER 7: A Longitudinal Study of Thrombosis and Bleeding Outcomes with Thromboprophylaxis in Pregnant Women at Intermediate and High risk of VTE

Summary: This longitudinal study in pregnant women at intermediate to high risk of VTE was designed to assess the risks of thrombosis and bleeding with thromboprophylaxis and its association with HIV. In this population, HIV was not an independent predictor of bleeding. Human immunodeficiency virus, however, could not be identified as a predictor of VTE as a result of the small number of recurrent events during the study period. The rates of thrombosis and bleeding in pregnancy and the postpartum period provided by this study can be used to inform women living with HIV of the associated risks.

Statement of originality document: Please see Appendix 8.2

A Longitudinal Study of Thrombosis and Bleeding Outcomes With Thromboprophylaxis in Pregnant Women at Intermediate and High Risk of VTE

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E. Schapkaitz, FCPATH¹ , E. Libhaber, PhD², A. Gerber, FCOG³, H. Rhemtula, FCOG³, J. Zamparini, FCP⁴, B.F. Jacobson, PhD¹, and H.R. Büller, PhD⁵

Abstract

Background: The efficacy and safety of thromboprophylaxis in pregnancy at intermediate to high risk of venous thromboembolism (VTE) is an area of ongoing research.

Aim: This study aimed to assess thrombosis and bleeding outcomes associated with thromboprophylaxis in women at risk of VTE.

Methods: A cohort of 129 pregnancies, who received thromboprophylaxis for the prevention of VTE, were identified from a specialist obstetric clinic in Johannesburg, South Africa. Intermediate-risk pregnancies, with medical comorbidities or multiple low risks, were managed with fixed low-dose enoxaparin antepartum and for a median (interquartile range) of 4 (4) weeks postpartum. High-risk pregnancies, with a history of previous VTE, were managed with anti-Xa adjusted enoxaparin antepartum and for a median of 6 (0) weeks postpartum. Pregnancy-related VTE was objectively confirmed. Major bleeding, clinically relevant nonmajor bleeding (CRNMB) and minor bleeding were defined according to the International Society on Thrombosis and Hemostasis Scientific Subcommittee.

Results: Venous thromboembolism occurred antepartum in 1.4% (95% CI: 0.04-7.7) of intermediate and 3.4% (95% CI: 0.4-11.7) of high-risk pregnancies. Bleeding events occurred in 7.1% (95% CI: 2.4-15.9) of intermediate and 8.5% (95% CI: 2.8-18.7) of high-risk pregnancies. Of these bleeding events, 3.1% (95% CI: 1.0-8.0) were classified as major bleeding. On univariate analysis, no independent predictors of bleeding were identified.

Conclusion: The rates of thrombosis and bleeding in this predominantly African population were consistent with similar studies and can be used to inform pregnant women of the benefits of anticoagulation and the risks of potential bleeding.

Keywords

pregnancy, low molecular weight heparin, venous thromboembolism, thromboprophylaxis, bleeding

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Introduction

Venous thromboembolism (VTE), which comprises deep vein thrombosis (DVT) and its complication (PE), is an important cause of pregnancy-related morbidity and mortality.¹⁻⁴ Worldwide, the prevalence differs considerably, with a higher reported frequency in populations of African ethnicity.^{2,5-8} The physiological changes of pregnancy are associated with a background risk for VTE. In addition, multiple risk factors which further increase the risk of VTE have been identified by a number of high-quality cohort and case-control studies.^{6,9-13} In particular, a personal history of thrombosis and inherited thrombophilia have both been

¹ Department of Molecular Medicine and Hematology, University of Witwatersrand Medical School, Johannesburg, South Africa

² Department of Research Methodology and Statistics, University of Witwatersrand Medical School, Johannesburg, South Africa

³ Department of Obstetrics, University of Witwatersrand Medical School, Johannesburg, South Africa

⁴ Department of Internal Medicine, University of Witwatersrand Medical School, Johannesburg, South Africa

⁵ Department of Vascular Medicine, University of Amsterdam, Amsterdam, the Netherlands

Corresponding Author:

Elise Schapkaitz, Department of Molecular Medicine and Hematology, Faculty of Health Sciences, University of Witwatersrand Medical School, PO Box 28985 Sandringham 2131, Johannesburg, South Africa.
Email: elise.schapkaitz@nhls.ac.za



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shown to be important risk factors for pregnancy-related VTE.^{6,14–16} The further increased risk among pregnant women of African ethnicity has been attributed to medical comorbidities including: peripartum cardiomyopathy, sickle cell disease, and human immunodeficiency virus (HIV) infection.^{4,17,18} HIV is of particular concern considering the increased prevalence among women in the reproductive age group as it confers at least a 2-fold increased risk of VTE.¹⁹ These and other clinical risk factors, such as maternal obesity and multiparity, have been associated with a low to intermediate risk of VTE.^{6,11,13}

Antepartum and postpartum thromboprophylaxis with low molecular weight heparin (LMWH) is recommended for the prevention of pregnancy-related VTE in women at a sufficiently high risk of thrombosis.^{20,21} Nonetheless, the efficacy and safety of thromboprophylaxis are limited by a paucity of randomized controlled studies in women at an increased risk of VTE.^{22–24} A recent systematic Cochrane review found insufficient evidence to make recommendations for thromboprophylaxis.²⁵ In this meta-analysis of 4 randomized controlled studies in 476 women, the use of antepartum heparin versus no treatment or placebo was not associated with a decreased VTE risk. The rates of bleeding in these studies were variably reported and thereby limited precise safety estimates of thromboprophylaxis.

Several research groups have carried out observational studies of thrombosis and bleeding outcomes associated with thromboprophylaxis in pregnant women. An earlier cohort study from the Netherlands evaluated 91 women at high and intermediate risk of VTE who received low-dose nadroparin.²⁶ Low-dose thromboprophylaxis was associated with recurrent VTE in women at high risk with a postpartum incidence of 7.0% (95% CI: 2.9–16.7) and an antepartum incidence of 1.8% (95% CI: 0.4–9.2). The rate of severe postpartum hemorrhage (PPH) (defined as blood loss of >1 000 mL) was 9.1% (95% CI: 4.7–16.9) and 6 women received transfusions. Recently, Cox et al published a 15-year cohort study of 123 women at intermediate to high risk for VTE who received low-dose enoxaparin thromboprophylaxis.²⁷ The rate of VTE was 1.2% (95% CI: 0.32–4.14) and these events occurred in the antepartum period. The rate of severe PPH was 9.3%, with these events occurring following cesarean delivery. Most recently, Rajaratnam et al have reported on their 10-year experience in 409 women at King's College Hospital.²⁸ In these women who received weight-adjusted enoxaparin thromboprophylaxis, the rate of VTE was 1.4% and the rate of severe PPH was 14.1%. These studies, however, applied inconsistent definitions of bleeding in pregnancy and the postpartum period. Furthermore, there is limited evidence for thromboprophylaxis among African women, in particular those with intermediate and multiple low-risk factors.^{29,30} Further research is required to determine the balance of pregnancy-related thrombosis and major bleeding risks in this pregnant population.

In view of these considerations, we conducted a descriptive longitudinal study at a single academic center in a cohort of pregnant women at intermediate and high risk of VTE to

assess the thrombosis and bleeding outcomes associated with antepartum and postpartum thromboprophylaxis.

Methods

Study Design and Population

A retrospective cohort study was conducted at the combined cardiac obstetric clinic at Charlotte Maxeke Johannesburg Academic Hospital, a quaternary-level maternity service in South Africa. This is a specialist referral clinic for women with cardiovascular disease including: congenital heart disease, pulmonary hypertension, coronary artery disease, cardiomyopathy, heart failure, arrhythmias, and VTE. Approximately 90 to 100 women attend this clinic per year. All women requiring antepartum thromboprophylaxis are managed by a multi-disciplinary team at the combined cardiac obstetric clinic. The clinical records of consecutive pregnant women, who received enoxaparin thromboprophylaxis for the prevention of VTE between March 1, 2017 and September 1, 2022, were reviewed. During the study period, 226 clinical records were identified. The study included 129 pregnancies which were classified as a high or intermediate risk for VTE according to local recommendations (Table 1). The intermediate-risk group included pregnancies with a high-risk inherited thrombophilia, antiphospholipid syndrome, medical comorbidities, or multiple low-risk factors (Table 2). Medical comorbidities included cardiomyopathy, heart failure, active systemic lupus erythematosus (SLE), pulmonary arterial hypertension, sickle cell disease, inflammatory polyarthropathy, and nephrotic syndrome. Peripartum and dilated cardiomyopathy with left ventricular (LV) ejection fraction <40%, were considered for thromboprophylaxis in the presence of one or more of the following risk factors: atrial fibrillation, LV thrombus, and/or systemic embolism.³¹ Multiple low-risk factors included >4 antepartum or ≥2 postpartum risk factors, namely age >35 years, body mass index ≥ 30 kg/m², parity ≥ 3, gross varicose veins, current systemic infection, paraplegia, pre-eclampsia with intra-uterine growth restriction, multiple pregnancies and human immunodeficiency virus-associated stroke.³² The high-risk group consisted of pregnancies with a history of clinical and radiological evidence for previous VTE. VTE was classified as unprovoked (in the absence of risk factors), provoked (within 3 months of a risk factor) or pregnancy or estrogen-related. Women who received thromboprophylaxis for the prevention of thrombo-embolism for valvular heart disease, congenital heart disease, arrhythmia, recurrent pregnancy loss, previous superficial venous thrombosis, and transient risk factors were excluded (n=97). The study was approved by the Wits Human Research Ethics Committee, Medical (M-220207).

Study Protocol

Data collection. Maternal demographics, obstetric and delivery characteristics were recorded. In the women living with HIV, data on HIV diagnosis, antiretroviral therapy (ART) and adherence were also recorded.

Table 1. Recommended Risk Stratification for Venous Thrombo-Embolism at Charlotte Maxeke Johannesburg Academic Hospital.³²

Antepartum Risk Factors	Postpartum Risk Factors	Recommendation
High risk Previous unprovoked or pregnancy or estrogen-related VTE	Anyone requiring antepartum prophylaxis Previous VTE High-risk inherited thrombophilia* Low-risk inherited thrombophilia and family history** Anti-phospholipid syndrome	Antepartum thromboprophylaxis Postpartum thromboprophylaxis for at least 6 weeks
Intermediate risk Single VTE related to a transient risk factor (not related to pregnancy or estrogen use). High-risk inherited thrombophilia* Nonobstetric surgery during pregnancy Medical comorbidities*** Antiphospholipid syndrome	Cesarean delivery in labor Readmission or prolonged admission (≥ 3 days) postpartum Surgery in the puerperium Medical comorbidities*** BMI ≥ 40 kg/m ²	Consider antepartum thromboprophylaxis Postpartum thromboprophylaxis for at least 2 weeks. If risk factors persist, consider extending thromboprophylaxis
Low Risk Low-risk inherited thrombophilia** Age > 35 years BMI ≥ 30 kg/m ² Parity ≥ 3 Smoker Family history of unprovoked or estrogen-related VTE in first-degree relative Gross varicose veins Current systemic infection Immobility Current pre-eclampsia with intra-uterine growth restriction Multiple pregnancies In vitro fertilization Dehydration/hyperemesis	Age > 35 years BMI ≥ 30 kg/m ² Parity ≥ 3 Smoker Elective cesarean delivery Family history of VTE Low-risk inherited thrombophilia Gross varicose veins Current systemic infection or peri-operative infection Immobility Multiple pregnancies Preterm delivery (< 37 weeks) Stillbirth Mid-cavity or rotational operative delivery Prolonged labor (> 24 h) Postpartum hemorrhage ($> 1\,000$ ml and surgery or blood transfusion)	Consider antepartum thromboprophylaxis if multiple (>4) risk factors present Postpartum thromboprophylaxis at least until discharge from hospital if multiple (≥ 2) risk factors present

Abbreviations: VTE, venous thrombo-embolism; BMI, body mass index.

*High-risk inherited thrombophilia: antithrombin deficiency, compound heterozygous or homozygous for low-risk thrombophilia.

**Low-risk inherited thrombophilia: protein S deficiency, protein C deficiency, heterozygous for Factor V Leiden or prothrombin gene G20210A mutations.

***Medical comorbidities: cancer, heart failure, active systemic lupus erythematosus, inflammatory polyarthropathy or inflammatory bowel disease; nephrotic syndrome; type I diabetes mellitus with nephropathy; sickle cell disease; current intravenous drug user.

Management. VTE risk assessment was performed at the first antepartum visit and repeated postpartum, following delivery and prior to discharge. Seventy pregnancies were classified as intermediate risk and 59 pregnancies were classified as high risk, antepartum as well as postpartum. Women at intermediate risk for VTE, were managed with fixed low dose daily subcutaneous enoxaparin (according to weight namely, 20 mg for <50 kg; 40 mg for 50-90 kg; 60 mg for 91-130 kg; and 80 mg for 131-170 kg) antepartum and continued for at least 2 weeks postpartum (Table 3).³² Thromboprophylaxis was extended in the presence of persistent risk factors. In those considered to be at high risk for VTE, enoxaparin was prescribed antepartum and continued until 6 weeks postpartum. Doses were adjusted with regular anti-Xa monitoring to achieve a prophylactic anti-Xa level of 0.2-0.6 units/mL. There were 9 (7.0%) pregnancies which were switched from extended duration anticoagulation at pregnancy diagnosis to enoxaparin

12-hly, adjusted to achieve a therapeutic anti-Xa level of 0.6-1.0 units/mL.

Outcomes. Pregnancy-related VTE was defined as VTE during pregnancy or 3 months postpartum. Symptomatic VTE was confirmed by compression ultrasound or computed tomography pulmonary angiogram. Postpartum hemorrhage referred to bleeding by visual estimation during the first 24 h (primary) or from 24 h to 6 weeks after delivery (secondary).³³ Major bleeding, clinically relevant nonmajor bleeding (CRNMB), and minor bleeding events were confirmed by an independent adjudication committee (ES, HR, and JZ).^{34,35}

Laboratory methods. Venous blood for anti-Xa measurement was collected in 3.2% sodium citrate (Becton-Dickinson, Oxford, the United Kingdom) at a mean $\pm$ standard deviation (SD) of 3.3 ± 0.4 h after the last enoxaparin injection.

Table 2. Indications for Thromboprophylaxis in Intermediate- and High-Risk Pregnancies (n = 129).

Indications for Thromboprophylaxis	n (%)
Intermediate-risk pregnancies for VTE	70 (54.2)
Medical comorbidities	50 (71.4)
Cardiomyopathies and heart failure*	24 (48.0)
Active systemic lupus erythematosus	14 (28.0)
Pulmonary arterial hypertension**	7 (14.0)
Sickle cell disease	3 (6.0)
Inflammatory polyarthropathy	2 (4.0)
Nephrotic syndrome	1 (2.0)
Multiple low-risk factors***	20 (28.6)
High-risk pregnancies for VTE	(59, 45.7)
Personal history of VTE	59 (45.7)
Unprovoked VTE	33 (55.9)
Pregnancy or estrogen-related VTE	23 (39.0)
Provoked VTE****	3 (5.1)
Recurrent VTE	10 (16.9)
Antiphospholipid syndrome with thrombosis	2 (3.4)
Protein S deficiency with thrombosis*****	9 (17.3)
Protein C deficiency with thrombosis*****	2 (3.8)

Abbreviations: VTE, venous thrombo-embolism.

*Peripartum and dilated cardiomyopathy and left ventricular (LV) ejection fraction <40%, or atrial fibrillation, or LV thrombus, or systemic embolism.

**World Health Organization (WHO) Group I = 3, WHO Group II = 1, WHO Group III = 2, WHO Group IV = 1.

***Including: Age >35 years, body mass index ≥ 30 kg/m², parity ≥ 3, gross varicose veins, current systemic infection, paraplegia, pre-eclampsia with intra-uterine growth restriction, multiple pregnancies, human immunodeficiency virus-associated stroke.

****Provoked and recurrent (n = 1), provoked and multiple low risk factors (n = 2).

*****Testing for proteins S and C in 52 high-risk pregnancies.

Anti-Xa analysis was performed on the STA-R Max[®] automated coagulation analyzer (Diagnostica Stago, Asnières sur Seine, France). Serial peak anti-Xa levels were available in 52 (88.1%) high-risk pregnancies. With pregnancy progression, 10 (19.2%) required a dose adjustment in the second trimester and 13 (25.0%) in the third trimester to achieve the target range.

Statistical Methods

A sample of 126 pregnancies was estimated, assuming a 2.0% incidence of bleeding or VTE during pregnancy and the postpartum period and considering a maximum incidence of 15%, at a confidence interval (CI) of 95% (Epi Info v7.2.0.1, Atlanta, Georgia, USA).³⁶ The statistical unit of analysis was pregnancy. Clinical and laboratory data was analyzed using Statistica 13.2 software (Palo Alto, California, the United States). Normally distributed continuous data are presented as mean ± SD and variables with non-Gaussian distribution as median (interquartile range [IQR]). Categorical data are presented as frequencies and percentages. Comparisons for continuous measurements were performed using a one-way ANOVA test with Sheffé's post hoc test or a Kruskal-Wallis test. Categorical variables were compared using chi-square test or Fisher's exact test. The median time to VTE, major bleeding, and CRNMB were estimated using the Kaplan-Meier method. Univariate Cox Proportional

Table 3. Antepartum, Peripartum, and Postpartum Management of Intermediate- and High-Risk Pregnancies.

	Intermediate Risk for VTE	High Risk for VTE
Number of pregnancies	70	59
Antepartum		
Gestational age enoxaparin commenced (weeks), median (IQR)*	24 (13)	12 (14)
Prophylactic daily enoxaparin dose, median (IQR)**	40 (20)	80 (40)
Therapeutic twice daily enoxaparin dose, median (IQR)**	-	80 (10)
Concomitant aspirin, 75-150 mg (n, %)	22 (31.4)	13 (22.0)
Peripartum****		
Last enoxaparin injection to delivery interval (hours), median (IQR)	28 (22)	24 (24)
First postpartum enoxaparin injection post-delivery interval (hours), median (IQR)	15 (12)	13 (11)
Postpartum*****		
Duration of postpartum enoxaparin (weeks), median (IQR)	4 (4)	6 (0)

Abbreviation: IQR, inter-quartile range; VTE, venous thrombo-embolism.

*In women receiving extended-duration anticoagulation; a switch was made to enoxaparin at a median of 8 (6) weeks' gestation.

**prophylactic enoxaparin n = 120; therapeutic enoxaparin n = 9.

In the women receiving therapeutic enoxaparin, enoxaparin was discontinued at a median of 18 (16) prior to delivery and reintroduced at 13 (28) h after delivery. In the women receiving prophylactic enoxaparin, enoxaparin was discontinued at a median of 24 (18) h prior to delivery and reintroduced at 16 (12) h after delivery.

***Prior to 16 weeks gestation until delivery.

****Of live births.

Hazard Regression analysis was used to identify predictors of bleeding events by calculating hazard ratios (HR with 95% CI). Statistical significance was set at a P value of <.05.

Results

Patient Characteristics

A total of 129 pregnancies in 127 women were eligible for inclusion. The characteristics of the 70 (54.3%) intermediate-risk and 59 (45.7%) high-risk pregnancies for VTE are described in Table 4. The majority were of African ethnicity (n = 123, 95.3%) in keeping with the demographics of the population served by our hospital. Thirty-five (27.1%) were living with HIV with a CD4 count of 501 (375) × 10⁶/L. The majority (n = 26, 74.3%) were virologically suppressed on first-line ART for a median duration of 5 (6) years. Adverse obstetric characteristics: chronic hypertension, pre-eclampsia with fetal growth restriction (FGR) and prolonged hospital admission were significantly increased in the intermediate-risk group whereas miscarriage was significantly increased in the high-risk group.

Table 4. Characteristics According to Risk Group.

Characteristics	Intermediate Risk for VTE	High Risk for VTE	P Value
Number of pregnancies	70	59	
Demographics			
Age at study entry (years), mean $\pm$ SD	32.8 $\pm$ 5.7	32.6 $\pm$ 5.4	.800
BMI (kg/m ²), mean $\pm$ SD	29.6 $\pm$ 8.3	31.1 $\pm$ 6.1	.242
Parity, median (IQR)	2.0 (2)	2.0 (2)	.969
HIV infected (n, %)	18 (25.7)	17 (28.8)	.693
Baseline laboratory characteristics			
White cell count ($\times 10^9$ /L), mean $\pm$ SD (ref: 3.9-12.6)	8.1 $\pm$ 3.1	7.2 $\pm$ 1.9	.046
Hemoglobin (g/L), mean $\pm$ SD (ref: 116-164)	119.1 $\pm$ 16.2	121.8 $\pm$ 13.0	.312
Platelet count ($\times 10^9$ /L), median (IQR) (ref: 186-454)	228.0 (112.0)	242.0 (87.0)	.618
eGFR (mL/min/1.73 m ²), median (IQR) (ref: > 60)	60 (0)	60 (0)	1.000
Obstetric characteristics			
Chronic hypertension (n, %)	24 (34.3)	7 (11.9)	.003
Pre-eclampsia and FGR (n, %)*	8 (11.8)	1 (2.0)	.048
Systemic infection (n, %)	6 (8.6)	3 (5.1)	.439
Prolonged hospital admission (n, %)	17 (24.3)	6 (10.2)	.037
Multiple pregnancy (n, %)	2 (2.9)	2 (3.4)	.862
Delivery characteristics			
Live births (n, %)	66 (94.3)	50 (86.2)	.138
Intra-uterine fetal death (n, %)	4 (5.7)	0 (0.0)	.126
Miscarriage (n, %)	0 (0.0)	5 (8.6)	.017
Termination of pregnancy (n, %)	0 (0.0)	3 (5.2)	.090
Gestational age delivery (weeks), mean $\pm$ SD**	36.5 $\pm$ 2.7	37.7 $\pm$ 1.7	.009
Birthweight (g), mean $\pm$ SD**	2633.7 $\pm$ 768.0	2938.7 $\pm$ 549.3	.023
Scheduled delivery (n, %)	50 (75.8)	39 (78.0)	.515
Mode of delivery**			
Normal vaginal delivery (n, %)	20 (30.3)	21 (42.00)	.192
Elective cesarean delivery (n, %)	28 (42.4)	18 (36.0)	.484
Emergency cesarean delivery (n, %)	18 (27.3)	11 (22.0)	.516
Neuraxial anesthesia (%)***	30 (65.2)	27 (93.1)	.006
Estimated blood loss at delivery (mL), median (IQR)			
Normal vaginal delivery	200 (120)	250 (250)	.160
Cesarean delivery	500 (200)	500 (250)	.972

Abbreviations: SD, standard deviation, IQR, inter-quartile range; BMI, body mass index; HIV, human immunodeficiency virus; FGR, fetal growth restriction; eGFR, estimated glomerular filtration rate; VTE, venous thrombo-embolism.

*In 118 pregnancies

**Of live births

***Of cesarean deliveries

Outcomes

The flow diagram in Figure 1 illustrates the thrombotic and major bleeding events in pregnancies at intermediate and high risk of VTE.

The incidence of antepartum VTE was 2.3% (95% CI: 0.5-6.9). In intermediate- and high-risk pregnancies, the

incidence of VTE was 1.4% (95% CI: 0.04-7.7) and 3.4% (95% CI: 0.4-11.7), respectively. A fatal PE, occurred in a 31-year-old, HIV-negative woman, at 19 weeks' gestation, with a personal history of postpartum PE and active SLE. She was receiving an inadequate dose of enoxaparin (anti-Xa = 0.1 IU/mL), the dose of which was increased 2 weeks prior to her event. Obstetric history was noteworthy for an intra-uterine fetal death at 30 weeks' gestation associated with anticardiolipin and anti-beta2-glycoprotein IgG positivity on a single occasion. A left-leg DVT occurred in a 36-year-old woman living with HIV, at 23 weeks gestation, with a personal history of an unprovoked DVT. The patient was adherent to ART (Zidovudine, Lamivudine, and Aluvia) for 4 years with a CD4 count of 230×10^6 /L and HIV viral load of 622 copies/mL. She was receiving low-dose enoxaparin from 8 weeks gestation. Compression ultrasound showed an acute proximal DVT of the femoral-popliteal system. The patient was switched to anti-Xa adjusted therapeutic enoxaparin for the remainder of the pregnancy. In the postpartum period, the patient was transitioned to long-term warfarin. Lastly, a PE, occurred in a 26-year-old, HIV-negative woman, at 32 weeks gestation, with a personal history of active SLE and prolonged hospital admission for disease exacerbation and systemic infection. She was receiving fixed low-dose enoxaparin from 28 weeks gestation.

The incidence of bleeding was 7.1% (95% CI: 2.4-15.9) and 8.5% (95% CI: 2.8-18.7) in intermediate- and high-risk pregnancies, respectively. There were 4 antepartum and 6 postpartum bleeding events of which 4 were major bleeding events (3.1%; 95% CI: 1.0-8.0), 3 were CRNMB events (2.3%, 95% CI: 0.5-6.9) and 3 were minor bleeding events (2.3%, 95% CI: 0.5-6.9; Table 5). On univariate logistic regression analysis, no independent predictors of bleeding were found (Table 6). Pre-eclampsia with FGR could not be calculated as the assumptions of proportionality were not met.

Peripartum, spinal, or epidural anesthesia were administered in 30 (62.5%) intermediate-risk and 27 (87.1%) high-risk cesarean deliveries, respectively, with no bleeding or neurological complications. There was a single (0.8%) wound hematoma post-delivery. There were 7 (5.4%) cases of excessive bruising and no hypersensitivity skin reactions at the injection sites. No women switched to another type of LMWH.

Figures 2 and 3 illustrate the timing of thrombotic and bleeding events, respectively, in intermediate- and high-risk pregnancies.

Discussion

Low rates of VTE have been reported previously with the use of thromboprophylaxis for intermediate risk factors.^{26-28,37-39} In this cohort, more than half of the pregnancies were classified as intermediate risk, which were managed with fixed low-dose enoxaparin. The incidence of VTE was 1.4%. In contrast to previously published studies which included thrombophilia and single VTE related to a temporary risk factor, the indications for thromboprophylaxis in this cohort, of

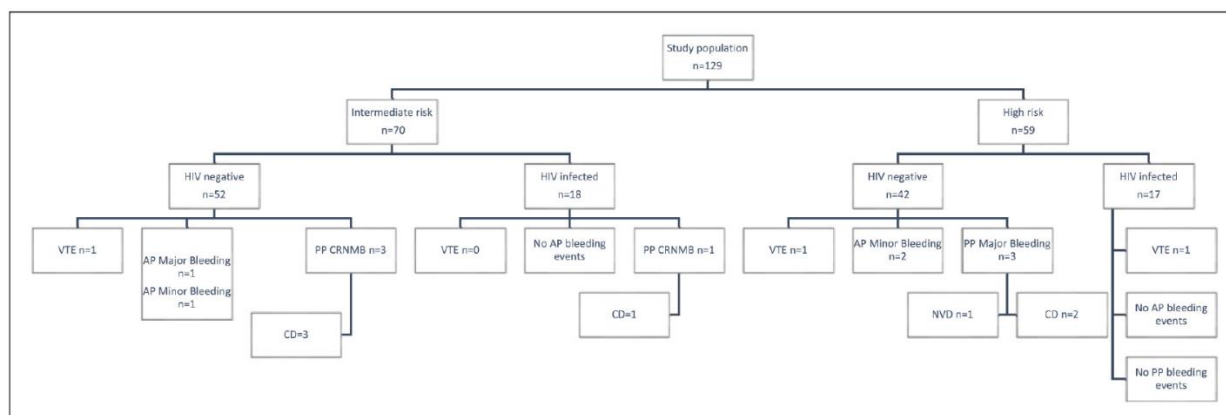


Figure 1. Schematic diagram of study outcomes.

Table 5. Description of Bleeding Events in Intermediate- and High-Risk Pregnancies.

Description	
Intermediate risk	
Major bleeding	Antepartum placenta praevia at 34 weeks
CRNMB	Multi-fibroid uterus and postpartum blood loss $\geq 1\ 000$ mL and first line treatment with uterotonics
	Postpartum blood loss $\geq 1\ 000$ mL and first line of treatment with uterotonics and tranexamic acid
	Uterine atony and postpartum blood loss $\geq 1\ 000$ mL and first line of treatment with uterotonics
Minor bleeding	Antepartum vaginal bleeding prompting a face-to-face evaluation at 34 weeks
High risk	
Major bleeding	Postpartum blood loss $\geq 1\ 000$ mL and transfusion and balloon tamponade
	Postpartum blood loss $\geq 1\ 000$ mL leading to transfusion
	Postpartum blood loss $< 1\ 000$ mL and transfusion
Minor bleeding	Antepartum vaginal bleeding prompting a face-to-face evaluation at 39 weeks
	Antepartum vaginal bleeding prompting a face-to-face evaluation at 29 weeks

Abbreviation: CRNMB, clinically relevant non major bleeding.

predominantly African ethnicity, were largely for medical comorbidities. Heart disease and active SLE in pregnancy are recognized risk factors for VTE which have been associated with at least a 6-fold increased risk of antepartum VTE. Similarly, sickle cell disease has been associated with at least a 4-fold increased risk of VTE.⁶ The incidence of sickle cell disease was, however, low in accordance with an earlier South African report.⁴⁰ Moreover, in this study cohort, the risks were further heightened by the presence of high-risk obstetric and delivery characteristics, which is consistent with international

Table 6. Univariate Cox Proportional Hazard Regression Analysis of Predictors of Bleeding.

Variable	HR	95% CI	P Value
High-risk pregnancies	1.29	0.37-4.46	.687
HIV	0.30	0.04-2.34	.250
Concomitant aspirin	2.63	0.76-9.07	.127
Cesarean delivery	2.17	0.46-10.21	.327
Spontaneous delivery	0.39	0.05-3.08	.372
Peripartum enoxaparin interval < 12 h prior to delivery	1.09	0.23-5.12	.916
Peripartum enoxaparin interval < 12 h post delivery	1.12	0.32-3.88	.855

Abbreviations: HR, hazard ratio; CI, confidence interval; HIV, human immunodeficiency virus. Assumptions of proportionality were not met for pre-eclampsia and fetal growth restriction.

cohorts.^{27,41} Studies to date have highlighted the wide variation in the associated risks of pregnancy-related thrombosis in pregnancies classified as intermediate risk.^{6,11,14} A randomized controlled trial is required to determine the safety and efficacy of postpartum and/or fixed low-dose antepartum thromboprophylaxis. This will assist in refining thromboprophylaxis indications and reducing the bleeding complications in intermediate-risk pregnancies.

Despite the use of anti-Xa-adjusted enoxaparin in pregnancies classified as high-risk, the incidence of thrombosis was 3.4%. In the majority of high-risk pregnancies, anti-Xa levels were monitored at regular intervals, in order to facilitate dose adjustments with advancing gestation. With pregnancy progression, 19.2% required a dose adjustment in the second trimester and 25.0% in the third trimester to achieve the target range. This supports prior observational studies.^{37,38,42,43} Owing to the low number of recurrent VTE events in high-risk pregnancies, the efficacy of anti-Xa monitoring could not be confirmed in this study. Regular anti-Xa monitoring too has several limitations. The timing of the assay is important for accurate interpretation

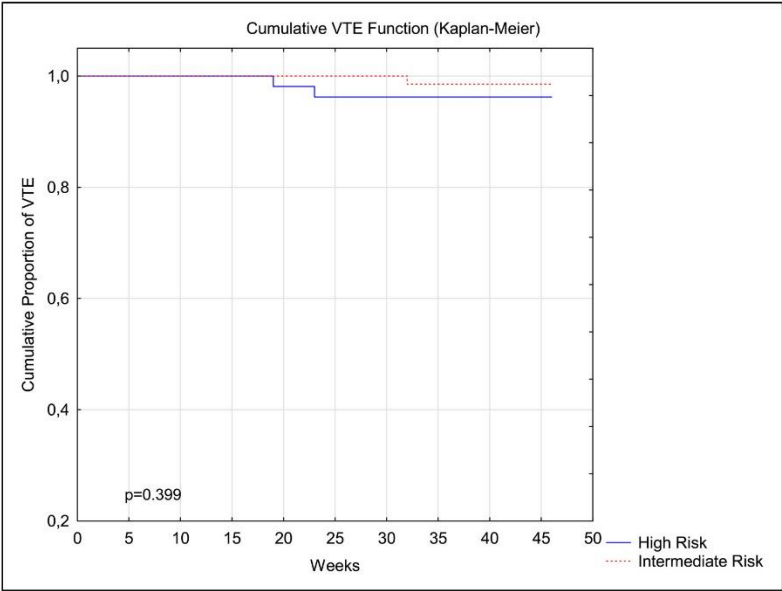


Figure 2. Thrombosis events in intermediate- and high-risk pregnancies. Kaplan–Meier curves are shown for the first occurrence of symptomatic venous thromboembolism in the overall study period.

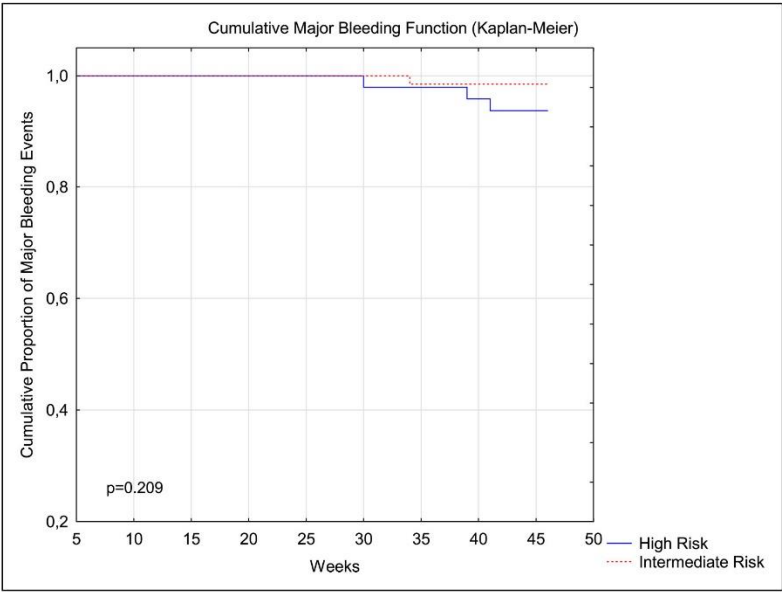


Figure 3. Major Bleeding events in intermediate- and high-risk pregnancies. Kaplan–Meier curves are shown for the first occurrence of adjudicated major bleeding events in the overall study period.

and there is no established prophylactic range for enoxaparin in pregnancy.⁴⁴ Furthermore, this specialized assay remains relatively expensive with limited availability in resource-poor settings.

VTE events occurred antepartum. There were no events in the postpartum period despite the associated higher risk of VTE.^{26,45} In intermediate-risk pregnancies, postpartum thromboprophylaxis was extended beyond the recommended 2 weeks in the presence of ongoing risk factors, for a median of 4 (4) weeks. High-risk pregnancies received postpartum thromboprophylaxis, as recommended, for a median of 6 (0) weeks. In the peripartum period, pregnancies were predominantly managed with scheduled delivery. Similar studies, however, have reported an increased risk of postpartum VTE and have as such proposed expectant management of labor to minimize the interruption of anticoagulation.^{46,47} Nevertheless, concerns exist over the risks of spontaneous labor with the use of intermediate prophylactic or therapeutic enoxaparin.²⁷ Further prospective studies are indicated to determine the safety of expectant management of labor in women receiving higher doses of LMWH.

In this study, recurrent VTE occurred in women with the following combinations of risk factors: SLE, non-criteria antiphospholipid syndrome and previous pregnancy-related PE; HIV and previously unprovoked DVT; and multiple low to intermediate risk factors. With regards to non-criteria antiphospholipid syndrome, the presence of antiphospholipid antibodies on the same occasion has been associated with recurrent thrombosis. According to a recent prospective study, the presence of 2 or more types of antiphospholipid antibodies on the same/different occasions were also predictors of recurrence in participants with a previously unprovoked VTE (HR: 4.5, 95% CI: 1.5-13.0).⁴⁸ Furthermore, in pregnant women living with HIV, we have previously shown at least a 2-fold increased risk of VTE, independent of HIV viral load, CD4 count, and ART.¹⁹ Current guidelines, however, do not consider HIV as an indication for antepartum or postpartum thromboprophylaxis.^{20,21,32,49} Accordingly, the most recent American Society of Haematology guideline advises against thromboprophylaxis in women with a single clinical risk factor owing to the low level of evidence.²¹ Nonetheless, owing to the small number of VTE events, this study could not assess predictors of recurrent thrombosis in order to guide VTE prevention.

Bleeding is an important safety concern in pregnant women receiving thromboprophylaxis. The incidence of bleeding in this cohort was 7.8%, of which major bleeding events accounted for 3.1%. This is consistent with similar studies. Most recently, major bleeding rates of 4.0% were reported in pregnant women with prior VTE randomized to intermediate dose and fixed low dose LMWH respectively by investigators of the multi-center, multi-national Highlow study.²⁴ Severe PPH rates ranging from 2.0% to 14.1% have been reported previously.^{26-28,42,50} Caution, however, is required when interpreting these studies, because of the use of nonstandardized definitions of bleeding.

This study evaluated predictors of bleeding, namely high-risk group, concomitant aspirin, comorbid HIV, pre-eclampsia

and FGR, cesarean delivery, spontaneous delivery, and peripartum enoxaparin interval. On univariate analysis, no independent predictors of bleeding were identified. Of note, cesarean delivery was not a risk factor for bleeding, despite the rate of cesarean deliveries of 64.7% at this specialist referral center for high-risk pregnancies. Furthermore, management with anti-Xa-adjusted enoxaparin in high-risk pregnancies was also not an independent predictor of bleeding. Future prospective studies are indicated to identify predictors of bleeding using standardized bleeding definitions.

Several limitations of this study warrant consideration. Firstly, the high-risk group was heterogeneous with regards to enoxaparin dosing and also included pregnancies with extended duration anticoagulation ($n=9$) who were prescribed therapeutic dose enoxaparin. These pregnancies were included as there is limited data on the associated risks of recurrent thrombosis and bleeding in African women. Secondly, bleeding was determined by visual estimation, which is increasingly recognized as an unreliable measure of bleeding. This may have resulted in an underestimation of bleeding events. Lastly, this study could not assess predictors of VTE as a result of the small number of recurrent events. In particular, HIV is a recognized risk factor for thrombosis, which merits further investigation.

In summary, this study adds to the current literature about the risks related to thrombosis and bleeding associated with antepartum and postpartum thromboprophylaxis in intermediate- and high-risk pregnancies. In this cohort, managed predominantly with scheduled delivery, there were no postpartum thrombosis events or reduced access to neuro-axial anesthesia. The rates of thrombosis and bleeding in this population of predominantly African ethnicity were consistent with similar studies and can be used to inform pregnant women of the benefits of anticoagulation and the risks of potential bleeding.

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Author Contributions

E Schapkaitz: protocol development, data collection and analysis, and manuscript writing; E Libhaber: protocol development, data analysis, and manuscript editing; A Gerber: protocol development, data collection, and manuscript editing; H Rhemtula: protocol development and manuscript editing; J Zamparini: manuscript editing; BF Jacobson: protocol development and manuscript editing; H R Büller: protocol development and manuscript editing

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ORCID iD

E. Schapkaitz  <https://orcid.org/0000-0002-1534-2930>

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CHAPTER 7: Conclusions

The findings described in this body of work in SA pregnant women with HIV firstly illustrated that the phenomenon of pregnancy related VTE can be attributed to HIV. This research, which is one of the first hospital-based studies to examine the significance of HIV as an antepartum and postpartum risk factor, found at least a two-fold increased risk for VTE among pregnant and postpartum women with HIV. In this present era, this risk of VTE was independent of opportunistic infections, ART and the degree of viral suppression. Traditional obstetric and venous risk factors were, however, linked to the risk of thrombosis. Risk factors including positivity for LAC, medical co-morbidities, chronic hypertension, a personal history of VTE, systemic infection, prolonged hospital admission and postpartum haemorrhage could be useful for identifying women with HIV, who may benefit from postpartum and/or antepartum thromboprophylaxis.

Secondly, this thesis advances the view that HIV infection per se may predispose to VTE in pregnant women on suppressive ART through an imbalance of maternal haemostasis. The studies in this research identified changes in coagulation and fibrinolysis which may contribute to the already prothrombotic state of pregnancy. This was confirmed by heightened markers of endothelial activation, namely the ratio of VWF propeptide to VWF antigen and impaired fibrinolysis, namely d-dimer and PAI-1. Similar to studies in non-pregnant HIV populations, the levels of these markers were not associated with a significant reduction with virological suppression as compared to the group with VL > 50 copies/mL. Moreover, an acquired increase in HMWM was observed in a subgroup of pregnant women with HIV. This more prothrombotic multimer pattern occurred in the presence of modestly reduced ADAMTS-13 activity levels, which suggests that ongoing endothelial activation is a predominant mechanism for the haemostatic imbalance in pregnant women on suppressive ART.

Thirdly, this thesis presented rates of thrombosis and bleeding with enoxaparin thromboprophylaxis which can be used to advise pregnant women with HIV of the benefits of anticoagulation and the risks of bleeding. In SA, PPH is the second most common direct cause of maternal mortality (129). As such, it is important to understand the risks of bleeding associated with thromboprophylaxis in order to inform maternal care as well as to anticipate the health care needs of pregnant women with HIV. Owing to the small number of events, HIV could not be assessed as a predictor of thrombosis and bleeding.

The studies in this thesis have shown that the risks facing pregnant women in the current HIV treatment era are complex, involving HIV related risk factors and traditional risk factors. Several important areas for future research have emerged from this thesis: This research showed a decline in the frequency of circulating aPL. However, whether APS contributes to the thrombotic complications in women with HIV on suppressive ART, requires further study. Moreover, it is possible that ongoing endothelial activation and impaired fibrinolysis contribute to thrombosis in pregnant women on suppressive ART. Research conducted in non-pregnant HIV populations has focused on the interactions between inflammation, endothelial activation and coagulation in the pathogenesis of thrombosis (11-13). A clear relationship between markers such as VWF and d-dimers and VTE has been established in non-pregnant HIV populations (15, 16). Markers of maternal haemostasis namely, the ratio of VWF propeptide to VWF antigen, d-dimer and PAI-1 warrant further study. A statistically powered longitudinal study is indicated to determine if markers of endothelial activation and impaired fibrinolysis are also associated with clinical VTE endpoints in pregnant women with HIV on suppressive ART. Lastly, the risks of thrombosis in pregnancies classified as intermediate risk are varied. A randomized controlled trial is thus indicated in intermediate risk HIV pregnancies in order to refine thromboprophylaxis indications and decrease the bleeding complications. This is central to advancing maternal care in pregnant women with HIV.

CHAPTER 8: Appendices

APPENDIX 8.1 Human Research Ethics Committee clearance documents



R14/49 Prof Elise Schapkaitz

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M181018

NAME: Prof Elise Schapkaitz
(Principal Investigator)
DEPARTMENT: Molecular Medicine and Haematology
Charlotte Maxeke Academic Hospital
National Health Laboratory Services

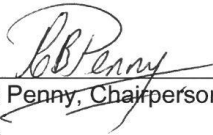
PROJECT TITLE: The effect of treated HIV infection on the haemostasis of pregnancy

DATE CONSIDERED: 26/10/2018

DECISION: Approved unconditionally

CONDITIONS:

SUPERVISOR: Prof BF Jacobson, E Libhaber and H Buller

APPROVED BY: 
Doctor CB Penny, Chairperson, HREC (Medical)

DATE OF APPROVAL: 05/12/2018

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on the Third Floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report.** The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in **October** and will therefore be due in the month of **October** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).


Principal Investigator Signature

05/12/2018
Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

 UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG	HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
--	--

Office of the Deputy Vice-Chancellor (Research & Post Graduate Affairs)

TO: Professor E Schapkaitz, et al
School of Pathology
Department of Molecular Medicine and Haematology
Charlotte Maxeke Johannesburg Academic Hospital

E-mail: Elise.Schapkaitz@nhls.ac.za

CC: Supervisor: Not applicable <>
and <HREC-Medical.ResearchOffice@wits.ac.za>

FROM: Iain Burns
Human Research Ethics Committee (Medical)
Tel: 011 717 1252

E-mail: Iain.Burns@wits.ac.za

DATE: 2020/02/14

REF: R14/49

PROTOCOL NO: M191038 (This is your ethics application study reference number. Please quote this reference number in all correspondence relating to this study)

PROJECT TITLE: *Pregnancy-related venous thrombo-embolism and HIV infection*

Please find attached the Clearance Certificate for the above project. I hope it goes well and that an article in a recognized publication comes out of it. This will reflect well on your professional standing and contribute to the Government funding of the University.



MSWorks2000/Iain0007/Clearscan.wps



R14/49 Professor E Schapkaitz, et al

**HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M191038**

NAME: Professor E Schapkaitz, et al
(Principal Investigator)
DEPARTMENT: School of Pathology
Department of Molecular Medicine and Haematology
Charlotte Maxeke Johannesburg Academic Hospital

PROJECT TITLE: Pregnancy-related venous thrombo-embolism and HIV infection

DATE CONSIDERED: 2019/10/25

DECISION: Approved unconditionally

CONDITIONS:

SUPERVISOR: Not applicable

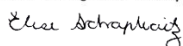
APPROVED BY: 
Dr CB Penny, Chairperson, HREC (Medical)

DATE OF APPROVAL: 2020/02/14

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on the 3rd Floor, Phillip Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.
I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to submit details to the Committee. I **agree to submit a yearly progress report**. When a funder requires annual re-certification, the application date will be one year after the date when the study was initially reviewed. In this case, the study was initially reviewed in **October** and will therefore reports and re-certification will be due early in the month of **October** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).


Principal Investigator Signature

2020/02/14
Date

PLEASE QUOTE THE CLEARANCE CERTIFICATE NUMBER IN ALL ENQUIRIES

 UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG	HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
---	--

2020/06/09

Professor E Schapkaitz, et al
School of Pathology
Department of Molecular Medicine and Haematology
Charlotte Maxeke Johannesburg Academic Hospital

Sent by e-mail to: Elise.Schapkaitz@nhls.ac.za

Dear Professor Schapkaitz

Re: Protocol Ref No: M191038
Protocol Title: *Pregnancy-related venous thrombo-embolism and HIV infection*
Principal Investigator: Professor E Schapkaitz, et al

Thank you for your letter of 2020/05/20. I apologise for the lockdown-generated delay in responding.

I confirm that your request to extend the data collection date in this study to 2020/06/30 has been noted and approved.

Thank you for keeping us informed.

Yours Sincerely



.....
Mr I Burns
For the Human Research Ethics Committee (Medical)



.....
Dr CB Penny, Chairperson, Human Research Ethics Committee (Medical)

UNIVERSITY OF THE
WITWATERSRAND,
JOHANNESBURG



HUMAN RESEARCH ETHICS
COMMITTEE (MEDICAL)

Professor E Schapkaitz, et al
School of Pathology
Department of Molecular Medicine and Haematology
Charlotte Maxeke Johannesburg Academic Hospital

2021/10/08

Sent by e-mail to: Elise.Schapkaitz@nhls.ac.za

Dear Professor Schapkaitz

Re: Protocol Ref No: M191038

Protocol Title: *Pregnancy-related venous thrombo-embolism and HIV infection*

Principal Investigator: Professor E Schapkaitz, et al

Thank you for your letter of 2021/09/27

I confirm that we have noted and approve of your proposal to add a retrospective record review of antiphospholipid syndrome, as a risk factor for venous and arterial thrombosis in HIV⁺ female patients attending the Antenatal Clinic at the Charlotte Maxeke Johannesburg Academic Hospital.

Thank you for keeping us informed.

Yours Sincerely

Mr I Burns

For the Human Research Ethics Committee (Medical)

Dr CB Penny, Chairperson, Human Research Ethics Committee (Medical)

 UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG	HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
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Office of the Deputy Vice-Chancellor (Research and Innovation)

TO: Professor E Schapkaitz; Drs E Gerber and H Rhemutla
School of Pathology
Department of Molecular Medicine and Haematology
Charlotte Maxeke Johannesburg Academic Hospital

E-mail: Elise.Schapkaitz@nhls.ac.za

CC: Supervisor: Not applicable
<>
and <HREC-Medical Research Office@wits.ac.za>

FROM: Mr Iain Burns
Human Research Ethics Committee (Medical)
Tel: 011 717 1252

E-mail: Iain.Burns@wits.ac.za

DATE: 2022/03/28

REF: R14/49

PROTOCOL NO: **M220207** (This is your ethics application reference number. Please quote it in all enquiries, oral or written, relating to this study.)

PROJECT TITLE: *The safety and efficacy of low molecular weight heparin thromboprophylaxis in pregnancy*

Please find attached the Clearance Certificate for the above project. I hope it goes well and that an article in a recognized publication comes out of it. This will reflect well on your professional standing and contribute to Government funding of the University.



MSWorks2000/Iain0007/Clearscan.wps



R49 Professor E Schapkaitz; Drs E Gerber and H Rhemutla

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M220207

NAME: Professor E Schapkaitz; Drs E Gerber and H Rhemutla
(Principal Investigator)

DEPARTMENT: School of Pathology
Department of Molecular Medicine and Haematology
Charlotte Maxeke Johannesburg Academic Hospital

PROJECT TITLE: *The safety and efficacy of low molecular weight heparin
thromboprophylaxis in pregnancy*

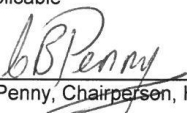
DATE CONSIDERED: 2022/02/25

DECISION: Approved unconditionally

CONDITIONS:

NOTE: If contact information regarding student study participants is required,
please contact the Registrar's office - <Nicoleen.Potgieter@wits.ac.za>

SUPERVISOR: Not applicable

APPROVED BY: 
Dr CB Penny, Chairperson, HREC (Medical)

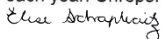
DATE OF APPROVAL: 2022/03/28

This Clearance Certificate is valid for 5 years from the date of approval. An extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office secretariat on the 3rd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated from the research protocol as approved, I/we undertake to submit details to the Committee. **I agree to submit a yearly progress report.** When a funder requires annual re-certification, the application date will be one year after the date when the study was initially reviewed. In this case, the study was initially reviewed in **February** and therefore reports and re-certification will be due in the month of **February** each year. Unreported changes to the study may invalidate the clearance given by the HREC (Medical).



Signature of Principal Investigator

2022/03/28

Date

UNIVERSITY OF THE
WITWATERSRAND
JOHANNESBURG



HUMAN RESEARCH ETHICS
COMMITTEE (MEDICAL)

2022/09/16

Professor E Schapkaitz; Drs E Gerber and H Rhemutla
School of Pathology
Department of Molecular Medicine and Haematology
Charlotte Maxeke Johannesburg Academic Hospital

Sent by e-mail to: Elise.Schapkaitz@nhls.ac.za

Dear Professor Schapkaitz

Re: Protocol Ref No: M220207

Protocol Title: *The safety and efficacy of low molecular weight heparin
thromboprophylaxis in pregnancy*

Principal Investigators: Professor E Schapkaitz; Drs E Gerber and H Rhemutla

Thank you for your e-mail of 2022/09/09.

I confirm that we have noted and approve of your proposal to extend your data collection window to include all records up to and including 2022/09/01.

Thank you for keeping us informed.

Yours Sincerely

.....
Mr I Burns
For the Human Research Ethics Committee (Medical)

.....
Dr CB Penny, Chairperson, Human Research Ethics Committee (Medical)

APPENDIX 8.2 Statement of originality documents

Declaration: Student's contribution to article(s) and agreement of co-author(s)

I, Elise Schapkaitz, student number [9701692H], declare that this Thesis is my own work and that I contributed adequately towards research findings published in the article(s) stated below which are included in my Thesis.

Signature of Student Elise Schapkaitz Date 14/03/23

Name of Primary Supervisor Barry Jacobson

Signature of Primary Supervisor Barry Jacobson Date 14 March 2023

Agreement by co-authors: By signing this declaration, the co-authors listed below agree to the use of the article(s) by the student as part of her Thesis. In cases where the student is not the 1st author of a published article, the primary supervisor must explain (under comments) why the student is entitled to use the paper for her degree purposes.

Article 1: Title: Pregnancy Related Venous Thromboembolism-Associated with HIV infection and Antiretroviral Therapy. Journal name, year, volume and page numbers: Semin Thromb Hemost. 2022 Sep

Authors	Name	Signature	Date
1 st author	Schapkaitz E	<u>Elise Schapkaitz</u>	<u>14/03/23</u>
2 nd author	Jacobson BF	<u>Barry Jacobson</u>	<u>14 March 2023</u>
3 rd author	Libhaber E	<u>E. Libhaber</u>	
4 th author			
5 th author			
6 th author			

Article 2: Title: Pregnancy-related venous thromboembolism and HIV infection. Journal name, year, volume and page numbers: Int J Gynaecol Obstet 2021 Oct;155(1):110-118.

Authors	Name	Signature	Date
1 st author	Schapkaitz E	<u>Elise Schapkaitz</u>	<u>14/03/23</u>
2 nd author	Libhaber E	<u>E. Libhaber</u>	<u>15/3/23</u>
3 rd author	Rhemtula H	<u>H. Rhemtula</u>	<u>15.03.2023</u>
4 th author	Gerber A	<u>A. Gerber</u>	<u>16 March 2023</u>
5 th author	Jacobson BF	<u>Barry Jacobson</u>	<u>16/03/2023</u>
6 th author	Chauke L	<u>L. Chauke</u>	<u>16/03/2023</u>
7 th author	Buller HR	<u>H. Buller</u>	<u>16/03/23</u>

Article 3: Title: Profile of antiphospholipid antibodies in HIV-infected and HIV-uninfected women with a history of thrombosis. Journal name, year, volume and page numbers: Int J Lab Hematol. 2022 Jun;44(3):635-642

Authors	Name	Signature	Date
1 st author	Schapkaitz E	<u>Elise Schapkaitz</u>	<u>14/03/23</u>
2 nd author	Libhaber E	<u>E. Libhaber</u>	<u>16/03/2023</u>
3 rd author	Jacobson BF	<u>Barry Jacobson</u>	<u>16/03/2023</u>
4 th author	Gerber A	<u>A. Gerber</u>	<u>16/03/2023</u>
5 th author	Rhemtula H	<u>H. Rhemtula</u>	<u>16/03/2023</u>
6 th author	Buller HR	<u>H. Buller</u>	<u>16/03/2023</u>

Article 4: Title: von Willebrand factor propeptide-to-antigen ratio in HIV-infected pregnancy: Evidence of endothelial activation. Journal name, year, volume and page numbers: J Thromb Haemost. 2021 Dec;19(12):3168-3176.

Authors	Name	Signature	Date
1 st author	Schapkaitz E	E. Schapkaitz	14/03/23
2 nd author	Libhaber E		
3 rd author	Jacobson BF		14 March 2023
4 th author	Meiring M	M. Meiring	13/03/2023
5 th author	Büller HR		7/03/23
6 th author			

Article 5: Title: Evaluation of markers of fibrinolysis and coagulation in pregnant women with human immunodeficiency virus. Journal name, year, volume and page numbers: Thromb Res. 2022 Jul 7;217:1-8.

Authors	Name	Signature	Date
1 st author	Schapkaitz E	E. Schapkaitz	14/03/23
2 nd author	Libhaber E		
3 rd author	Jacobson BF		14 March 2023
4 th author	Toman M		8.3.2023
5 th author	Gerber A	A. Gerber	25.02.2023
6 th author	Büller HR		7/03/23

Article 6: Title: A Longitudinal Study of Thrombosis and Bleeding Outcomes with Thromboprophylaxis Pregnant Women at Intermediate and High risk of VTE Journal name, year, volume and page numbers: Clin Appl Thromb Hemost. 2023 March.

Authors	Name	Signature	Date
1 st author	Schapkaitz E	E. Schapkaitz	14/03/23
2 nd author	Libhaber E		
3 rd author	Gerber A	A. Gerber	25.02.2023
4 th author	H Rhemtula		12/03/23
5 th author	Zamparini J		15/03/2023
6 th author	Jacobson BF		14 March 2023
7 th author	Büller HR		27/03/23

APPENDIX 8.3 Declaration



PLAGIARISM DECLARATION TO BE SIGNED BY ALL HIGHER DEGREE STUDENTS

SENATE PLAGIARISM POLICY: APPENDIX ONE

I Elise Schapkaiz (Student number: 9701692h) am a student registered for the degree of PhD in the academic year 2023.

I hereby declare the following:

- I am aware that plagiarism (the use of someone else's work without their permission and/or without acknowledging the original source) is wrong.
- I confirm that the work submitted for assessment for the above degree is my own unaided work except where I have explicitly indicated otherwise.
- I have followed the required conventions in referencing the thoughts and ideas of others.
- I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or that I have failed to acknowledge the source of the ideas or words in my writing.
- I have included as an appendix a report from "Turnitin" (or other approved plagiarism detection) software indicating the level of plagiarism in my research document.

Signature: *Elise Schapkaiz* Date: 01/04/2023

APPENDIX 8.4 Similarity report

Intro lit rev and discussion- 3.docx

by Elise Schapkaitz

Submission date: 14-Mar-2023 11:54AM (UTC+0200)
Submission ID: 2036916584
File name: Intro_lit_rev_and_discussion.docx (212.35K)
Word count: 3920
Character count: 22587

Intro lit rev and discussion-3.docx

ORIGINALITY REPORT

13%

SIMILARITY INDEX

3%

INTERNET SOURCES

13%

PUBLICATIONS

0%

STUDENT PAPERS

PRIMARY SOURCES

- | | | |
|-------|--|----|
| 1 | Elise Schapkaitz, Elena Libhaber, Haroun Rhemtula, Annika Gerber, Barry F Jacobson, Lawrence Chauke, Harry R Büller. "Pregnancy related venous thrombo - embolism and HIV infection", International Journal of Gynecology & Obstetrics, 2021
<small>Publication</small> | 2% |
| <hr/> | | |
| 2 | "Posters", Journal of Thrombosis and Haemostasis, 07/2009
<small>Publication</small> | 2% |
| <hr/> | | |
| 3 | Disorders of Thrombosis and Hemostasis in Pregnancy, 2015.
<small>Publication</small> | 2% |
| <hr/> | | |
| 4 | Elise Schapkaitz, Barry F. Jacobson, Elena Libhaber. "Pregnancy Related Venous Thromboembolism-Associated with HIV Infection and Antiretroviral Therapy", Seminars in Thrombosis and Hemostasis, 2022
<small>Publication</small> | 1% |

5	Elise Schapkaitz, Elena Libhaber, Barry F. Jacobson, Marketa Toman, Annika Gerber, Harry R. Büller. "Evaluation of markers of fibrinolysis and coagulation in pregnant women with human immunodeficiency virus", Thrombosis Research, 2022 Publication	1 %
6	www.researchgate.net Internet Source	1 %
7	M. Christy Lamont, Christopher McDermott, Andrew J. Thomson, Ian A. Greer. "United Kingdom recommendations for obstetric venous thromboembolism prophylaxis: Evidence and rationale", Seminars in Perinatology, 2019 Publication	1 %
8	onlinelibrary.wiley.com Internet Source	1 %
9	Patricia E. A. M. Mercelina-Rournans. "Coagulation and fibrinolysis in smoking and nonsmoking pregnant women", BJOG An International Journal of Obstetrics and Gynaecology, 8/1996 Publication	1 %
10	Middeldorp, S.. "Thrombosis in women: what are the knowledge gaps in 2013?", Journal of Thrombosis and Haemostasis, 2013. Publication	<1 %

11	Greer, I.A.. "Prevention of venous thromboembolism in pregnancy", Best Practice & Research Clinical Haematology, 200306 Publication	<1 %
12	jogh.org Internet Source	<1 %
13	Handbook of Thromboprophylaxis, 2011. Publication	<1 %
14	Anne - Mette Hvas, Jorgen Ingerslev, Jannie Dalby Salvig. "Thrombophilia risk factors are associated with intrauterine foetal death and pregnancy - related venous thromboembolism", Scandinavian Journal of Clinical and Laboratory Investigation, 2009 Publication	<1 %
15	www.frontiersin.org Internet Source	<1 %
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17	Elise Schapkaitz, Elena Libhaber, Barry F. Jacobson, Muriel Meiring, Harry R. Büller. "von Willebrand factor propeptide - to - antigen ratio in HIV - infected pregnancy: Evidence of	<1 %

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