

https://dspace.org/

Profile of antiphospholipid antibodies in HIVinfected and HIVuninfected women with a history of thrombosis

Elise Schapkaitz; Elena Libhaber; Barry Jacobson; Annika Gerber; Haroun Rhemtula ...

https://hdl.handle.net/10539/36717
WOS

Downloaded from a DSpace repository. https://dspace.org/

Check for updates

Received: 13 October 2021 | Accepted: 22 January 2022

DOI: 10.1111/ijlh.13805

ISLH International Journal of Laboratory Hematology WILEY

ORIGINAL ARTICLE

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

Elise Schapkaitz[1] | Elena Libhaber[2] | Barry F. Jacobson[1] | Annika Gerber[3] | Haroun Rhemtula[3] | Harry R. Büller[4]

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

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

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

[4]Department 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

Funding information
National Research Foundation, Grant/Award Number: 121943

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]

 © 2022 John Wiley & Sons Ltd

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/m^2. 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 aβ2GP1antibodies IgG and IgM), performed preconception 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 x *g* for 15 min) within 30 min of collection. Serum for aCL and aβ2GP1 IgG and IgM antibodies was separated by centrifugation at 3500 × *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 aβ2GP1 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, aβ2GP1 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 ±*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 ±*SD* age of the study population was 32 ± 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] × 10^6/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

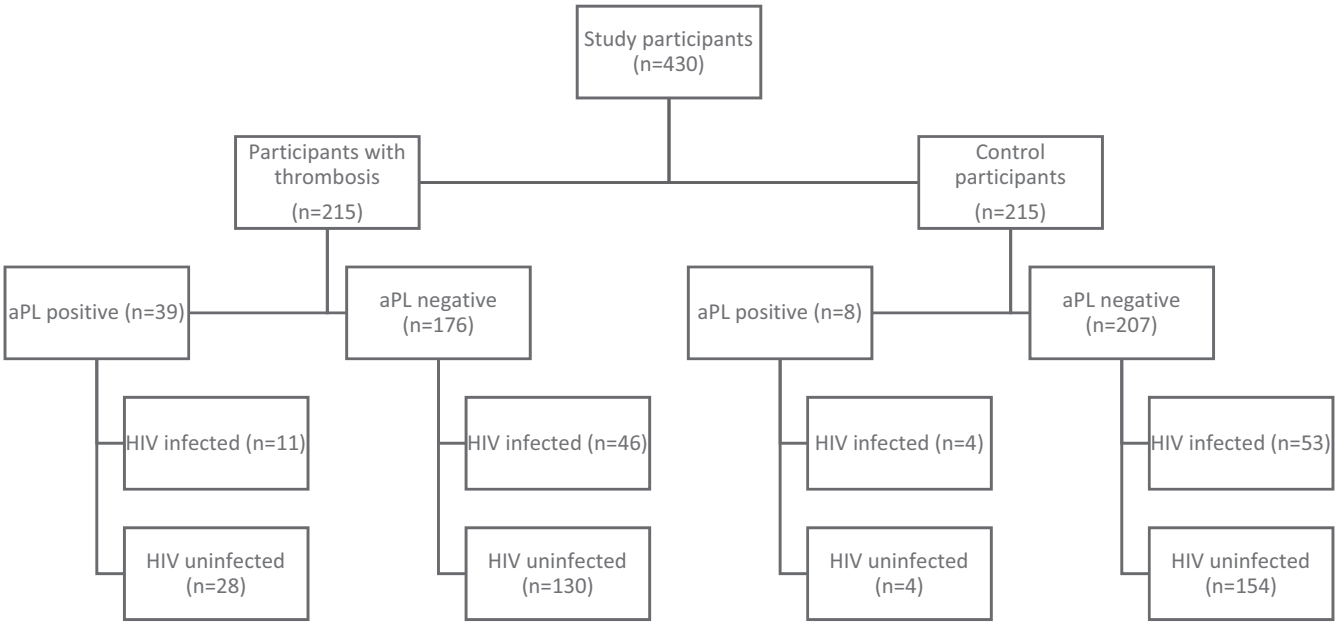


FIGURE 1 Flow diagram of patient selection

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.

[a]Thrombophilia 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).

[b]Baseline aPL positivity refers to positivity on a single occasion for LAC, aCL or aβ2GP1antibodies IgG and IgM.

[c]AIIRD 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).

[d]Testing 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/m^2 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 ±*SD*	31 ± 5	32 ± 6	0.257
Parity, mean ±*SD*	2 ± 1	2 ± 1	0.710
BMI (kg/m^2), mean ±*SD*[a]	25.8 ± 5.0	29.8 ± 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 ≥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 (×10^9/L), mean ±*SD* (ref: 3.9–12.6)	6.8 ± 3.1	7.8 ± 2.9	0.076
Haemoglobin (g/L), mean ±*SD* (ref: 116–164)	116.3 ± 22.7	123.3 ± 18.0	0.037
Platelet count (×10^9/L), mean ±*SD* (ref: 186–454)	270.9 ± 129.7	284.9 ± 99.7	0.455
Mean platelet volume (fL), mean ±*SD* (ref: 7.3–11.3)	9.9 ± 2.3	10.1 ± 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.

[a]AIIRD 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 × 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 aβ2GP1 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%) aβ2GP1 IgG antibodies. In the HIV-infected control participants ($n = 57$), one (1.8%) had aCL IgM antibodies and three (5.3%) had aβ2GP1 IgM antibodies. LAC positivity was significantly associated with thrombosis ($p < 0.003$). In contrast, aCL and aβ2GP1 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%, aβ2GP1 IgG of 0%–37% and aβ2GP1 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, aβ2GP1 IgG and aβ2GP1 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 aβ2GP1 IgM antibodies at a combined rate of 7%. These were not associated with LAC or IgG antibodies. The presence of single aCL and aβ2GPI 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 aβ2GPI 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.

ACKNOWLEDGEMENTS

This work was supported by the National Research Foundation (grant no 121943). We thank the patients of the antenatal clinic at CMJAH for participating and the nursing sisters who staff the antenatal clinic at CMJAH.

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

REFERENCES

1. Duarte-Garcia A, Pham MM, Crowson CS, et al. The epidemiology of antiphospholipid syndrome: a population-based study. *Arthritis Rheumatol*. 2019;71(9):1545-1552.
2. Devreese KMJ, Ortel TL, Pengo V, de Laat B. Subcommittee on Lupus Anticoagulant/Antiphospholipid A. Laboratory criteria for antiphospholipid syndrome: communication from the SSC of the ISTH. *J Thromb Haemost*. 2018;16(4):809-813.
3. Miyakis S, Lockshin MD, Atsumi T, et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). *J Thromb Haemost*. 2006;4(2):295-306.
4. Sammaritano LR, Bermas BL, Chakravarty EE, et al. 2020 American College of Rheumatology guideline for the management of reproductive health in rheumatic and musculoskeletal diseases. *Arthritis Care Res (Hoboken)*. 2020;72(4):461-488.
5. Sevim E, Zisa D, Andrade D, et al. Characteristics of antiphospholipid antibody positive patients in AntiPhospholipid Syndrome Alliance for Clinical Trials and InternatiOnal Networking. *Arthritis Care Res (Hoboken)*. 2020.74(2):324-335.
6. Abuaf N, Laperche S, Rajoely B, et al. Autoantibodies to phospholipids and to the coagulation proteins in AIDS. *Thromb Haemost*. 1997;77(5):856-861.
7. Becker AC, Libhaber E, Sliwa K, et al. Antiphospholipid antibodies in black south africans with hiv and acute coronary syndromes: prevalence and clinical correlates. *BMC Res Notes*. 2011;4:379.
8. Guerin J, Feighery C, Sim RB, Jackson J. Antibodies to beta2-glycoprotein I–a specific marker for the antiphospholipid syndrome. *Clin Exp Immunol*. 1997;109(2):304-309.
9. Loizou S, Singh S, Wypkema E, Asherson RA. Anticardiolipin, anti-beta(2)-glycoprotein I and antiprothrombin antibodies in black South African patients with infectious disease. *Ann Rheum Dis*. 2003;62(11):1106-1111.
10. Petrovas C, Vlachoyiannopoulos PG, Kordossis T, Moutsopoulos HM. Anti-phospholipid antibodies in HIV infection and SLE with or without anti-phospholipid syndrome: comparisons of phospholipid specificity, avidity and reactivity with beta2-GPI. *J Autoimmun*. 1999;13(3):347-355.

11. Sedlacek D, Ulcova-Gallova Z, Milichovska L, Novakova P, Rokyta Z. Seven antiphospholipid antibodies in HIV-positive patients: correlation with clinical course and laboratory findings. *Am J Reprod Immunol*. 2003;50(6):439-443.
12. Galrao L, Brites C, Atta ML, et al. Antiphospholipid antibodies in HIV-positive patients. *Clin Rheumatol*. 2007;26(11):1825-1830.
13. Martinez V, Diemert MC, Braibant M, et al. Anticardiolipin antibodies in HIV infection are independently associated with antibodies to the membrane proximal external region of gp41 and with cell-associated HIV DNA and immune activation. *Clin Infect Dis*. 2009;48(1):123-132.
14. Abdel-Wahab N, Talathi S, Lopez-Olivo MA, Suarez-Almazor ME. Risk of developing antiphospholipid antibodies following viral infection: a systematic review and meta-analysis. *Lupus*. 2018;27(4):572-583.
15. Johnstone FD, Kilpatrick DC, Burns SM. Anticardiolipin antibodies and pregnancy outcome in women with human immunodeficiency virus infection. *Obstet Gynecol*. 1992;80(1):92-96.
16. Ndakotsu MA, Salawu L, Durosinmi MA. Lupus anticoagulant in Nigerian patients living with human immunodeficiency virus/acquired immunodeficiency syndrome. *J Microbiol Immunol Infect*. 2009;42(1):69-73.
17. Turhal NS, Peters VB, Rand JH. Antiphospholipid syndrome in HIV infection: report on four cases and review of the literature. *ACI Internat*. 2001;13:268-271.
18. Chen JY, Ribaudo HJ, Souda S, et al. Highly active antiretroviral therapy and adverse birth outcomes among HIV-infected women in Botswana. *J Infect Dis*. 2012;206(11):1695-1705.
19. Fowler MG, Qin M, Fiscus SA, et al. Benefits and risks of antiretroviral therapy for perinatal HIV prevention. *N Engl J Med*. 2016;375(18):1726-1737.
20. Stringer EM, Kendall MA, Lockman S, et al. Pregnancy outcomes among HIV-infected women who conceived on antiretroviral therapy. *PLoS One*. 2018;13(7):e0199555.
21. Santosa WB, Staines-Urias E, Tshivuila-Matala COO, Norris SA, Hemelaar J. Perinatal outcomes associated with maternal HIV and antiretroviral therapy in pregnancies with accurate gestational age in South Africa. *Aids*. 2019;33(10):1623-1633.
22. Zash R, Souda S, Chen JY, et al. Reassuring birth outcomes with tenofovir/emtricitabine/efavirenz used for prevention of mother-to-child transmission of HIV in Botswana. *J Acquir Immune Defic Syndr*. 2016;71(4):428-436.
23. Kulkarni M, Bowman E, Gabriel J, et al. Altered monocyte and endothelial cell adhesion molecule expression is linked to vascular inflammation in human immunodeficiency virus infection. *Open Forum Infect Dis*. 2016;3(4):ofw224.
24. Howard JFB, Rokx C, Smit C, et al. Incidence of a first venous thrombotic event in people with HIV in the Netherlands: a retrospective cohort study. *Lancet HIV*. 2019;6(3):e173-e181.
25. Schapkaitz E, Libhaber E, Rhemtula H, et al. Pregnancy-related venous thromboembolism and HIV infection. *Int J Gynaecol Obstet*. 2021;155(1):110-118.
26. Devreese KMJ, de Groot PG, de Laat B, et al. Guidance from the Scientific and Standardization Committee for lupus anticoagulant/antiphospholipid antibodies of the International Society on Thrombosis and Haemostasis: update of the guidelines for lupus anticoagulant detection and interpretation. *J Thromb Haemost*. 2020;18(11):2828-2839.
27. Devreese KM, Pierangeli SS, de Laat B, et al. Testing for antiphospholipid antibodies with solid phase assays: guidance from the SSC of the ISTH. *J Thromb Haemost*. 2014;12(5):792-795.
28. Palomo I, Alarcon M, Sepulveda C, Pereira J, Espinola R, Pierangeli S. Prevalence of antiphospholipid and antiplatelet antibodies in human immunodeficiency virus (HIV)-infected Chilean patients. *J Clin Lab Anal*. 2003;17(6):209-215.
29. Stimmler MM, Quismorio FP Jr, McGehee WG, Boylen T, Sharma OP. Anticardiolipin antibodies in acquired immunodeficiency syndrome. *Arch Intern Med*. 1989;149(8):1833-1835.
30. Chayoua W, Kelchtermans H, Gris JC, et al. The (non-)sense of detecting anti-cardiolipin and anti-beta2glycoprotein I IgM antibodies in the antiphospholipid syndrome. *J Thromb Haemost*. 2020;18(1):169-179.
31. Savelli SL, Roubey RAS, Kitzmiller KJ, et al. Opposite profiles of complement in Antiphospholipid Syndrome (APS) and Systemic Lupus Erythematosus (SLE) among patients with Antiphospholipid Antibodies (aPL). *Front Immunol*. 2019;10:885.
32. Decavele AS, Schouwers S, Devreese KM. Evaluation of three commercial ELISA kits for anticardiolipin and anti-beta2-glycoprotein I antibodies in the laboratory diagnosis of the antiphospholipid syndrome. *Int J Lab Hematol*. 2011;33(1):97-108.
33. Persijn L, Decavele AS, Schouwers S, Devreese K. Evaluation of a new set of automated chemiluminescense assays for anticardiolipin and anti-beta2-glycoprotein I antibodies in the laboratory diagnosis of the antiphospholipid syndrome. *Thromb Res*. 2011;128(6):565-569.
34. Litvinova E, Darnige L, Kirilovsky A, Burnel Y, de Luna G, Dragon-Durey MA. Prevalence and significance of non-conventional antiphospholipid antibodies in patients with clinical APS criteria. *Front Immunol*. 2018;9:2971.
35. Pengo V, Del Ross T, Ruffatti A, et al. Lupus anticoagulant identifies two distinct groups of patients with different antibody patterns. *Thromb Res*. 2018;172:172-178.
36. van den Dries LW, Gruters RA, Hovels-van der Borden SB, et al. von Willebrand Factor is elevated in HIV patients with a history of thrombosis. *Front Microbiol*. 2015;6:180.
37. Jong E, Louw S, van Gorp EC, Meijers JC, ten Cate H, Jacobson BF. The effect of initiating combined antiretroviral therapy on endothelial cell activation and coagulation markers in South African HIV-infected individuals. *Thromb Haemost*. 2010;104(6):1228-1234.
38. Funderburg NT, Mayne E, Sieg SF, et al. Increased tissue factor expression on circulating monocytes in chronic HIV infection: relationship to in vivo coagulation and immune activation. *Blood*. 2010;115(2):161-167.
39. Hodkinson KE, Mahlangu JN. Deep-vein thrombosis in the era of high HIV and tuberculosis prevalence: a prospective review of its diagnosis and treatment in a quaternary centre. *S Afr Med J*. 2017;107(10):859-863.
40. Becker AC, Jacobson B, Singh S, et al. The thrombotic profile of treatment-naive HIV-positive Black South Africans with acute coronary syndromes. *Clin Appl Thromb Hemost*. 2011;17(3):264-272.
41. Becker AC, Sliwa K, Stewart S, et al. Acute coronary syndromes in treatment-naive black South africans with human immunodeficiency virus infection. *J Interv Cardiol*. 2010;23(1):70-77.
42. Allie S, Stanley A, Bryer A, Meiring M, Combrinck MI. High levels of von Willebrand factor and low levels of its cleaving protease, ADAMTS13, are associated with stroke in young HIV-infected patients. *Int J Stroke*. 2015;10(8):1294-1296.

SUPPORTING INFORMATION

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

How to cite this article: Schapkaitz E, Libhaber E, Jacobson BF, Gerber A, Rhemtula H, Büller HR. Profile of antiphospholipid antibodies in HIV-infected and HIV-uninfected women with a history of thrombosis. *Int J Lab Hematol*. 2022;44:635–642. doi:10.1111/ijlh.13805