Trends in clinical presentation and treatment outcomes in a South African TTP cohort

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DECLARATION

I, Nokubonga Vundla, hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university. I empower the university to reproduce for the purpose of research either the whole or any portion of the contents in any manner whatsoever.

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FORMAT

This is a publication-ready format manuscript. We aim to submit this manuscript to the South African Journal of HIV Medicine.

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LIST OF ABBREVIATIONS

ADAMTS13	A disintegrin and metalloprotease with thrombo-spondin type 1 motif, member 13
AIDS	Acquired immune deficiency syndrome
ART	Antiretroviral therapy
CD4	Cluster of differentiation 4
DIC	Disseminated intravascular coagulation
FFP	Fresh frozen plasma
GSH	Groote Schuur Hospital
HIV	Human immunodeficiency virus
HREC	Human Research Ethics Committee
HUS	Haemolytic uraemic syndrome
IQR	Interquartile range
LDH	Lactate dehydrogenase
LTFU	Lost to follow-up
MAHA	Micro-angiopathic haemolytic anaemia
MCV	Mean corpuscular volume
mL/kg	Milliliters per kilogram
NHLS	National Health Laboratory Service
PEX	Plasma exchange
PI	Plasma infusion
SA	South Africa
SLE	Systemic lupus erythematosus
TMA	Thrombotic microangiopathy
TNF-α	Tumour necrosis factor alpha
TNF-β	Tumour necrosis factor beta
TTP	Thrombotic thrombocytopenic purpura
µmol/L	Micromole per liter
vWF	von Willebrand factor
WHO	World Health Organization

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TITLE

Trends in clinical presentation and treatment outcomes in a South African TTP cohort

ABSTRACT

Background: HIV is the most common cause of secondary thrombotic thrombocytopenic purpura (TTP) in South Africa.

Objectives: To assess the clinical presentations and outcomes of patients treated for HIV-associated and idiopathic TTP.

Methods: We conducted a retrospective cohort study of patients consecutively diagnosed with TTP from 2010 to 2020 at Groote Schuur Hospital. Study participants were identified by reviewing patient files and Western Cape Blood Services records. Kaplan-Meier curves and log-rank tests were used to evaluate remission rates overall and, by HIV status and treatment group. Logistic regression models were used to identify predictors of remission and relapse.

Results: 139 patients were included, 85.6% of whom were HIV positive. There were no significant differences in the TTP pentad features by HIV status. Most patients achieved remission (71.9%) with an overall median time of 8 days. Remission occurred significantly earlier in those treated with FFPs only, suggesting less severe disease (median=8 days [IQR 6-10]), compared to those requiring the addition of plasma exchange suggesting more severe disease (median=12 days [IQR 8-22]). The overall mortality in the 10-year period was 38.9%, with 10.8% of the surviving patients relapsing after a median of 169 days (IQR 146-281) following the initial TTP event. There were no significant differences in remission status, time to remission, mortality or relapse by HIV status. All HIV positive patients who relapsed had defaulted their antiretroviral therapy (ART).

Conclusion: HIV status did not affect patient outcomes in our cohort. ART is important in preventing HIV-associated TTP and relapse.

What this study adds: A well-defined demographic of patients with TTP in the ART era in South Africa.

Key words: Thrombotic thrombocytopenic purpura, haemolytic anaemia, thrombotic microangiopathy, HIV-associated TTP, ADAMTS13, treatment outcomes, plasma infusion, plasma exchange.

INTRODUCTION

Thrombotic thrombocytopenic purpura (TTP) is a rare but life threatening condition that belongs to a heterogenous group of disorders termed thrombotic microangiopathies (TMAs)(1). TMA was first described in 1924 by Moschcowitz (2) and is characterized by multisystemic microvascular deposition of platelet-rich thrombi resulting in consumptive thrombocytopenia and haemolytic anaemia. Microvascular thrombosis and anaemia result in target organ ischaemia and dysfunction of variable severity(3, 4).

Severe ADAMTS13 (a disintegrin and metalloprotease with thrombo-spondin type 1 motif, member 13) deficiency has classically been considered a specific biomarker for TTP(1). Severe deficiency of ADAMTS13 results in the increase of ultra-large von Willebrand factor multimers in the circulation, which drives platelet capturing, thrombi formation and platelet consumption(5, 6).

The annual incidence of TTP is reported to be as low as one case per million(4, 6). Congenital TTP due to ADAMTS13 mutations(7) make up only 5% of TTP cases(4). The vast majority of acquired TTP occur as a result of autoantibodies formed against ADAMTS13 which can be primary (idiopathic) or secondary to other conditions like human immunodeficiency virus (HIV), systemic lupus erythematosus (SLE), post transplantation or pregnancy(7).

The diagnosis of TTP during the initial stages largely relies on laboratory evidence of a TMA. In clinical practice, most patients present with a triad of thrombocytopenia, fragmentation hemolysis and an elevated lactate dehydrogenase (LDH) which is sufficient to make a diagnosis of TTP(6, 8). The diagnosis of TTP is confirmed by severely low ADAMTS13 activity (<10% of normal)(4, 9). However, in the HIV-associated group, a normal ADAMTS13 level does not exclude TTP(10). ADAMTS13 activity results are usually unavailable during the initial stages of diagnosis and management, since the testing is only performed at highly specialised reference coagulation laboratories and is often batch tested(11).

HIV infected patients have a significantly increased risk of developing acquired TTP as compared to the general population(12). Jokela et.al. first described HIV-associated TTP in 1987 which is now a clinically recognized manifestation of untreated HIV infection(5, 13). In South Africa (SA), the majority of TTP cases are associated with HIV and in most cases, TTP is the initial presentation of HIV infection(14). The pathogenesis of TTP in the HIV setting is still poorly understood. In HIV-associated TTP, autoantibodies directed against ADAMTS13 are detected in only 50% of patients(3, 5) compared to 75% of acquired, HIV unaffected TTP cases(15). Patients with advanced HIV disease and high viral loads predominantly have normal ADAMTS13 levels(3). It is currently uncertain if patients with laboratory and/or clinical features of HIV-associated TTP but normal ADAMTS13 activity rather

represent a subgroup of patients with a TTP-like syndrome or HIV-associated TMA(3, 16). Although advanced HIV (low CD4 count and acquired immuno-deficiency syndrome) is considered an added risk factor for TTP, a decline in HIV-associated TTP in the anti-retroviral therapy (ART) era has not been demonstrated in the South African setting(4, 14), for reasons which are unclear. Since the induction of ART in the early phase of HIV infection in 2015 as per the "Test and Treat" strategy(17), it is uncertain if the annual incidence of HIV-associated TTP has declined.

The clinical pentad of TTP was first described by Amarosi and Ultmann in 1966. The pentad, which represents the most severe form of the disease, consists of thrombocytopenia, microangiopathic haemolytic anaemia (MAHA), fever, renal impairment and neurological dysfunction(3, 15). All 5 clinical features, however, are only observed in approximately 10% of patients(6, 15). The incidence of the aforementioned clinical complications may be difficult to define in the HIV-associated TTP setting as patients often have baseline organ dysfunction relating to HIV infection and/or ART(3). The clinical presentation and pattern of organ involvement in HIV-associated TTP requires further investigation and refinement.

The current standard of care for TTP in SA includes either daily therapeutic plasma exchange (PEX) with plasma replacement, or daily large volume (30 ml/kg/day) plasma infusion (PI), in combination with oral corticosteroids(18). This treatment has dramatically reduced the mortality of patients with HIV associated TTP from 90% to 10-20%(19). Treatment protocols vary among the different care centres in SA with respect to the volume and frequency of fresh frozen plasma (FFP) as well as the use of PEX with FFPs as initial treatment. Most centres have reported their experiences with managing TTP in retrospective studies and these studies looked at cohorts treated prior to, or very early during, the 'Test and Treat' era of HIV management(14, 19, 20).

At our centre in Cape Town, TTP is initially treated with daily with 30mL/kg of FFP with each patient receiving about 6-8 units of FFPs per day. This treatment protocol was implemented after the findings by Novitzky et al. that FFP appears non-inferior to PEX with a 95% response rate reported in HIV-associated TTP(20). The findings by Novitzky et al. may reflect the fact that the majority of HIV-associated TTP do not show demonstrable anti-ADAMTS13 antibodies and replacement of ADAMTS13 by PI alone is therefore sufficient.

In this study we assessed the clinical presentations and outcomes of patients treated for HIV associated TTP in a public, tertiary health care facility in SA.

METHODS

This was a single-centre, retrospective cohort study conducted at the Clinical Haematology Unit at Groote Schuur Hospital (GSH), a tertiary health care facility in Cape Town, SA. The study was approved by the University of Cape Town Human Research Ethics Committee (HERC REF 112/2021) .139 patients diagnosed with idiopathic and HIV-associated TTP over a 10-year period between 01 January 2010 and 31 December 2020 were included in the study. Those patients with an identifiable cause of secondary TTP e.g. pregnancy and autoimmune diseases were excluded from the study.

The study participants were identified by reviewing inpatient and outpatient files as well as a list compiled by the Western Cape Blood Services (WCBS) of all patients who received FFP during the study period at GSH with keywords of TTP, MAHA and TMA provided on the request form by the clinician. This list was correlated with patient medical records to identify patients with TTP. The diagnosis of TTP was made on the results of laboratory investigations and required the presence of thrombocytopenia and fragmentation haemolysis with the absence of any other identifiable cause(s) such as disseminated intravascular coagulation (DIC) for these findings. Idiopathic TTP cases were identified by reviewing the clinical notes and laboratory results. Idiopathic TTP was defined as TTP in the absence of identifiable cause for TTP such as HIV infection.

Patient Data

Data was collected from patient records and the local laboratory information system. Demographic and clinical data collected included patient age, gender, HIV status, ART status, neurological dysfunction, and presence or absence of fever at diagnosis as well as the treatment provided. Regarding management outcomes, the time to remission, duration of hospital stay, relapse and mortality were noted. Mortality was further subdivided into the probable causes of demise and whether death occurred during the index hospital admission, after admission or during disease relapse.

Therapy

Patients were treated with supportive measures, daily large volume FFP (30 mL/kg/day, 8-10 units per day) via a central or peripheral line, and corticosteroid therapy. Diuretics to manage or prevent fluid overload were given based on clinical indication. FFP was given daily until clinical improvement in neurological function and laboratory end points were met (platelet count >150 x 10^9/L and LDH level <450 U/L on two consecutive days) after which the FFP was tapered down by 320 mL (1 unit) every 3 days. As adjunctive therapy, ART naïve HIV positive patients were commenced on ART as per SA's national ART guidelines(20).

Patients refractory to the above regimen and patients who were fluid overloaded and could not tolerate large volume PIs were offered PEX with FFPs(14, 20). On days when PEX was not available, mainly on weekends, FFPs were given at 30 mL/kg/day(20). Haemodialysis was offered as required per acute dialysis indications.

Laboratory investigations

The laboratory results generated at presentation and collected for this study included full blood count, peripheral blood smear for red cell fragment identification and enumeration, LDH, renal function testing (urea and creatinine), HIV serology and, if HIV positive, the absolute CD4+ T- cell count and HIV viral load. ADAMTS13 antigen and antibody tests were requested prior to FFPs in very few patients and these results were not included in the analysis. Significant red cell fragmentation was defined as a red cell fragment count >1%, significant thrombocytopenia as a platelet count <90 x 10^9/L and a raised LDH as >190 IU/L. Creatinine >100 µmol/L was regarded as a marker of renal dysfunction(14, 20, 21). Advanced HIV was defined by an absolute CD4+ T-cell count of <200 cells/µL or stage IV AIDS-defining condition as per World Health Organization (WHO) guidelines (22)

Clinical Outcome

Duration of hospital stay was noted and complications such as nosocomial infections were documented. Remission was defined as platelet count >150 x 10^9/L and LDH level <450 U/L on at least two consecutive days. Relapse was defined as reoccurrence of TTP after FFP treatment was concluded. Refractory TTP was defined as failure of improvement of the laboratory parameters *viz* platelet count, anaemia and LDH(20, 21). The causes of death were documented as per treating clinicians' reports.

Data Capturing and Analysis

All data were entered into the Clinical Haematology REDCap database. Data were exported from REDCap® into STATA® Version 14 (Stata Corporation, College Station, Texas, USA) for analysis. Patient characteristics were described by frequencies (%), means and standard deviations or medians and interquartile ranges. Categorical data was compared using Chi-squared and Fisher's exact tests. The Mann-Whitney U test was used to compare continuous variables as they were not normally distributed. Kaplan-Meier curves were also used to display remission rates for the total cohort, by HIV status and by treatment group. Remission rates between groups were compared using the log-rank test. Univariable and multivariable logistic regression models were used to identify predictors of remission, time to remission and relapse. Statistical significance was set at $p<0.05$ for all analyses.

Ethical Considerations

The study was approved by the University of Cape Town Human Research Ethics Committee (HREC approval number 112/2021) and institutional approval was obtained from Groote Schuur Hospital. A waiver of consent for data collection is in place for patients included in the Clinical Haematology REDCap database prior to 2019 and informed consent is obtained for patients included from 2019 onwards.

RESULTS

The data for 139 consecutive patients admitted with HIV-associated and idiopathic TTP during the study period were reviewed. A flow chart of the study participants and their outcomes are presented in Figure 1. The baseline characteristics, treatment and outcomes are presented in Tables 1 and 2. The majority of patients had HIV-associated TTP (85.6%) and the median age of the cohort was 34.0 years (IQR 28.6-40.0) with most patients being female (73.4%). Of the HIV positive patients, only 1 patient (0.8%) was on ART and virally suppressed, 49.6% were ART naïve, and 49.6% had defaulted ART at the time of presentation. The median absolute CD4+ T-cell count for the HIV positive cohort was 154.5 cells/µL (IQR 86.0-242.0) (normal reference value 500 – 1500 cells cells/µL). There were no significant differences in age or gender between the HIV positive and negative (idiopathic TTP) subgroups. The number of TTP cases per year, by HIV status is presented in Supplementary Figure 1 and shows that the annual number of TTP cases has not declined.

Features of classic TTP pentad

Of the classic TTP pentad (fever, thrombocytopenia, haemolytic anaemia, renal dysfunction, and neurologic dysfunction), renal dysfunction (creatinine above 100 µmoL/L) was least common, occurring in <50% of the study population (Table 1). Almost all patients presented with thrombocytopenia (99.3%) and haemolytic anaemia (97.8%). Neurologic dysfunction (88.5%) and fever, with temperature exceeding 37.4 ℃ (73.4%) were also common. There were no significant differences between the HIV positive and negative subgroups. However, more patients with HIV-associated TTP presented with neurological dysfunction (89.9% vs. 79.0%) showing a trend towards statistical significance (P=0.166). Compared to patients who achieved remission, a significantly greater proportion of patients who did not achieve remission presented with neurological dysfunction (84.0% vs. 100%, P=0.008) suggesting that neurological dysfunction was a marker for severe TTP (Supplementary Table 1). No further significant differences between pentad features and remission status were observed.

Laboratory data

Key baseline laboratory results are presented in Table 1. There were no significant differences between the results recorded for the HIV positive and HIV negative subgroups. ADAMTS13 levels were obtained for 6 patients (4.3%) and meaningful statistical analyses were therefore not possible using this variable.

Treatment and outcomes

Most patients (81.3%) were treated with therapeutic FFP as per local protocol (Table 2). Only 25 patients (18%) required the addition of PEX as their laboratory parameters had not improved. Regardless of the treatment protocol used (FFP only vs FFP with PEX), the majority of patients (71.9%) achieved remission. No significant differences in treatment protocol and remission status were noted between the HIV positive and negative subgroups. Univariate and multivariate logistic regression showed no statistical relationship between patient factors assessed (age, gender, and HIV status) or treatment protocol and remission status (Table 3).

The overall median time to remission was 8 days (IQR 6-12) (Figure 2A). There was no significant difference in time to remission by HIV status (Table 2 and Figure 2B). Remission occurred significantly earlier in those treated with FFPs only (median=8 days [IQR 6-10]) compared to those requiring the addition of PEX (median=12 days [IQR 8-22) (Figure 2C). Univariable and multivariable linear regression models identified treatment protocol as a predictor of time to remission (Table 4). Compared to patients treated with FFP only, patients treated with FFP and PEX took 5.9 days longer to achieve remission on average, holding all other variables constant (P=0.012). The laboratory results for all patients who achieved remission, despite HIV status, showed normal renal function and platelet counts at time of discharge (Table 2).

After the first TTP episode, there was a total of 54 (38.9%) deaths, 36 (30.3%) among the HIV positive subgroup and 7 (36.8%) among the HIV negative subgroup. The median length of hospital stay was 20 days (IQR 15-27) for those who survived. Among this group, 15 (10.8%) relapsed after a median of 169 days (IQR 146-281) from discharge. No significant differences were noted between HIV positive and negative subgroups in terms of mortality, the number of patients who relapsed or the time to relapse. No significant predictors of disease relapse were identified in univariable or multivariable logistic regression models (Table 5). However, a trend towards statistical significance for the positive association between time to remission and TTP relapse was noted (P=0.084).

Severe TTP was recorded as the main cause of death in both HIV positive and negative subgroups. Additionally, 7 (5.9%) HIV positive patients succumbed to sepsis while sepsis as a cause for death, was not recorded for the HIV negative subgroup (Table 2).

Figure 1: Flow chart of study patients and their outcomes

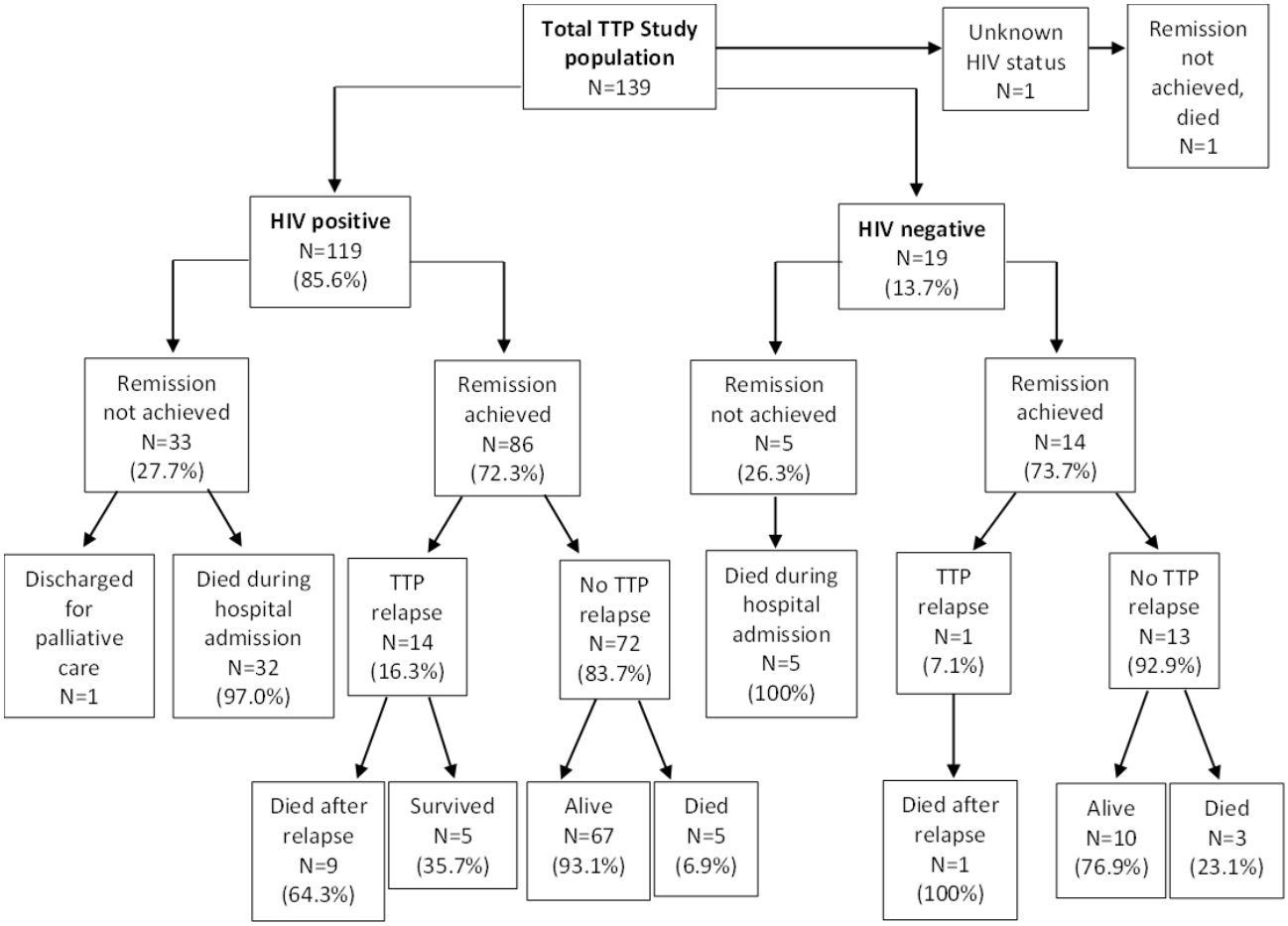

Figure 1: Flow chart of study patients and their outcomes

Note: percentages are calculated using the total of the group above as the denominator.

TTP, thrombotic thrombocytopenic purpura; HIV, human immunodeficiency virus.

One patient was not added to the "Remission Rates by HIV Status Figure 2(B) as he had end stage renal failure and was not a candidate for haemodialysis. He was subsequently sent home for palliative care.

Table 1: Baseline characteristics of patients diagnosed with TTP between 2010-2020

Characteristic	Total (N=139)	HIV-associated TTP (n=119)	HIV negative TTP (n=19)	*P*-value
Demographic data				
Age (years)	34.0 (28.6-40.0)	33.6 (28.3-38.8)	41.2 (32.6-49.0)	0.014
Sex				
Male	37 (26.6)	30 (25.2)	7 (36.8)	0.288
Female	102 (73.4)	89 (74.8)	12 (63.2)	
Features of classic TTP pentad				
Fever	102 (73.4)	90 (75.6)	12 (63.2)	0.250
Thrombocytopenia	138 (99.3)	118 (99.2)	19 (100)	0.688
Haemolytic anaemia	136 (97.8)	116 (97.5)	19 (100)	0.484
Renal dysfunction	62 (44.6)	53 (44.5)	8 (42.1)	0.843
Neurologic dysfunction	123 (88.5)	107 (89.9)	15 (79.0)	0.166
All 5 features	30 (21.6)	29 (24.4)	1 (5.3)	0.061
Laboratory data				
Creatinine (54 – 97 µmol/L), n=135	97.0 (73.0-154.0)	98 (73.0-161.5)	89.5 (72.0-139.0)	0.777
Haemoglobin (12.5 – 16 g/dL)	5.7 (4.8-6.9)	5.7 (4.7-6.9)	6.1 (5.0-8.6)	0.171
Platelets (150 – 450 $x10^9$/L)	14.0 (8.0-24.0)	14.0 (8.0-24.0)	14.0 (9.0-24.0)	0.894
LDH (140 – 200 IU/L), n=132	1453 (985-2121)	1385 (943-2121)	1602.5 (1254-2255)	0.269
White cell count (4.5 – 11.0 $x10^9$/L)	9.2 (6.5-13.1)	8.9 (6.4-13.0)	9.7 (8.2-14.4)	0.292
MCV (50 – 98 fL)	89.0 (83.0-97.0)	89.0 (83.0-98.0)	88.0 (81.0-93.0)	0.453
CD4 count (500 – 1500 cells/mm^3), n=116	-	154.5 (86.0-242.0)	-	-
ART status				
ART naïve	-	59 (49.6)	-	-
Defaulted ART		59 (49.6)		
On ART, virally suppressed		1 (0.8)		

Values expressed as median (IQR) or n (%)

LDH, lactate dehydrogenase; MCV, mean corpuscular volume; ART, antiretroviral therapy; TTP, thrombotic thrombocytopenic purpura; HIV, human immunodeficiency virus.

Table 2: Treatment and outcomes of TTP patients by HIV status

Characteristic	Total (N=139)	HIV-associated TTP (n=119)	HIV negative TTP (n=19)	*P*-value
Treatment				
FFP only	113 (81.3)	98 (83.0)	14 (73.7)	0.327
FFP and PEX	25 (18.0)	20 (17.0)	5 (26.3)	
Died before treatment	1 (0.7)	1 (0.8)	0 (0)	
Remission				
No. achieving remission	100 (71.9)	86 (72.3)	14 (73.7)	0.898
No. achieving remission with FFP only	81 (72.3)	72 (73.5)	9 (64.3)	0.472
No. achieving remission with FFP & PEX	19 (76.0)	14 (70.0)	5 (100)	0.160
Time to remission (days)				
Overall	8 (6-12)	8 (6-12)	8 (5-11)	0.708
FFP only	8 (6-10)	8 (6-10)	7 (5-10)	0.423
FFP & PEX	12 (8-22)	13 (10-23)	11 (7-14)	0.377
Laboratory data at remission				
Creatinine (54 - 97 µmol/L), n=80	70.5 (58.0-87.5)	70.5 (55.5-87.5)	71.0 (66.5-107.0)	0.325
Haemoglobin (12.5 - 16 g/dL)	9.7 (8.5-10.5)	9.7 (8.4-10.6)	10.0 (8.7-10.3)	0.945
Platelets (4.5 – 11 x 10^9/L)	283.0 (227.5-363.5)	286.0 (231.0-368.0)	259.0 (200.0-300.0)	0.338
LDH (140 - 280 IU/L), n=132	308.5 (263.0-344.5)	308.5 (260.0-344.5)	316.0 (283.5-410.0)	0.200
Haematocrit (40 - 55%), n=45	0.29 (0.25-0.33)	0.29 (0.25-0.33)	0.30 (0.26-0.32)	0.742
White cell count (4.5 - 11), n=99	7.2 (5.3-9.3)	7.0 (5.0-9.1)	7.4 (6.7-10.1)	0.144
MCV (80-98Fl), n=97	94.0 (90.0-101.0)	95.0 (90.0-102.0)	92.0 (89.0-100.0)	0.435
Outcome after initial episode				
Survived	94 (67.6)	83 (69.8)	11 (57.9)	0.303
Died	45 (32.4)	36 (30.3)	8 (42.1)	
Length of stay (days)				
Survived	20 (15-27)	20 (14-27)	24 (17-41)	0.352
Died during admission	5 (2-11)	5 (2-11)	3 (2-16)	0.561
Number who relapsed	15 (10.8)	14 (11.8)	1 (5.3)	0.398
Time to relapse (days)	169 (146-281)	186 (149-281)	87 (87-87)	0.247
Final Outcome				
Death	54 (38.9)	45 (37.8)	8 (42.1)	0.496
Discharged from Haematology	76 (54.7)	67 (56.3)	9 (47.4)	
Lost to follow up	1 (0.7)	1 (0.8)	0 (0)	
Presumed dead	3 (2.2)	2 (1.7)	1 (5.3)	
Relocated	5 (3.6)	4 (3.4)	1 (5.3)	
Cause of death				
Severe TTP	44 (81.5)	36 (80.0)	7 (87.5)	0.341
Sepsis	7 (13.0)	7 (15.6)	0 (0)	
Other	2 (3.7)	1 (2.2)	1 (12.5)	
Unknown	1 (1.9)	1 (2.2)	0 (0)	

1 patient died before treatment was administered.

FFP, fresh frozen plasma; PEX, plasma exchange; LDH, lactate dehydrogenase; MCV, mean corpuscular volume; HIV, human immunodeficiency virus.

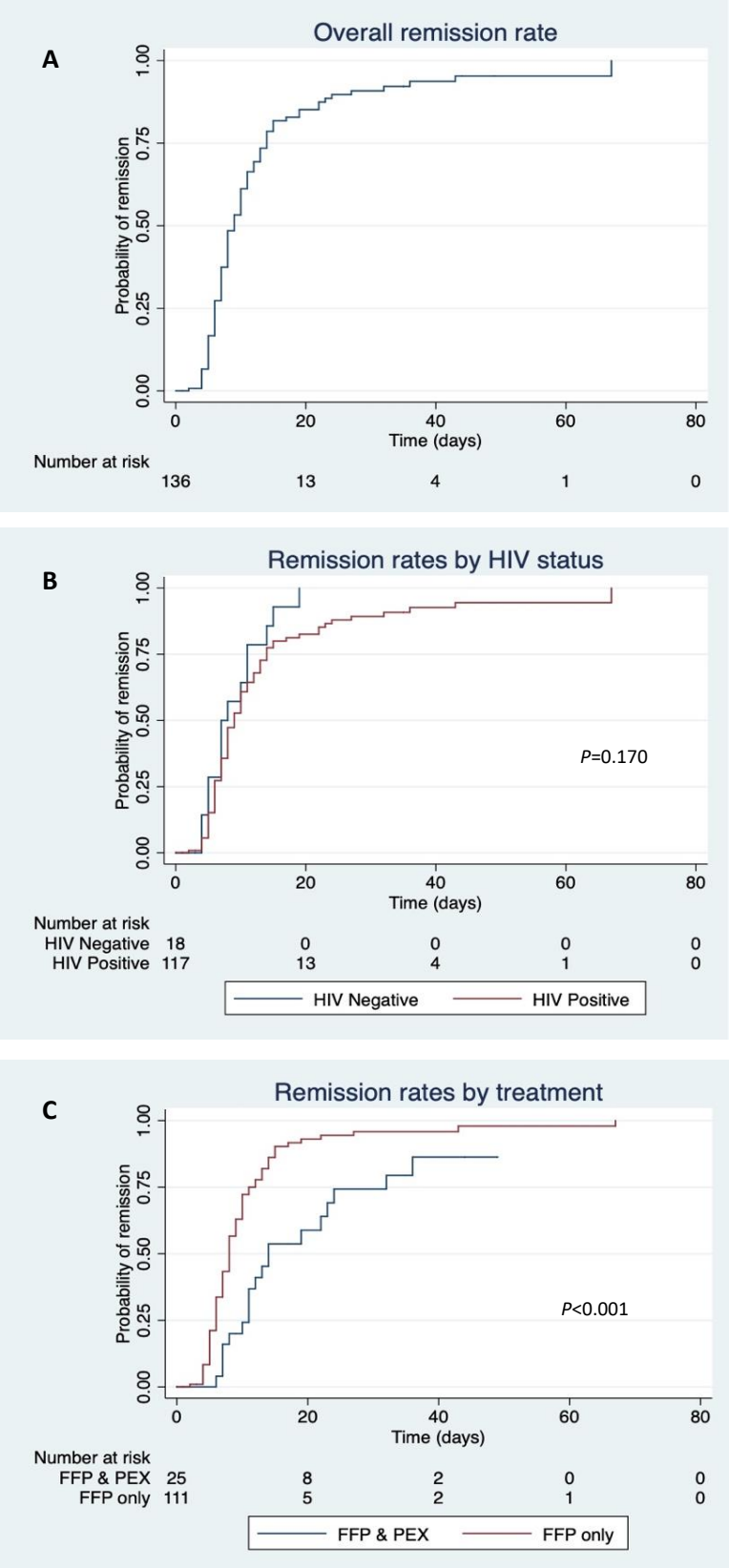

Figure 2: Remission rate of (A) the total TTP cohort, (B) by HIV status and (C) by type of treatment received.

FFP, fresh frozen plasma; PEX, plasma exchange; HIV, human immunodeficiency virus.

*3 patients died on their date of diagnosis which means that they are automatically omitted from the survival curves as their follow-up time is 0 days (total 139-3=136). The reason Figure 2A and 2C have one more person (N=136) than Figure 2B (N=135) is because they include the patient with a missing HIV status.

Table 3: Univariable and multivariable logistic regression models for the outcome variable;

Univariable analysis	**Odds ratio**	***P*-value**	**95% CI**
Age	1.01	0.768	0.97-1.04
Sex (female)	1.12	0.792	0.49-2.56
HIV status (positive)	0.93	0.898	0.31-2.79
Treatment (FFP & PEX)	1.25	0.662	0.46-3.42
Multivariable analysis			
Age	1.00	0.867	0.96-1.04
Sex (female)	1.23	0.634	0.53-2.86
HIV status (positive)	0.97	0.963	0.31-3.02
Treatment (FFP & PEX)	1.21	0.716	0.44-3.36

remission.

FFP, fresh frozen plasma; PEX, plasma exchange; HIV, human immunodeficiency virus.

Univariable analysis	**Co-efficient**	***P*-value**	**95% CI**
Age	0.02	0.788	-0.15-0.19
Sex (female)	-0.37	0.857	-4.40-3.66
HIV status (positive)	1.88	0.464	-3.20-6.96
Treatment (FFP & PEX)	5.45	0.015	1.08-9.82
Multivariable analysis			
Age	0.01	0.928	-0.17-0.18
Sex (female)	-0.41	0.841	-4.42-3.60
HIV status (positive)	3.17	0.235	-2.10-8.43
Treatment (FFP & PEX)	5.86	0.012	1.34-10.39

Table 4: Univariable and multivariable linear regression models for the outcome variable; time to remission (n=100).

FFP, fresh frozen plasma; PEX, plasma exchange; HIV, human immunodeficiency virus.

Univariable analysis	Odds ratio	*P*-value	95% CI
Age	0.95	0.145	0.89-1.02
Sex (female)	0.70	0.535	0.22-2.19
HIV status (positive)	2.40	0.412	0.30-19.39
Treatment (FFP & PEX)	1.15	0.841	0.30-4.41
Time to remission	1.05	0.084	0.99-1.10
Multivariable analysis			
Age	0.95	0.140	0.88-1.02
Sex (female)	0.50	0.285	0.14-1.78
HIV status (positive)	2.07	0.521	0.22-19.23
Treatment (FFP & PEX)	0.96	0.961	0.22-4.24
Time to remission	1.05	0.102	0.99-1.11

Table 5: Univariable and multivariable logistic regression models for the outcome variable; relapse.

FFP, fresh frozen plasma; PEX, plasma exchange; HIV, human immunodeficiency virus.

DISCUSSION

This was a single centre, retrospective study which evaluated the clinical features, laboratory data and clinical outcomes of 139 patients diagnosed with idiopathic and HIV-associated TTP over a period of 10 years during the ART era. The last study on TTP performed at our centre in Cape Town was in 2005 before ART became widely available (20). Despite a decline of 47% in the number of new annual HIV infections between 2000 and 2019, the number of annual patients presenting with HIV-associated TTP remains unchanged in our centre.

Of the patients included in the study, the majority (85.6%) were HIV positive which is in line with previous studies on TTP in SA(14, 20). As of 2017, SA had the largest HIV burden in the world with an estimated 8 million South Africans living with HIV which accounts for the larger proportion of HIV-associated TTP seen in our setting (23). This is in contrast to idiopathic TTP which is rare not only in SA but globally, with an annual incidence estimated at one per million population(6). HIV infection unfortunately increases the likelihood of acquiring TTP up to 40-fold(24).

The median age of HIV-associated TTP patients at diagnosis was 34 years, which is similar to the study by Masoet et al. which reported a median age of 33.7 years at diagnosis(14). This is also in keeping

with national findings on Statistics SA, that HIV mainly affects young adults between the ages of 20-34 years(23). In the group with HIV-associated TTP, 73.4% were female which is like previous studies(14, 21, 25).

In the HIV-associated TTP group, 49.6% were ART naïve (representing first diagnosis and presentation of HIV) while the other 49.6% had defaulted their ART at the time of presentation. The number of patients who presented after defaulting ART is significantly more than the 21.9% reported in 2019 by Masoet et al.(14). SA has the largest ART program in the world, so it was surprising that half of our HIV infected patients presented after defaulting on ART. This suggests a possible gap in the methods of counselling at the time of ART initiation and at follow-up at primary health care level(14, 17).

Despite SA having the largest ART program in the world, 50% of our patients were ART naïve at presentation but this could be explained by the history of the ART program in the country. Before the year 2010, patients with absolute CD4+ T cell counts below 200 cells/µL or WHO-defined stage IV HIV (AIDS-defining illness) were started on ART whereas by 2013, the absolute CD4+ T cell count whereby ART was commenced was increased to 350 cells/µL. It was only in 2015 that HIV positive patients, irrespective of their absolute CD4 counts, were started on ART as soon as clinically possible. With this improvement in the ART program, the number of patients presenting with AIDS defining illnesses has declined(17, 23). The median absolute CD4+ T cell count for the HIV positive cohort was 154.5 cells/µL (500 – 1500 cells/µL) which suggests that HIV-associated TTP is largely a disease of untreated HIV. In our study, only one patient was HIV positive with a suppressed viral load at the time of presentation. In this patient, the TTP was attributed to newly diagnosed SLE.

Neurological dysfunction occurred more commonly in the HIV positive subgroup (89.9%) compared to the HIV negative group (79%) but this was not statistically significant. Compared to the study by Masoet et al.(14), neurological dysfunction was reported in 78% of the cohort with no significant difference in the HIV negative and the HIV positive groups. The reason for the higher percentage of HIV associated TTP with neurological dysfunction in our study is unclear.

Similar numbers of HIV positive patients (72.3%) and HIV negative patients (73.7%) achieved remission. Time to remission was also similar for the HIV positive and negative groups. In the HIV positive group, 98 (83%) received FFP infusion only while 20 (17%) required both FFP infusion and PEX. In the retrospective study by Masoet et al., no significant differences were reported for remission rates in HIV-associated vs HIV unaffected TTP patients (56.1% vs 54.5%, respectively) and for HIV-associated TTP patients treated with FFP infusion only vs PEX with FFP. ART initiation was the only predictor for remission identified in that study(14).

Patients who were treated with FFPs only went into remission after a median of 8 days from admission while those who required both FFPs and PEX had a median recovery time of 12 days. It is, however, not surprising that patients who required both PEX and FFPs had a longer time to recover because PEX is reserved for those with resistant disease. In comparison to the study by Masoet et al., the above time to remission was shorter (2 days in the FFPs only group, and 4.5 days in the PEX and FFPs group)(14). These differences are likely due to differences in treatment protocols. In the Masoet study, PEX was only available every second day whereas in our centre PEX is given daily except on weekends where large volume FFPs are administered. Additionally, our centre uses larger plasma volumes of FFPs (30mL/kg/day) compared to 20mL/kg/day in the study by Swart et al.(19), but there has not been a South African study that compares 30mlL/kg/day to 20mL/kg/day of plasma infusions. Such a study would be useful in preventing over and underdosing of plasma infusions.

Neurological dysfunction was significantly associated with remission status. It was more common in those who did not achieve remission (100%) compared to those who achieved remission (84%). Similarly, Swart et al. reported impaired consciousness as a significant predictor of mortality compared to remission(19). Our study did not differentiate the different types of neurological dysfunctions as done in the Swart study. This may have impacted our findings as patients with mild delirium would be expected to recover much quicker than patients who have had a stroke or ongoing seizures. Further study in our centre would be required where neurological dysfunction is further defined and more closely assessed.

During the study period, 45 (37.8%) patients with HIV-associated TTP demised and 2 (1.7%) were presumed dead based on laboratory results at a subsequent admission at another health care facility. These results are comparable to the study by Masoet et al. who reported a mortality of 43.9%. In this study, all patients received PI only unless the patients had refractory disease and was given PEX on alternate days(14). The majority (81.5%) of those who died during the first admission, succumbed to severe disease and this is expected as TTP is a disease with high mortality which remains at 20% despite adequate treatment(3, 26). Due to the differences in treatment protocols across SA, the comparison of treatment outcomes at different centres is difficult.

The limitations of this study included its retrospective study design, which resulted in some of the clinical information being lost. Additionally,ADAMTS-13 levels were not routinely requested for all patients. Pregnant patients with thrombocytopenia and fragmentation haemolysis due to pregnancy-related hypertensive disorders were investigated and followed for clinical resolution to exclude TTP. However, some of these received high-doses of FFPs during the first few days of admission, which may have obscured TTP-related disease. On the other hand, TTP may have been over diagnosed especially

in the group of patients who had features suggestive of a TMA but were not reviewed by Clinical Haematology to confirm a diagnosis of TTP. Due to the manner that our data was collected, it's unclear how severity of TTP was diagnosed i.e., neurological dysfunction versus renal dysfunction versus thrombocytopenia.

SUGGECTED FUTURE STUDIES

A prospective multicentre study on TTP in South Africa would help standardize the treatment protocols across South Africa. Neurological dysfunction was a marker for severe disease in this study but was not further defined and assessed. Therefore, further studies in this regard may be of value to identify patients who require escalation of treatment at diagnosis.

CONCLUSION

To date this is the largest study of HIV-associated TTP patients conducted in SA during the ART era. Even in the modern era of medicine, TTP regardless of patient HIV status, has a high mortality. A contributing factor to mortality is the limited access to FFPs which are only available at tertiary centres in SA, leading to a delay in the initiation of treatment. ART remains the most important therapy in preventing TTP and other AIDS defining conditions. The ART program in SA still needs to improve on the counselling at ART initiation as a significant proportion of our patients relapsed after defaulting on ART. Even though HIV-associated TTP prevalence remained unchanged in our study, the mortality from TTP has improved. Hospital stay can be shortened by prompt diagnosis and commencement of FFPs.

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COMPETING INTERESTS

None

AUTHOR CONTRIBUTIONS

Prof Estelle Verburgh conceptualized the study. Prof Verburg and Dr Jenique Bailly supervised the study. Dr Nokubonga Vundla collected the data and wrote the manuscript. Jenna Oosthuizen and Karryn Brown assisted with data management, conducted the data analysis, and edited the manuscript. Prof Verburgh and Dr Bailly edited the manuscript and reviewed and corrected the final version.

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DATA AVAILABILITY STATEMENT

Data from the study is available from the corresponding author on request.

DISCLAIMER

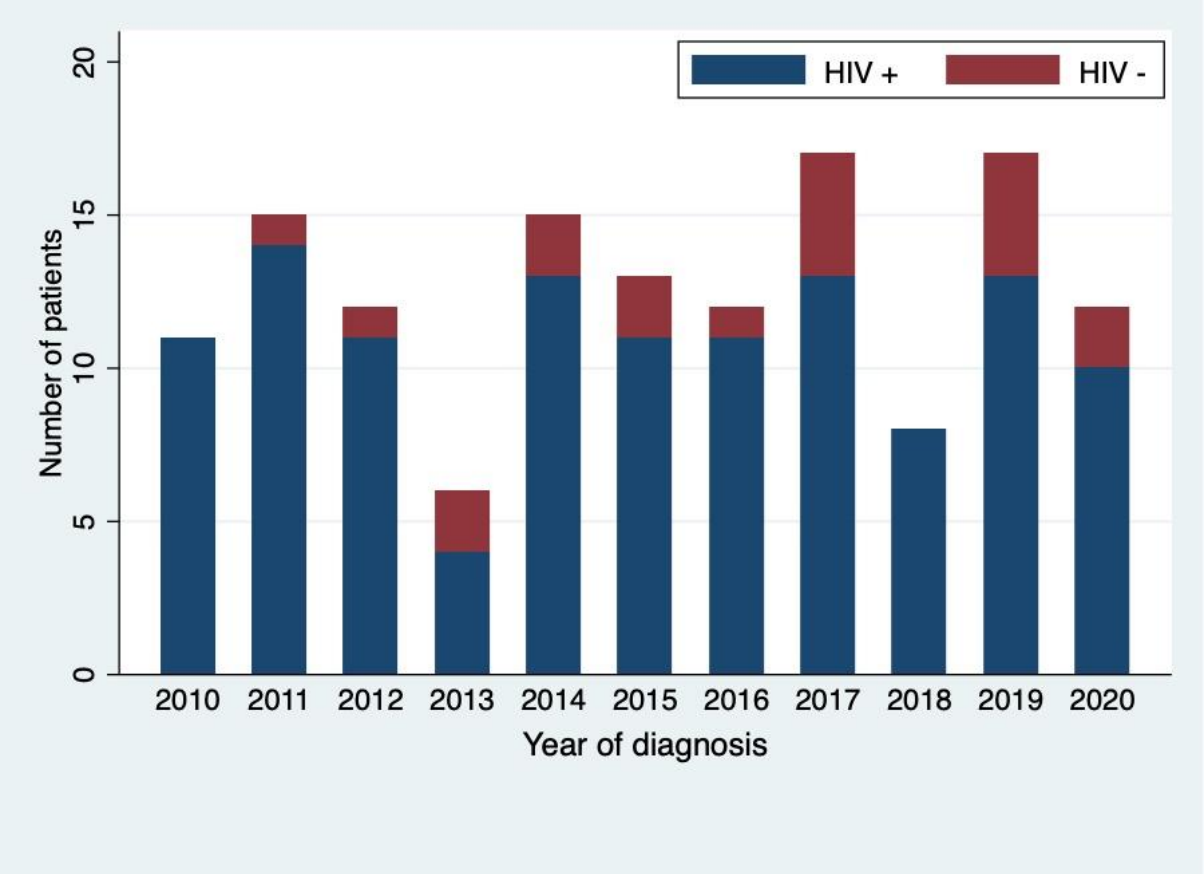
The views expressed in the submitted article are the authors own and not an official position of the institution or funder.

REFERENCES

1. Sukumar S, Lammle B, Cataland SR. Thrombotic Thrombocytopenic Purpura: Pathophysiology, Diagnosis, and Management. J Clin Med. 2021;10(3).
2. Moschcowitz E. An acute febrile pleiochromic anemia with hyaline thrombosis of the terminal arterioles and capillaries: an undescribed disease. Arch Intern Med (Chic). 1925(36(1):89–93).
3. Gunther K, Garizio D, Dhlamini B. The pathogenesis of HIV-related thrombotic thrombocytopaenic purpura – is it different? ISBT Science Series. 2006;1(1):246-50.
4. Louw S, Gounden R, Mayne ES. Thrombotic thrombocytopenic purpura (TTP)-like syndrome in the HIV era. Thromb J. 2018;16:35.
5. Meiring M, Webb M, Goedhals D, Louw V. HIV-associated Thrombotic Thrombocytopenic Purpura–what we know so far. Eur Oncol Haematol. 2012;8(2):89-91.
6. Sadler JE. What's new in the diagnosis and pathophysiology of thrombotic thrombocytopenic purpura. Hematology. 2015;2015(1):631-6.
7. Park YA, Hay SN, Brecher ME. ADAMTS13 activity levels in patients with human immunodeficiency virus-associated thrombotic microangiopathy and profound CD4 deficiency. Journal of Clinical Apheresis. 2009;24(1):32-6.
8. Moake J, Chintagumpala M, Turner N, McPherson P, Nolasco L, Steuber C, et al. Solvent/detergent-treated plasma suppresses shear-induced platelet aggregation and prevents episodes of thrombotic thrombocytopenic purpura. Blood. 1994;84(2):490-7.
9. van Marle AC, Joubert J, Meiring SM. Comparison of ADAMTS13 and Von Willebrand factor levels and activities, and plasminogen levels, in plasma products currently available for the treatment of thrombotic thrombocytopenic purpura in South Africa. Transfus Apher Sci. 2019;58(1):72-8.
10. Gunther K, Garizio D, Nesara P. ADAMTS13 activity and the presence of acquired inhibitors in human immunodeficiency virus-related thrombotic thrombocytopenic purpura. Transfusion. 2007;47(9):1710-6.
11. Magnette A, Chatelain M, Chatelain B, Ten Cate H, Mullier F. Pre-analytical issues in the haemostasis laboratory: guidance for the clinical laboratories. Thromb J. 2016;14:49.
12. van Marle AC, Joubert J, Meiring SM. Comparison of ADAMTS13 and Von Willebrand factor levels and activities, and plasminogen levels, in plasma products currently available for the treatment of thrombotic thrombocytopenic purpura in South Africa. Transfusion and Apheresis Science. 2019;58(1):72-8.
13. Jokela J, Flynn T, Henry K. Thrombotic thrombocytopenic purpura in a human immunodeficiency virus (HIV)-seropositive homosexual man. American Journal of Hematology. 1987;25(3):341-3.
14. Masoet A, Bassa F, Chothia M-Y. HIV-associated thrombotic thrombocytopaenic purpura: A retrospective cohort study during the anti-retroviral therapy era. Journal of Clinical Apheresis. 2019;34(4):399-406.
15. Joly BS, Coppo P, Veyradier A. Thrombotic thrombocytopenic purpura. Blood. 2017;129(21):2836-46.
16. Hart D, Sayer R, Miller R, Edwards S, Kelly A, Baglin T, et al. Human immunodeficiency virus associated thrombotic thrombocytopenic purpura--favourable outcome with plasma exchange and prompt initiation of highly active antiretroviral therapy. Br J Haematol. 2011;153(4):515-9.
17. Johnson LF, Meyer-Rath G, Dorrington RE, Puren A, Seathlodi T, Zuma K, et al. The Effect of HIV Programs in South Africa on National HIV Incidence Trends, 2000–2019. JAIDS Journal of Acquired Immune Deficiency Syndromes. 2022;90(2):115-23.
18. Coppo P, Cuker A, George JN. Thrombotic thrombocytopenic purpura: Toward targeted therapy and precision medicine. Res Pract Thromb Haemost. 2019;3(1):26-37.
19. Swart L, Schapkaitz E, Mahlangu JN. Thrombotic thrombocytopenic purpura: A 5-year tertiary care centre experience. J Clin Apher. 2019;34(1):44-50.

20. Novitzky N, Thomson J, Abrahams L, Du Toit C, McDonald A. Thrombotic thrombocytopenic purpura in patients with retroviral infection is highly responsive to plasma infusion therapy. British Journal of Haematology. 2005;128(3):373-9.
21. Novitzky N, Jacobs P, Rosenstrauch W. The treatment of thrombotic thrombocytopenic purpura: plasma infusion or exchange? British Journal of Haematology. 1994;87(2):317-20.
22. Organization WH. WHO case definitions of HIV for surveillance and revised clinical staging and immunological classification of HIV-related disease in adults and children: World Health Organization; 2007.
23. Mabaso M, Maseko G, Sewpaul R, Naidoo I, Jooste S, Takatshana S, et al. Trends and correlates of HIV prevalence among adolescents in South Africa: evidence from the 2008, 2012 and 2017 South African National HIV Prevalence, Incidence and Behaviour surveys. AIDS Research and Therapy. 2021;18(1):97.
24. Malak S, Wolf M, Millot GA, Mariotte E, Veyradier A, Meynard J-L, et al. Human Immunodeficiency Virus-Associated Thrombotic Microangiopathies: Clinical Characteristics and Outcome According to ADAMTS13 Activity. Scandinavian Journal of Immunology. 2008;68(3):337-44.
25. Louw S, Gounden R, Mayne ES. Thrombotic thrombocytopenic purpura (TTP)-like syndrome in the HIV era. Thrombosis Journal. 2018;16(1):35.
26. Hovinga JAK, Vesely SK, Terrell DR, Lämmle B, George JN. Survival and relapse in patients with thrombotic thrombocytopenic purpura. Blood. 2010;115(8):1500-11.

SUPPLEMENTARY TABLES AND FIGURES



Supplementary Figure 1: Number of TTP cases per year, by HIV status

Supplementary Table 1: Proportions of individuals with features of the classic TTP pentad by remission status.

Features of classic TTP pentad	Remission achieved (n=100)	Remission not achieved (n=39)	P-value
Fever	71 (71.0)	31 (79.5)	0.309
Thrombocytopenia	99 (99.0)	39 (100)	0.531
Haemolytic anaemia	97 (97.0)	39 (100)	0.274
Renal dysfunction	40 (40.0)	22 (56.4)	0.080
Neurologic dysfunction	84 (84.0)	39 (100)	0.008

APPENDICES

Haematology Patient Registry Consent Form

Human Research Ethics Committee Approval

Groote Schuur Hospital Approval