CLINICAL PRESENTATION, MANAGEMENT AND OUTCOME OF THROMBOTIC THROMBOCYTOPENIC PURPURA AT THE CHARLOTTE MAXEKE JOHANNESBURG ACADEMIC HOSPITAL

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DECLARATION

I, LÉANNE SWART, declare that this research report is my own work. It is being submitted for Master of Medicine (in the branch of Haematology) to the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

…….

Léanne Swart

16th day of October 2017 in Johannesburg.

PRESENTATIONS

Léanne Swart, Elise Schapkaitz, Johnny N Mahlangu. Clinical Presentation, Management And Outcome Of Thrombotic Thrombocytopenic Purpura At Charlotte Maxeke Johannesburg Academic Hospital. Poster presented at PathReD 2017 Congress, Emperors Palace, Johannesburg, South Africa, 23-24 June 2017.

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ABSTRACT

This is a retrospective study of the clinical and laboratory features, management, and outcome of 41 adults diagnosed with thrombotic thrombocytopenic purpura (TTP) at the Charlotte Maxeke Johannesburg Academic Hospital.

There were 45 TTP events during the five-year review period, 41 of which met the inclusion criteria. Most study patients were of black ethnicity (95.1%) and female gender (78.1%). TTP was most commonly secondary to human immunodeficiency virus infection (78.0%). Neurological (82.9%) and bleeding (78.1%) symptoms were frequent. Management included initial plasma infusion (78.1%), therapeutic plasma exchange (87.8%), intensive care admission (41.5%), renal dialysis (12.2%), highly active anti-retroviral therapy (78.3%), corticosteroids (61.0%) and other immunosuppressive agents (4.9%). The median (range) number of therapeutic plasma exchanges was 10.0 (7.0-15.0). The relapse rate was low (8.9%), however refractory disease (70.7%) was frequent. Haemoglobin level, platelet count, lactate dehydrogenase, red cell distribution width and creatinine level were reliable therapeutic end-points ($P < 0.05$).

The high mortality rate (29.3%) emphasises the importance of early diagnosis, referral and management of TTP.

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NOMENCLATURE

ADAMTS13: a disintegrin-like and metalloprotease with thrombospondin type 1 motifs

CMJAH: Charlotte Maxeke Johannesburg Academic hospital

DIC: disseminated intravascular coagulopathy

EFS: event free survival

ELISA: enzyme-linked immunosorbent assay

FFP: fresh frozen plasma

FRET: fluorescence resonance energy transfer

HAART: highly active anti-retroviral therapy

HELLP: pregnancy associated syndrome of haemolysis, elevated liver enzyme levels, and low platelet levels

HIV: human immunodeficiency virus

HSCT: haemopoietic stem cell transplantation

HUS: haemolytic uraemic syndrome

ICSH: International Council for Standardisation in Haematology

ICU: intensive care unit

Ig: **i**mmunoglobulin

LDH: lactate dehydrogenase

MAHA: microangiopathic haemolytic anaemia

NHLS: National Health Laboratory Service

RDW: red cell distribution width

SLE: systemic lupus erythematosus

TMA: thrombotic microangiopathy

TPE: therapeutic plasma exchange

TTP: thrombotic thrombocytopenic purpura

ULVWF: ultra large von Willebrand factor

VWF: von Willebrand factor

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1. INTRODUCTION

1.1 Thrombotic thrombocytopenic purpura background

Thrombotic thrombocytopenic purpura (TTP) is a thrombotic microangiopathy (TMA) which was first described in 1924 (1) and became a recognised clinical syndrome in 1947 (2). The first reports of successful treatment of acute TTP with plasma infusion or therapeutic plasma exchange (TPE) are as recent as the late 1970s (1). TTP is a relatively uncommon disorder (3), with an annual prevalence of 10 per million population in the developed world. However when this haematological emergency is left untreated, the mortality approaches 90% (4), even in adequately resourced settings (5). Once TTP is diagnosed, attempts to identify and manage secondary (acquired) causes are made, and if no identifiable cause is found, TTP is presumed to be primary (idiopathic) in nature. Most cases are autoimmune mediated. Congenital TTP is hereditary (non-immune mediated) and exceedingly rare with an estimated prevalence of 1 per million population (5).

In the developing world, the clinical presentation, diagnosis and treatment of TTP is poorly documented with only a handful of case reports or case series published in the literature (3, 6). The incidence of TTP in Africa is currently unknown.

1.2 Definition of thrombotic thrombocytopenic purpura

TTP is a syndrome characterised by haemolytic anaemia, consumptive thrombocytopenia and microvascular thrombosis. Historically, a diagnostic pentad of criteria was recognised as defining TTP. The pentad includes thrombocytopenia, microangiopathic haemolytic anaemia (MAHA), neurological abnormalities, renal failure and fever (3). However, studies have demonstrated that a minority of patients present with the classic pentad (5, 7) and this is often a feature of advanced disease. The association of renal failure, neurological abnormalities and fever is variable (8). Other associated organ dysfunction may include cardiac, gastrointestinal and renal abnormalities. Renal abnormalities include elevated creatinine level, decreased estimated glomerular filtration rate and abnormal urinalysis. Oliguria or renal failure, requiring renal replacement

therapy, is however not typically a feature (9). Presently, a diagnostic triad includes thrombocytopenia, schistocytosis (red cell fragments >1%) and elevated lactate dehydrogenase (LDH) level in the absence of another identifiable cause(s) of these features (8).

1.3 Aetiology and associations of thrombotic thrombocytopenic purpura

TTP can be classified as primary (idiopathic) or secondary (acquired), based on the presence or absence of specific associated clinical conditions. Congenital TTP (Upshaw-Schulman syndrome) is due to hereditary gene mutations (10) of the metalloprotease enzyme, a disintegrin-like and metalloprotease with thrombospondin type 1 motifs (ADAMTS13).

In primary TTP, no underlying association or disease is identifiable and most cases show the presence of severe antibody mediated ADAMTS13 deficiency (11, 12). Circulating neutralising auto-antibodies, referred to as neutralising inhibitors (13), inhibit the proteolytic activity of ADAMTS13. Non-neutralising inhibitors, in the form of immunoglobulin (Ig)G or IgM/IgA isotypes, are thought to cause ADAMTS13 deficiency through antibody mediated clearance (opsonisation) or other unknown mechanisms (13).

TTP may be associated with a variety of clinical conditions, and these are considered secondary (acquired) forms of TTP. The associated conditions include human immunodeficiency virus (HIV) infection, pregnancy, autoimmune disease (e.g. systemic lupus erythematosus [SLE] and scleroderma), malignancy, haemopoietic stem cell transplantation (HSCT) and the use of certain drugs (14). Acquired ADAMTS13 deficiency may result from inhibitory auto-antibodies, usually of IgG subtype (15). However, not all patients with secondary (acquired) TTP have severe ADAMTS13 deficiency (<10% of protease activity). The absence of detectable antibodies suggests other factors play a role in the pathogenesis of secondary TTP, particularly in the setting of HIV-associated TTP, and these are discussed in the next section on the pathogenesis of TTP.

1.3.1 Pathogenesis of thrombotic thrombocytopenic purpura

The metalloprotease, ADAMTS13, is produced in the liver and vascular endothelium and is responsible for cleaving ultra large von Willebrand factor (ULVWF) in plasma (16). The platelet-adhering function of von Willebrand factor (VWF) is dependent on its multimeric size, which is regulated by ADAMTS13 at the VWF A2 domain (10). ADAMTS13 deficiency characteristically causes capillary and arteriolar thrombi when VWF multimers attach to exposed subendothelial connective tissue (17) and aggregate platelets under high shear fluid stress. VWF is both endothelial and megakaryocyte derived, and is stored in endothelial Weibel-Palade bodies and platelet α-granules, respectively (18). Activated endothelial cells secrete substantial amounts of VWF. Identified stimuli that induce endothelial VWF release include infection, pregnancy and trauma.

Thrombocytopenia is the result of excessive platelet adhesion and consumption in microvascular thrombi. Mechanical red cell fragmentation occurs through the partially occluded microvasculature at high shear flow, causing a MAHA (19). High LDH levels are due to a combination of ischaemia and red cell haemolysis (19). Microvascular thrombosis in TTP leads to ischaemia and target organ dysfunction; mainly in brain, kidney, myocardium, adrenal glands, digestive tract and pancreas. Common presenting symptoms include weakness, gastrointestinal symptoms, purpura and transient focal neurological abnormalities (20).

1.3.1.1 Congenital thrombotic thrombocytopenic purpura

Over 150 ADAMTS13 mutations (18) on chromosome 9q34 are known to cause congenital ADAMTS13 deficiency; these mutations affect the biosynthesis, intracellular trafficking and secretion and/or proteolytic activity of ADAMTS13. Bi-allelic mutations are required to cause severe ADAMTS13 deficiency and heterozygous carriers are usually asymptomatic (21). Congenital TTP is however exceedingly rare and accounts for <5% of TTP cases (19). The absence of ADAMTS13 antibody detection, on inhibitor assays, may help distinguish congenital TTP form antibody mediated forms (21). The

diagnosis is confirmed by genetic testing (15). A chronic relapsing course often occurs after an acute episode, which may be precipitated by pregnancy, heavy alcohol consumption (22, 23), vaccination, dehydration or infection (9); as these induce the release of VWF stores from the Weibel-Palade bodies.

1.3.1.2 Idiopathic and acquired thrombotic thrombocytopenic purpura

Both primary (idiopathic) and secondary (acquired) TTP may result from antibody mediated ADAMTS13 deficiency. Autoimmune production of anti-ADAMTS13 antibodies lead to ADAMTS13 deficiency, which results in increased circulating ULVWF multimers (24). It has been proposed that in antibody mediated TTP, the loss of immunological tolerance is elicited by antigens that show molecular mimicry to ADAMTS13 (16). However, other recognised secondary conditions are associated with TTP in the absence of ADAMTS13 deficiency; these include the use certain medications, allogeneic HSCT, advanced malignancy and HIV infection (25).

Pregnancy-associated TTP occurs secondary to a decrease in ADAMTS13 levels with considerable risk of disease relapse during subsequent pregnancies (15). Antibody mediated TTP has been described with the use of drugs such as quinine (15), hormonal therapy (5), ticlodipine (26), interferon (27), simvastatin (5), and trimethoprim (28). However, drug-associated TTP is uncommon and represents <15% of TTP cases (21). Inflammatory-associated conditions contribute to acute episodes in both acquired and hereditary TTP (29, 30). Complement system dysfunction has also been suggested as a contributing factor in the pathogenesis of TTP, in some studies, and therefore the atypical form of haemolytic uraemic syndrome (HUS) may often be difficult to distinguish clinically from TTP (21). Transplant associated microangiopathy occurs after HSCT. Risk factors include chemotherapy, infections, immunosuppressive drugs and graft versus host disease, which mediate endothelial toxicity (15). The absence of ADAMTS13 deficiency is characteristic of transplant associated microangiopathy. In malignancy associated microangiopathies there is no significant decrease in ADAMTS13 levels, however, endothelial damage with increased release of ULVWF multimers is recognised (31). In secondary HIV-associated TTP, chronic inflammation mediates microthrombi via

endothelial injury, activation of the coagulation cascade and VWF multimer release (6). HIV induces cytokine production, namely tumour necrosis factor-α, interleukin -1 and -6 (32), as well as VWF and tissue factor expression (33), which results in endothelial cell dysfunction. The direct effect of HIV appears more relevant than an underlying immune component in the pathophysiology of HIV-associated TTP (34). Therefore, virally mediated endothelial damage is favoured in the pathogenesis (35). Literature reports associations of normal ADAMTS13 levels, TPE resistance and increased mortality in HIV-associated TTP (6, 36-38). The prompt initiation of highly active anti-retroviral therapy (HAART) down-regulates inflammation (39), restores immunological function and resolves an autoimmune component, when present (40).

In South Africa, HIV-associated TTP is typically observed in African females with advanced HIV disease (3). TTP may be the initial presenting feature of HIV infection or occur in those non-compliant with HAART and with low CD4 counts (41, 42). In HIV-associated TTP, ADAMTS13 activity and anti-ADAMTS13 IgG titres do not correlate with clinical severity (5). The heterogeneity of ADAMTS13 activity and inhibitors have been reported in a South African study of HIV-related TTP (6). Since ADAMTS13 defects are not consistently identified in HIV-associated TTP (43), routine testing is not available in many institutions in Southern Africa. Secondary HIV-associated TTP constitutes only a small proportion of TTP in developed countries (9), however, in Southern Africa, TTP is thought to occur largely in association with HIV, likely reflecting the high HIV burden.

1.4 Diagnosis of thrombotic thrombocytopenic purpura

In clinical practice, TTP is suspected when unexplained MAHA, thrombocytopenia and associated organ dysfunction are present. The initial diagnosis of TTP is therefore based on clinical history and examination, together with laboratory findings of a low platelet count and schistocytes (red cell fragments) on the peripheral blood smear. A significant schistocyte count is recognised as ≥ 1% (18), assessed on microscopy (44). A rapid diagnosis of TTP is required to allow for emergency management (10), and attempts are made to exclude conditions associated with secondary TTP.

1.4.1 The role of ADAMTS13 assays and ADAMTS13 genetic analysis

ADAMTS13 assays include ADAMTS13 activity, ADAMTS13 antigen and ADAMTS13 auto-antibody (inhibitor) detection. ADAMTS13 gene sequencing is available for mutational analysis in congenital TTP.

The principle of ADAMTS13 activity measurement in test plasma (45) is a direct or indirect measurement of VWF cleavage (9). Patient plasma is incubated with peptide substrate based on the ADAMTS13 cleavage site in VWF. The residual VWF is measured by electrophoresis, fluorescence resonance energy transfer (FRET) technique, or immunoassay. An ADAMTS13 activity assay level of <10% typically confirms the diagnosis of TTP (16). Normal to moderately reduced ADAMTS13 (10-40% of normal) levels are frequently associated with secondary disease conditions such as HIV infection, chemotherapy, metastatic cancer and HSCT (41). The absence of severe ADAMTS13 deficiency (<10%) may be helpful to distinguish complement driven HUS from TTP, in the appropriate clinical context; in order to rapidly initiate TPE in TTP and eculizamab for HUS (10). HUS is thought to arise from dysregulated alternative complement pathway regulation.

ADAMTS13 inhibitory auto-antibody tests are performed by measuring residual ADAMTS13 activity after incubation with equal amounts of patient and normal pool plasma (13). Non-neutralising antibodies are detected using Western blotting or enzyme-linked immunosorbent assays (ELISA). Newer assays use recombinant ADAMTS13 in a simplified ELISA (Technozyme ADAMTS13 INH kit, Technoclone, Vienna, Austria). Inhibitory assays are most useful in distinguishing congenital TTP from antibody mediated forms (21), and may also guide the use of immunosuppressive agents and determine disease prognosis.

1.4.2 Limitations and variables in ADAMTS13 testing

The ADAMTS13 activity and auto-inhibitory assays have several limitations for emergency testing: The greatest limitation remains the laboratory turnaround time (2),

because of the batch testing at a specialised reference laboratory. Furthermore, testing is not routinely available in diagnostic laboratories in South Africa. Numerous inter-lab and inter-platform variables exist in ADAMTS13 activity measurement. This results in discrepant results between different assays (2), and inhibitor detection is also difficult to standardise (9). The inhibitor assay is non-specific and ADAMTS13 auto-antibodies may also be detected in other immune mediated disorders (e.g. SLE), hypergammaglobinemia (13) and even in a small portion of healthy individuals (9). The ADAMTS13 activity assay is also non-specific and the reported causes of ADAMTS13 deficiency include sepsis, disseminated intravascular coagulopathy (DIC), liver disease and *Plasmodium falciparum* infection (46-48). For therapeutic monitoring, the same ADAMTS13 assay should be used throughout.
Indirect ADAMTS13 activity assays measure protease activity under static conditions and denaturing agents are required to promote the susceptibility of VWF multimers to ADAMTS13 cleavage in the absence of physiological fluid shear.

Thus, for the diagnosis of acute TTP, ADAMTS13 assays are not essential and TPE initiation should not be delayed for this reason (4). The diagnosis of TTP is therefore based on clinical signs and symptoms, surrogate laboratory markers, including thrombocytopenia, red cell fragmentation and elevated LDH levels (8). Other TMAs such as HUS, pregnancy associated syndrome of haemolysis, elevated liver enzyme levels, and low platelet levels (HELLP) and eclampsia, DIC and malignant hypertension are excluded and clotting assays are usually normal in TTP. A prior history of TTP also raises suspicion (9) of disease relapse.

1.5 Management of thrombotic thrombocytopenic purpura

1.5.1 Plasma infusion and therapeutic plasma exchange

The standard of treatment for primary and secondary TTP relies on daily TPE (49) and a rapid diagnosis of TTP is required for emergency management (10). In contrast, congenital TTP may be treated with plasma infusion at a dose of 20-40 mL/kg, and TPE is considered unnecessary (18). The rationale for using TPE is, to replace ADAMTS13

and to remove possible circulating autoantibodies and ULVWF multimers (13), which are responsible for the associated organ dysfunction. The availability of TPE has dramatically improved the survival during acute TTP (50), with reported over-all survival rates of 80-85% (49). Therefore, early clinical and laboratory recognition is of paramount importance. Pregnancy-associated TTP is considered a high-risk, and a multi-disciplinary approach is required as soon as pregnancy is confirmed.

Supportive therapy, such as renal dialysis may be indicated for acute renal failure. In well-resourced settings, the practice is to manage TTP in the intensive care unit (ICU) environment. The prevention of renal and neurological abnormalities is an important goal of TTP therapy in adequately resourced settings (10).

The optimal duration and number of daily TPEs required varies per individual clinical condition (13). Disease recurrence and refractoriness pose other challenges during the management of TTP. These issues raise concern around the cost of TTP management in developing countries.

Plasma infusion and TPE is associated with potentially serious complications and remains expensive (1). TPE is more effective than plasma infusion, as a large amount of plasma can be replaced without causing fluid overload, together with the simultaneous removal of ULVWF multimers (51) and possible circulating antibodies to ADAMTS13 (1). In the attempt to reduce the risk of early relapse, daily TPE should continue for a minimum of two days after achieving the therapeutic endpoints, which define remission (19). Studies have shown that cryosupernatant is at least as effective as fresh frozen plasma (FFP) (52, 53). Cryosupernatant also provides a source of ADAMTS13.

1.5.2 Adjunctive therapy

The use of immunosuppressive corticosteroids as an adjunct to TPE in the context of acquired TTP is rational given that there is an autoimmune pathogenesis (51). A corticosteroid (such as oral prednisone 1mg/kg/day) is started at presentation if an immune component is suspected and is considered when there is a sub-optimal response

or clinical deterioration despite TPE. Superior outcome has not been observed in studies where steroids were given in combination to TPE, versus TPE alone (54). Other immunosuppressive agents such as cyclosporine, azathioprine or cyclophosphamide have been used in the setting of relapsed and refractory disease.

Immunomodulatory therapy with rituximab is recommended as an adjunct in refractory non-HIV associated TTP (55). Rituximab acts by decreasing anti-ADAMTS13 antibodies (51), through peripheral B-cell depletion. A delayed onset of action is therefore expected. Rituximab has reportedly been used to prevent relapses in acquired TTP (56), however further studies are required for the assessment of its efficacy. Rituximab may be administered at a dose of 375mg/m^2 once weekly for 4 consecutive weeks in TTP.

HAART initiation should be expedited in the HIV positive patient and administered immediately after TPE to maximise drug exposure (34).

New agents are in clinical trials. For example, caplacizumab is an anti-VWF nanobody that inhibits VWF-GPIb-IX-V interaction. Phase II studies have demonstrated promise in its use as an adjunct to TPE in the management of acquired TTP (57). Recombinant active ADAMTS13 shows potential of reducing plasma infusion requirements in the congenital form of TTP in clinical trials (58).

1.5.3 Assessment of treatment response

Treatment response is defined by haematological recovery, normalisation of LDH levels and clinical improvement (59). Monitoring of ADAMTS13 activity and/or inhibitor levels are generally not recommended to determine treatment response for acquired TTP, owing to conflicting study results (60, 61). Although high ADAMTS13 inhibitor levels have been described as disease predictors in primary immune mediated TTP, these levels have not been defined (9). Predictors of TTP severity in other studies included the need for intubation and ventilation, older age, neurological features (62), cardiac symptoms and/or raised troponin levels (63). The failure of improvement of routine laboratory parameters, such a platelet count, LDH level and haemoglobin level despite TPE are

indicative of refractory disease, however these parameters have not been found to predict disease severity (9).

TTP recurrence remains high with an expected rate of 20-50%, and most relapses occur within the first two years (13). A single centre study found that ADAMTS13 activity measurement, during the remission of secondary TTP, does not appear to be predictive of relapse (64). In contrast, risk factors for disease relapse in primary immune mediated TTP appear to be ADAMTS13 deficiency (particularly <10%), persistently severe ADAMTS13 deficiency and persistent anti-ADAMTS13 antibodies (9), when assessed during disease remission. Thus, in the absence of a reliable and well-defined marker of TTP disease activity, the assessment of surrogate laboratory markers and clinical features remain important (59).

1.5.4 Complications associated with therapeutic plasma exchange

During the plasma exchange procedure, a filtration device or historically a centrifugation device is used to remove protein bound substances from blood. In TTP, patient plasma is exchanged with FFP or cryosupernatant. A central venous catheter secures venous access and citrate anticoagulant or heparin may be used for line patency and to prevent filter clotting. The most common adverse reactions include, hypocalcaemia related to anticoagulant use; minor allergic reactions/anaphylaxis related to replacement fluids; hypotension related to a low haematocrit prior to plasma exchange, as well as venous catheter dysfunction, line infection and thrombosis (65). Red blood cell transfusions are suggested prior to TPE in patients with TTP, to prevent hypotensive episodes (65), as is the correction of electrolyte imbalances (e.g. hypocalcaemia and hypokalaemia), prior to subsequent TPE.

1.6 Aim of research

The management of TTP in Southern Africa varies with regards to use of plasma infusion or TPE; the respective volumes and frequencies; plasma product and adjunctive therapies, including the use of adjunctive corticosteroids and other immunosuppressive agents.

There are no national guidelines for the management of TTP in South Africa. TTP is usually managed at tertiary/quaternary hospitals where TPE and specialised care is available. Early recognition and referral from primary and secondary health care facilities are cornerstones of management in TTP. Experience with the management of TTP at academic centres in Southern Africa has been published (3, 6, 66). Comparison between studies is difficult owing to the differences in inclusion and exclusion criteria and treatment protocols. Most of these studies were done in the pre-HAART era, with limited or no access to HAART. The more recent experience in which most study patients are routinely initiated on HAART has not yet been described in our clinical academic setting. Whilst anecdotally there is a perception that mortality from TTP has decreased in the recent years, this has not been formally documented. The aim of this retrospective review is to address these questions.

The specific aim of this research study is to review TTP at the Charlotte Maxeke Johannesburg Academic hospital (CMJAH). The specific objectives are:

1. To review the clinical presentation of patients with TTP at the CMJAH.
2. To review the presentation and follow-up laboratory parameters of TTP at the CMJAH.
3. To review the treatment of TTP at the CMJAH.
4. To evaluate the treatment outcomes and overall survival of patients managed for TTP at the CMJAH.

2. PATIENTS AND METHODS

2.1 Study setting

CMJAH is a quaternary care public health facility with approximately 1088 beds, offering comprehensive specialist and sub-specialist medical services including all medical, surgical, paediatric, pathology, obstetrical and gynaecological specialities and sub-specialities. The haematology service includes a 24-bed capacity ward and multiple outpatient facilities in areas 295, 495 and 454 at the CMJAH. Patients with suspected TTP are admitted, diagnosed and treated in ward 595 and may be transferred to the intensive or high care unit at the CMJAH. Most patients admitted to the CMJAH are referred from peripheral primary or secondary healthcare facilities.

2.2 Ethical issues and permissions

This study was approved by the University of the Witwatersrand Human Research Ethics Committee (M160420; see Appendix B). Permission to conduct the study was sought and granted by the Hospital Management, the Head of the Haematology ward and the National Health Laboratory Service (NHLS).

2.3 Study population

The study population included all adult patients with TTP who presented and were managed at the CMJAH from 1st January 2012 to 31st December 2016.

2.3.1 Inclusion criteria

Patients were included in the study if they were 18 years and older and presented with clinical and laboratory features of TTP. The clinical features recorded included neurological signs and symptoms, renal dysfunction and fever. The laboratory features of a microangiopathy were defined by the presence of thrombocytopenia (platelet count of $<100 \times 10^9$/L) and MAHA (in particular, the presence of red cell fragments on the peripheral blood smear and elevated LDH levels).

2.3.2 Exclusion criteria

Patients with other identifiable causes of TMA such as HUS, pregnancy associated syndrome of HELLP and eclampsia, DIC and malignant hypertension were excluded. Patients under the age of 18 years, as well as patients who were managed outside the CMJAH were also excluded.

2.4 Study Design

This is a retrospective uncontrolled hospital record review.

2.5 Data collection

Patients that presented and were treated for TTP at the CMJAH from January 2012 to December 2016 were identified from the ward statistical records in the Department of Haematology. The diagnosis of TTP was confirmed in each patient by reviewing the medical records and by identifying the diagnostic triad or pentad. Data was collected on data collection sheets (Appendix C). The data was anonymised and coded once all relevant information was collected. Data was, as far as possible, collected at three points, namely presentation, relapse and follow-up. At each time point, the clinical, laboratory, management and treatment outcome data was collected.

2.5.1 Clinical parameters

Clinical information was obtained from the inpatient and outpatient files in the Department of Haematology. Clinical data recorded included demographics (age, gender, height, weight and self-identified ethnicity), the presenting clinical signs and symptoms including the presence of neurological dysfunction (focal neurological signs, seizures, headache, altered consciousness and intracranial haemorrhage), fever (temperature >38 °C) and renal dysfunction (elevated serum creatinine level). Other clinical features included symptomatic thrombocytopenia (purpura, bruising, epistaxis, gum bleeding, menorrhagia, haematuria and gastrointestinal bleeding), gastrointestinal manifestations

(nausea and vomiting, abdominal pain and diarrhoea) as well as symptomatic anaemia (fatigue, weakness and shortness of breath).

2.5.2 Laboratory parameters

Laboratory information was obtained from the NHLS laboratory information system (TrakCare®, Intersystems, Massachusetts, USA and/or DisaLab, Laboratory Systems Technologies, Johannesburg, South Africa). The laboratory parameters collected included the full blood count parameters, namely haemoglobin level, platelet count and red cell distribution width (RDW) that were measured using the ADVIA 2120 haematology analysers (Siemens Healthcare Diagnostics, Deerfield, IL, USA). The ADVIA 2120 is an automated haematological analyser based on flow cytometric principles. Red blood cells and platelets are counted and sized based on light scatter. The RDW is calculated from the histogram of the haemoglobin concentration and the cell-by-cell measurement of red cell volume. The measurement of haemoglobin concentration is a modification of the cyanmethaemoglobin method as proposed by the International Council for Standardisation in Haematology (ICSH). The manually reviewed peripheral blood smears were assessed by at least two observers using optical microscopy in accordance with ICSH guidelines (44). In an optimal area of the peripheral blood film, using 50x magnification; at least 1000 red cells were assessed and schistocytes were expressed as a percentage. Biochemical parameters collected namely, LDH, and urea and creatinine levels that were measured using the ADVIA 1800 chemistry analysers (Siemens Healthcare Diagnostics, Deerfield, IL, USA). Haemolytic assays, namely the direct Coombs test was done using a manual method named DC-Screening II (BIORAD Laboratories, California, USA), % reticulocytes were measured using the ADVIA 2120 haematology analysers and haptoglobin level was measured using the ADVIA 1800 chemistry analysers. Clotting factor assays namely, D-dimer, prothrombin time and partial thromboplastin time were done on the STA-R evolution coagulation analysers (Diagnostica Stago, Paris, France). HIV serology was assessed using the ADVIA Centaur analysers (Siemens Healthcare Diagnostics, Deerfield, IL, USA). The absolute CD4 count was measured using the FACS Calibur analysers (Becton Dickinson, San Jose, CA, USA) and the viral load measured using the COBAS 8800 analysers (Roche Molecular

Systems, Branchburg, NJ, USA). The NHLS reference ranges were as follows: haemoglobin, 13.4 - 17.5 g/dL; RDW, 12.1 - 16.3%; platelet count, 171 - 388 x 10^9/L; LDH, 100 – 190 U/L; urea, 2.1 - 7.1 mmol/L; creatinine, 64 - 104 umol/L; international normalised ratio, <1.3; activated partial thromboplastin time, 31.0 - 48.0 seconds; fibrinogen, 2.0 - 4.0 g/L; D-Dimer (quantitative), 0.00 - 0.25 mg/L; absolute CD4, 332 – 1642 cells/uL, HIV viral load, linear range of 20 - 10,000,000 RNA copies/mL; haptoglobin, 0.40 - 2.40 g/L and reticulocyte production index, 1 - 2 is an adequate response, < 1 is an inadequate bone marrow response and > 2 is indicative of haemolysis, recent bleeding or nutritional support.
All these laboratory tests are part of the routine diagnostic workup of TTP. No additional tests were collected from the study patients as part of this research project.

2.5.3 Treatment

The treatment and patient outcomes (last date of follow up) were obtained from the patient records. Treatment at CMJAH mainly included supportive therapy and daily TPE. TPE was initiated at 1.0 volume plasma exchange and continued until remission was achieved (see definition of remission below). In study patients with a suspected diagnosis of TTP, in whom the diagnosis of TTP was delayed, supportive plasma infusion (20 mL/kg) was started until TPE could be initiated, depending on the fluid tolerance of the patient. Platelet transfusions were generally contraindicated due to the risk of thrombosis. During TPE intensification for refractory disease, the TPE exchange volume (with FFP) was increased to 1.5 before the use of cryosupernatant, at a volume of 1.0 plasma exchange was considered. In patients who achieved a desired clinical and laboratory end-point, TPE was thereafter continued for two more days and then stopped without tapering. Close monitoring of the haematological parameters (haemoglobin level and platelet count), LDH level and peripheral blood smear schistocytes were performed daily during the acute episode, and subsequently on an outpatient basis after inpatient discharge.

Therapies adjunctive to TPE included some or all of the following: serologically HIV positive study patients, not yet on anti-retroviral agents, were started on a regimen of

HAART in accordance with the South African National Antiretroviral guidelines (67). Prednisone at a starting dose of 1mg/kg day was reserved for refractory cases and additional immunomodulatory therapy (rituximab at a dose of 375mg /kg/m^2/week x 4 doses) were considered in refractory or relapsing study patients at the treating physician's discretion.

2.5.4 Evaluation of treatment response

The following definitions were used in the study:

Remission: defined as a platelet count of >150 x 10^9/L, serum LDH levels of <450 U/L and the absence of schistocytes on the peripheral blood smear microscopy for two consecutive days in a clinically-well patient.

Relapse: defined as the recurrence of clinical signs and/or symptoms and laboratory features of TTP, which included a platelet count of <150 x 10^9/L, LDH levels of >450 U/L and the presence of schistocytes on the peripheral blood smear microscopy, after 30 days of stable remission.

Refractory TTP: was defined as failure of a platelet count to increase after three consecutive days of TPE or the clinical deterioration despite adequate TPE treatment.

Death/mortality: TTP associated mortality was defined as any death that occurred from the time of diagnosis to within 30 days after completion of TPE.

2.6. Data analysis

Data was captured on the data collection sheets (Appendix C). After data was anonymised and coded, it was transcribed to an Excel® spread sheet (Micorsoft® Office 2010, Redmond, USA) and analysed using Statistica® software (Version 13.2, 2016, Tulsa, Oklahoma, USA). Qualitative data was described. The Shapiro-Wilk test was used to assess the normality of distribution of continuous variables. Continuous parameters which follow a statistically normal (Gaussian) distribution were expressed as means (SD)

and non-parametric parameters were expressed as medians with their respective ranges. Categorical parameters were expressed as frequencies (%). Statistical comparisons were performed using chi-squared and the two-tailed Fischer exact test for categorical variables. For statistical comparisons of continuous variables, the parametric paired t-test or t-test, and non-parametric Wilcoxon Matched Pairs test or Mann-Whitney U test were used depending upon normality between the groups. Statistical significance was set at a *P* value of < 0.05. Event free survival (EFS) was defined from the date of the diagnosis until the date of death or last follow-up. EFS rates were estimated using Kaplan-Meier survival analysis.

3. RESULTS

3.1 Patient characteristics

During the five-year study period, 46 patients were treated for TTP at the CMJAH. Forty-one patients were eligible for inclusion in the study, after elimination of patients who did not meet inclusion criteria (n=3) and patients with incomplete records (n=2). The 41 TTP patients included 32 (78.1%) females and 9 (22.0%) males, with a female to male ratio of 3.5:1. The mean (SD) age was 34.3 years (7.9 years). Most of the study population were of black ethnicity (n=39/41, 95.1%). Forty-five separate clinical events were recorded for these 41 patients, of which 41 (91.1%) were initial disease presentations and four (8.9%) were relapsed episodes. Twenty-seven (60.0%) cases were referred to CMJAH, from peripheral/regional hospitals for the management of TTP, all of which were initial disease presentations.

3.2 Clinical characteristics

Eight of 41 (19.5%) study patients had fever at presentation. The clinical characteristics at initial disease presentation are shown in Table 3.1. Thirty-four (82.9%) patients presented with neurological features. This included new onset focal neurological signs, seizures, headache and/or altered consciousness. Of these, 27 (79.4%) patients returned to baseline on the day of last follow-up. Thirty-two (78.1%) patients presented with bleeding manifestations, of which haematuria was the most commonly observed feature (n=15/32; 46.9%). Intracranial haemorrhage was not documented in any of the study patients. Gastrointestinal manifestations were seen in 13 of 41 (31.7%) patients, and these included nausea, vomiting and/or abdominal pain. All bleeding and gastrointestinal manifestations were resolved by discharge in those patients who achieved remission. Three of 41 (7.3%) patients presented with thrombotic stroke, radiologically confirmed by computerised tomography of the brain.

Table 3.1: Clinical characteristics of TTP patients at initial disease presentation

Clinical manifestation	Number of study patients (N =41)
Neurological manifestations, n (%)	34 (82.9)
Focal neurological signs, n (%)	4 (9.8)
Seizures, n (%)	12 (29.3)
Headache, n (%)	17 (41.5)
Altered consciousness, n (%)	25 (61.0)
Bleeding manifestations, n (%)	32 (78.1)
Haematuria, n (%)	15 (36.6)
Petechiae, n (%)	8 (19.5)
Bruising, n (%)	9 (22.0)
Epistaxis, n (%)	3 (7.3)
Menorrhagia, n (%)	7 (17.1)
Gum bleeding, n (%)	6 (14.6)
Malaena stools, n (%)	9 (9.8)
Haematemesis, n (%)	1 (2.4)
Gastrointestinal manifestations, n (%)	13 (31.7)
Nausea and vomiting, n (%)	12 (29.3)
Abdominal pain, n (%)	2 (4.9)
Thrombotic manifestations, n (%)	3 (7.3)
Cerebrovascular incident, n (%)	3 (7.3)

Some study patients presented with more than 1 clinical sign/symptom and therefore the total % does not add to 100%.

Identifiable secondary associations at initial presentation in the 41 patients included auto-immune pathology in two (4.9%), pregnancy in four (9.8%) and one (2.4%) patient was both HIV positive and pregnant. Thirty-one (75.6%) study patients were HIV positive in the absence of any other identifiable secondary association. Three (7.3%) study patients had no obvious underlying disease association and were classified as having idiopathic (primary) TTP.

3.3 Laboratory characteristics

All forty-one (100%) patients included in this study, presented with a diagnostic triad which included significant red cell fragmentation on the reviewed peripheral blood smear. Only four of 41 (9.8%) patients presented with the classic pentad features of TTP. All (100%) patients in this study presented with anaemia. Of these, 19 of 41 patients (46.3%) reported symptomatic anaemia which included fatigue, weakness and/or shortness of breath. The mean (SD) haemoglobin level at presentation was 6.5 g/dL (1.8 g/dL). The median (range) LDH level was 1664.0 U/L (921.0-2904.0 U/L) and the median (range) platelet count was 15.0 x 10^9/L (range 9.0-22.0 x 10^9/L). The median (range) time to target LDH and platelet values was 9.0 days (8.0-13.0 days). Renal dysfunction (serum creatinine level > 104 μmol/L) was present in 23 of 41 (56.1%) study patients. A median (range) reticulocyte production index of 1.4 (0.7-3.9) was observed. A negative direct Coombs test was confirmed in 16 (39.0%) study patients. The coagulation assay results at initial disease presentation are shown in Table 3.2. All study patients were screened for DIC and none were diagnosed with DIC. The D-dimer was elevated in all study patients with a median (range) level of 4.9 mg/L (1.7-10.9 mg/L).

Table 3.2: The coagulation assay results of TTP patients at initial disease presentation

Laboratory parameter	Result (N =41)
PTT (seconds), median (range)	35.1 (30.5-41.4)
PT (seconds), mean (SD)	16.7 (1.8)
INR, mean (SD)	1.1 (0.1)
D-dimer (mg/L), median (range)	4.9 (1.7-10.9)

PTT, partial thromboplastin time; PT, prothrombin time; SD, standard deviation; INR, international normalised ratio.

Among the 32 (78.0%) study patients confirmed to be HIV positive, the median (range) absolute CD4 count was 137.0 x 10^6/L (59.0-194.0 x 10^6/L) with a median (range) HIV viral load of 219420.0 viral copies/mL (24782.0-558995.0 viral copies/mL). Only 8 study patients were HIV negative. One patient refused an HIV test and demised later. The

laboratory parameters at initial disease presentation and last day of follow up in the inpatient setting are shown in Table 3.3.

Table 3.3: Laboratory parameters of TTP patients at initial disease presentation and last day of follow up

Laboratory parameter	Result at presentation (N=41)	Result at last day of follow up (N=41)	*P* value
Haemoglobin (g/dL), mean (SD)	6.5 (1.8)	11.0 (2.7)	0.000*
Platelet count (x 10^9/L), median (range)	15.0 (9.0-22.0)	223.0 (28.0-301.0)	0.000#
LDH (U/L), median (range)	1664.0 (921.0-2904.0)	258.0 (220.0-1530.0)	0.001#
RDW (%), median (range)	25.5 (20.2-31.6)	16.9 (14.2-20.8)	0.000#
Serum Creatinine (µmol/L), median (range)	117.0 (83.0-160.0)	79.0 (63.5-167.5)	0.004#

SD, standard deviation; LDH, lactate dehydrogenase; RDW, red cell distribution width.
*Paired t test. #Wilcoxon Matched Pairs test. *P* value <0.05.

Table 3.4: Laboratory parameters of TTP patients at disease relapse

Laboratory parameter	Result (N=4)
Haemoglobin (g/dL), mean (SD)	9.8 (2.7)
Platelet count (x 10^9/L), median (range)	12.0 (11.0-283.0)
LDH (U/L), median (range)	753.0 (604.0-1379.0)

SD, standard deviation; LDH, lactate dehydrogenase.

The diagnostic triad was present in three of four (75.0%) study patients at relapse. One (25%) of the relapsed patients, known with SLE, presented with malaena stools, anaemia and significant red cell fragmentation on the reviewed peripheral blood smear and this was considered to be relapsed disease despite a normal platelet count. The supporting laboratory investigations at disease relapse are shown in Table 3.4. Of the relapse

patients, one (25.0%) had renal dysfunction due to TTP. Of the HIV-positive (n=2/4; 50.0%) relapsed patients, one (50.0%) was not virologically supressed

3.4 Treatment protocol

3.4.1 Supportive therapy

A median (range) of four units (3.0-4.0 units) of FFP at a dose of 20mL/kg were administered in 32 of 41 (78.0%) study patients, as an initial supportive measure until TPE could be initiated. HIV positive patients were transfused a similar amount of FFP compared to HIV negative patients, (*P = 0.237*). All 41 (100%) patients required packed red cell transfusions, during initial disease presentation, with a median (range) of four units (3.0-7.0 units) transfused. None of the study patients received a platelet transfusion. Other supportive care included ICU or high care admission in 17 of 41 (41.5%) patients and renal dialysis in 5 of 41 (12.2%) patients. Due to resource limitations, not all patients with TTP were considered eligible for ICU or high care admission at the CMJAH. Both intensive/high care admission and the initiation of renal dialysis were at the discretion of the consulting intensive care physician or nephrologist at the time, and as permitted by the availability of limited resources. The median (range) hospital stay during the initial TTP episode was 17.0 days (12.5-25.5 days).

3.4.2 Therapeutic plasma exchange

The median (range) time to TPE initiation was one day (1.0-2.0 days) in the 36 of 41 (87.8%) study patients who received TPE. The following reasons for delayed TPE initiation were identified in this study: Twenty-seven (65.9%) initial disease presentations were referred to CMJAH from peripheral/regional hospitals. The transfer and transport logistics contributed to the delay in initiation of TPE. In addition, plasma exchange is performed by the South African National Blood Services apheresis unit, and therefore timing of initiation depends on the availability of equipment, blood products and a skilled nurse to perform the procedure. TPE is initiated once patients are optimised with regards to haemoglobin, calcium and potassium levels, which contributed to further delay in the commencement of TPE.

A plasma exchange volume of 1.0 was carried out in the 36 of 41 (87.8%) initial presentation cases, with a median (range) duration of 10.0 days (7.0-15.0 days) of TPE. TPE was administered once daily at the CMJAH. Once the desired clinical and laboratory end-points defining remission were achieved (which are a platelet count of >150 x 10^9/L, LDH levels of <450 U/L and the absence of schistocytes on the reviewed peripheral blood smear), in a clinically-well patient, TPE was continued for a further two days and then stopped.

3.4.3 Therapies adjunctive to therapeutic plasma exchange

Adjunctive immunosuppressive corticosteroid therapy in the form of oral prednisone or intravenous hydrocortisone or dexamethasone were administered in 25 of 41 (61.0%) patients, at the discretion of the treating physician at the time of admission. Study patients with autoimmune pathologies (n=2/41) were treated with additional immunosuppressive therapies, namely methotrexate and azathioprine for SLE and mixed connective tissue disease, respectively. No patients received rituximab during initial disease presentation or subsequent relapse. Figure 3.1 illustrates HAART in patients with HIV-associated TTP. Twenty-five (78.1%) of the HIV positive patients received HAART.

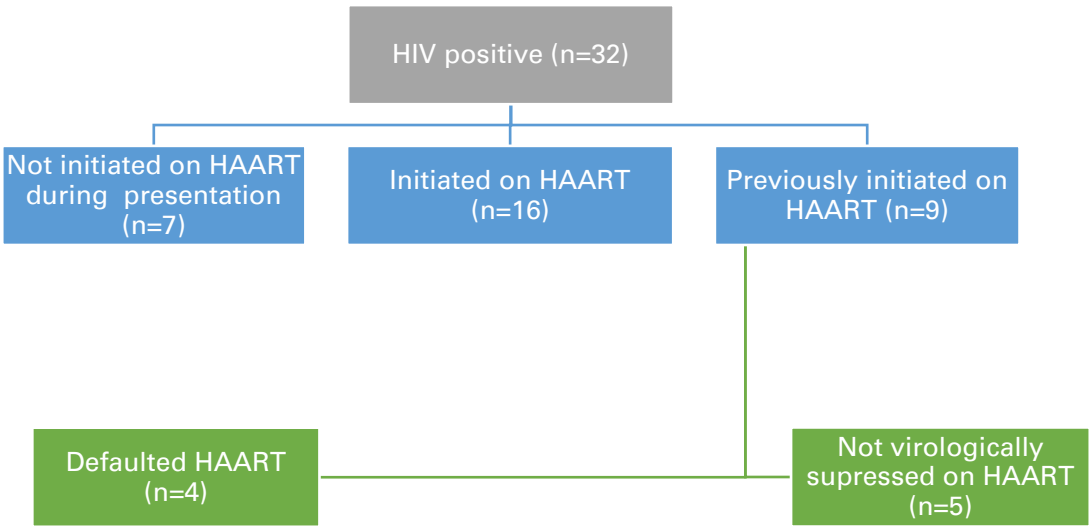

Figure 3.1: HAART in HIV-associated TTP at initial presentation

Anticoagulant therapy was not prophylactically prescribed in the TTP patients. One (2.44%) study patient was anticoagulated following iatrogenic deep vein thrombosis induced by femoral line placement for TPE. This study patient was treated with oral warfarin (5 mg daily) after therapeutic subcutaneous enoxaparin (60 mg 12 hourly) bridging.

3.5 Treatment response

3.5.1 Remission group

After TPE, remission was achieved in 24 of 36 (66.7%) study patients. Figure 2 illustrates the median survival time of 516 days on Kaplan-Meier survival analysis.

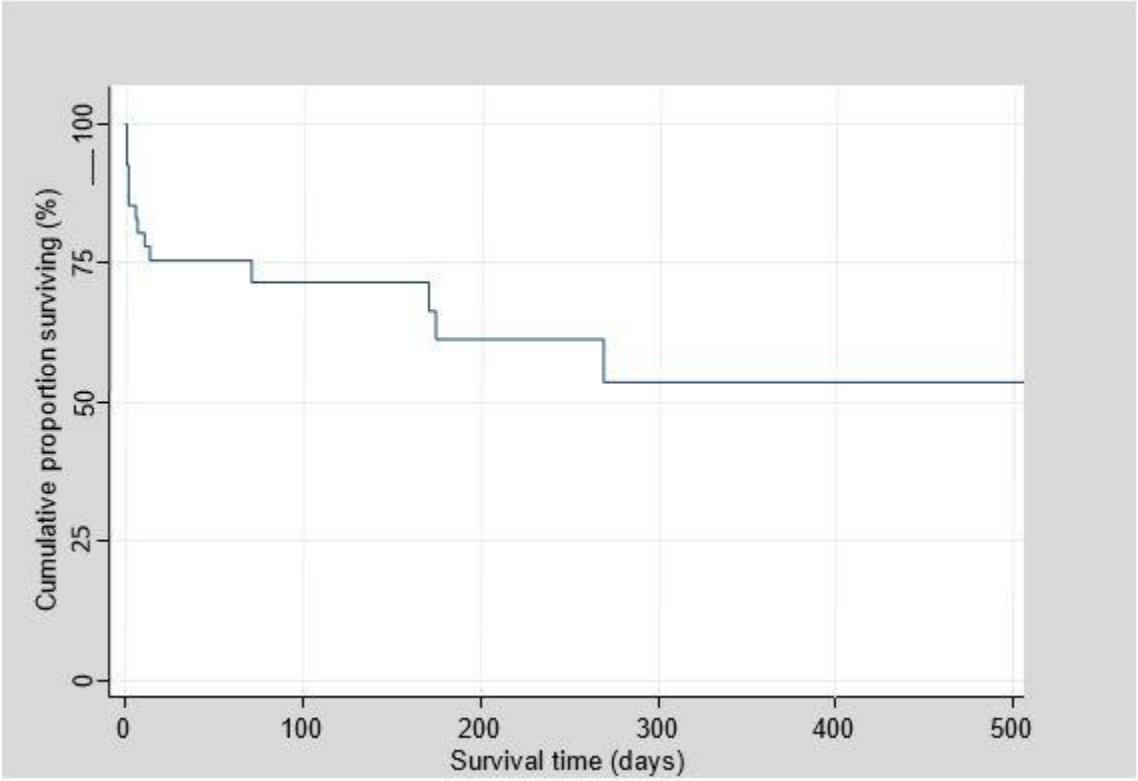

Figure 3.2: Kaplan-Meier graph of survival estimation in 41 TTP patients

Twenty (83.3%) study patients presented for follow-up at CMJAH haematology outpatient department. None of these study patients had features of the TTP pentad or the diagnostic triad on the last follow up day. Episodic headache was reported in 7 of 20

(35.0%) patients, and this was considered non-specific in origin. These 20 patients were in stable remission and did not require further TPE or other supportive inpatient therapies such as renal dialysis or blood product transfusion. Oral prednisone was continued in 10 of 20 (50.0%) patients with dose tapering. One (5.0%) patient continued therapy with both oral prednisone and azathioprine as part of the management of mixed connective tissue disease. Figure 3.3 illustrates the underlying associations in TTP patients who achieved complete remission. Most HIV positive patients (n=14/15, 93.3%) were on HAART. The remaining five of 20 (25.0%) patients were HIV negative of which only one of 20 (5%) was idiopathic TTP.

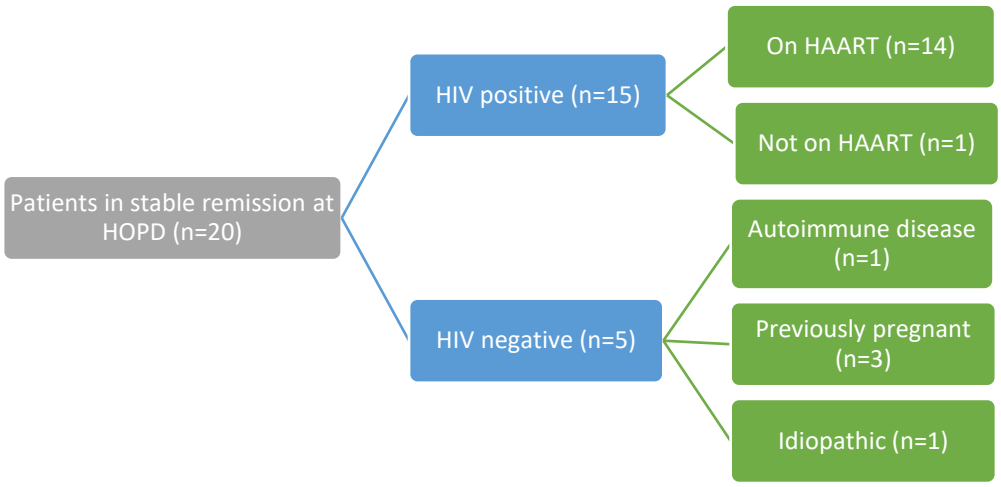

Figure 3.3: The secondary associations in TTP patients who achieved complete remission

3.5.2 Disease relapse

During the study period, four of 41 (9.8%) study patients had a single relapse at a mean (SD) of 98.8 days (107.68 days), after achieving stable remission. All relapses (n=4) occurred within the first year of initial disease presentation. The median (range) admission duration for TTP relapse was 19.0 days (18.0-32.0 days).

Headache was the only presenting symptom for three of four (75.0%) study patients and one of four (25.0%) study patients had laboratory features of TTP relapse in the absence of clinical symptoms. Identified secondary disease associations included HIV (n=2/4;

50.0%) and auto-immune pathology (n=2/4; 50.0%), namely as SLE and mixed connective tissue disease.

Supportive FFP was transfused in one of four (25.0%) in the relapse group before TPE could be initiated. The median (range) time to TPE initiation, in this group, was 0.0 days (0.0-4.0 days) and TPE was initiated with FFP at a plasma exchange volume of 1.0. A median (range) of 11.5 daily (4.0-16.0 daily) TPEs were administered in this group. Remission was achieved in two of four (50.0%) relapsed patients, with a follow-up of 324 and 297 days respectively.
Four (100%) were eligible for intensive/high care admission at the discretion of the treating physician at the time. None received renal dialysis for TTP. Adjunctive therapies included corticosteroids in three of four (75.0%) relapsed cases and HAART in two of four (50.0%) relapsed cases. Oral prednisone together with azathioprine and chloroquine was given to one (25.0%) study patient as part of the management of mixed connective tissue disease. None were treated with rituximab during disease relapse.

3.5.3 Refractory episodes

As per study definition, there were 29 refractory TTP events which comprised 26 of 41 (89.7%) initial disease presentations and three of four (10.3%) relapsed presentations. Fourteen of 29 (48.3%) patients in this group were referred from peripheral/regional hospitals at initial presentation. Temporary supportive FFP was transfused in 79.3% (n=23/29) of all refractory TTP events. The median (range) time to TPE initiation in refractory TTP was one day (range 1.0-2.0 days) at initial disease presentation. Of the relapsed disease presentations, all (n=3) were also refractory at initial disease presentation. A comparison of the initial disease presentation (clinical, laboratory and treatment) characteristics in the TTP refractory and non-refractory groups are shown in Tables 3.5, 3.6 and 3.7 respectively.

Table 3.5: A comparison of clinical characteristics at initial disease presentation in the TTP refractory and non-refractory groups

Clinical parameter at initial disease presentation	Result in refractory cases (N=26)	Result in non-refractory cases (N=11)	*P* value
Neurological features, n (%)	21 (80.8)	8 (72.7)	0.650**
Bleeding symptoms, n (%)	22 (84.6)	8 (72.7)	1.000*

* Chi-squared test. ** Two tailed Fischer exact test. *P* value <0.05.

Table 3.6: A comparison of laboratory parameters at initial disease presentation in the TTP refractory and non-refractory groups

Laboratory parameter at initial disease presentation	Result in refractory cases (N=26)	Result in non-refractory cases (N=11)	*P* value
HIV reactive, n (%)	20 (76.9)	8 (72.7)	0.678**
Renal dysfunction, n (%)	15 (57.7)	4 (36.4)	0.281**
Haemoglobin (g/dL), mean (SD)	6.6 (1.6)	6.5 (2.4)	0.092***
Platelet count (x 10^9/L), median (range)	15.5 (9.0-26.0)	12.0 (21.7-32.6)	0.308#
LDH (U/L), median (range)	1791.0 (1179.0-2745.0)	1190.0 (910.0-3801.0)	0.687#
RDW (%), median (range)	27.5 (20.1-31.9)	24.6 (21.7-32.6)	0.866#

HIV, human immunodeficiency virus; SD, standard deviation; LDH, lactate dehydrogenase; RDW, red cell distribution width. **Two tailed Fischer exact test. *** Parametric t test. # Mann-Whitney U test. *P* value <0.05.

Table 3.7: A comparison of treatment characteristics at initial disease presentation in the TTP refractory and non-refractory groups

Treatment characteristics at initial disease presentation	**Result in refractory cases (N=26)**	**Result in non-refractory cases (N=11)**	***P* value**
Supportive FFP, n (%)	19 (73.1)	9 (81.8)	0.593**
Cryoprecipitate use for TPE, n (%)	7 (26.9)	0 (0.0)	0.756**

FFP, Fresh frozen plasma; TPE, therapeutic plasma exchange. ** Two tailed Fischer exact test. *P* value <0.05.

TPE was intensified to a plasma exchange volume of 1.5 with continued use of FFP in all refractory episodes (n=29) prior to considering cryosupernatant as exchange fluid. Cryosupernatant at an exchange volume of 1.0 was subsequently used in nine of 29 (31.0%) refractory episodes; two (6.9%) of which were relapsed episodes and seven (24.1%) of which were initial presentation episodes. Corticosteroid therapy was administered in 18 of 29 (62.1%) refractory episodes. In addition, all (n=3) relapsed cases were also treated with oral prednisone, at initial disease presentation, however none received other adjunctive immunosuppressants or rituximab. Azathioprine was administered in two of 29 (6.9%) refractory cases and none received rituximab therapy. All adjunctive therapies were initiated at the discretion of the treating physician at the time.

Figure 3.4 illustrates the secondary associations in TTP refractory cases. The major secondary association was HIV infection (n=22, 75.9%) in all refractory episodes. Refractory episodes were associated with mortality in nine of 26 (34.6%) initial disease presentation episodes and two of three (66.7%) relapsed episodes.

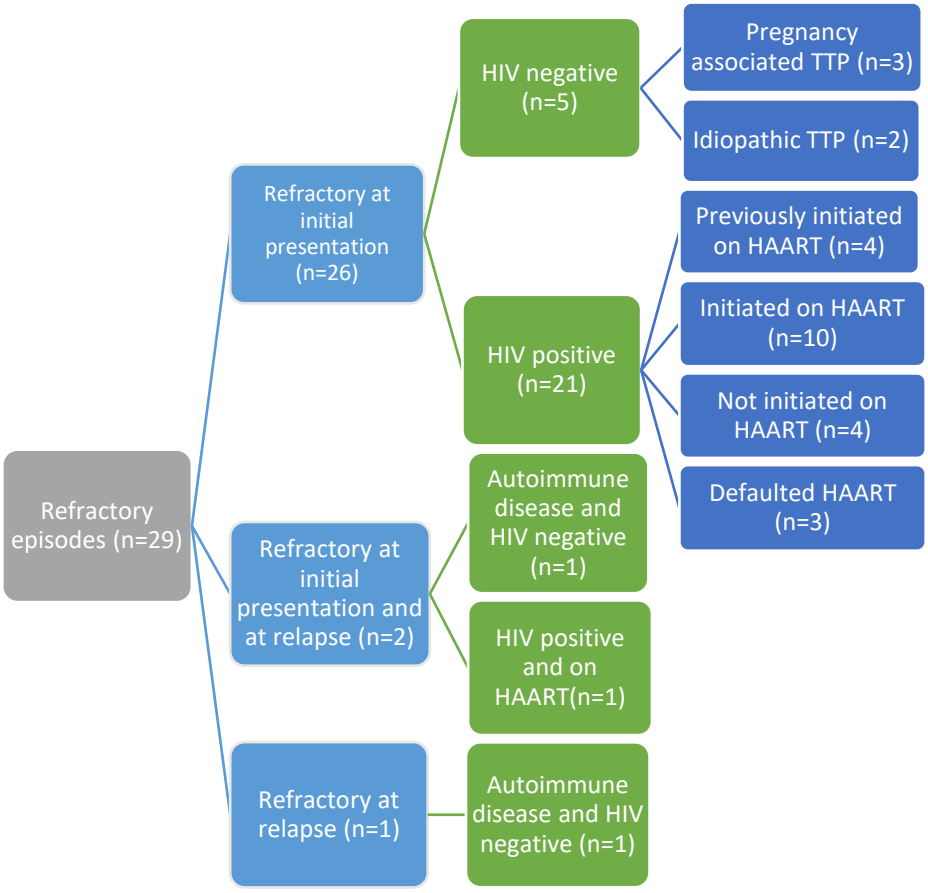

Figure 3.4: The secondary associations in TTP refractory cases

3.5.4 Mortality

3.5.4.1 Thrombotic thrombocytopenic purpura related mortality

TTP related mortality was observed in 12 of 41 (29.3%) study patients during inpatient management. Of these, 11 (91.7%) deaths occurred during initial disease presentation and one (8.3%) death during disease relapse. In the mortality group, seven of the 12 (58.3%) patients were referred to CMJAH for TTP management from peripheral/regional hospitals. The median (range) time to TPE initiation was 2.0 days (1.0-2.0 days), and death occurred at a median (range) of 2.0 days (1.0-9.0 days) after presentation to CMJAH. The most commonly identifiable secondary disease association was HIV infection (n=10/12). One patient was both HIV positive and pregnant. No secondary cause could be identified in one patient prior to death.

Table 3.8: A comparison of clinical characteristics at initial disease presentation

Clinical characteristics at initial disease presentation	Result in remission group (N=24)	Result in mortality group (N=12)	*P* value
Neurological features, n (%)	18 (75.0)	12 (100.0)	0.079**
Impaired consciousness, n (%)	12 (50.0)	11 (91.7)	0.025**

**Fischer-exact test. *P* value <0.05.

Table 3.9: A comparison of laboratory parameters at initial disease presentation in TTP remission and mortality groups

Laboratory parameters at initial disease presentation	Result in remission group (N=24)	Result in mortality group (N=12)	*P* value
HIV reactive, n (%)	18 (75.0)	10 (83.3)	0.200*
CD4 count (x 10^6/L), median (range)	163.5 (118.0-220.0)	50.5 (20.0-138.0)	0.025#
Renal dysfunction, n (%)	11 (45.8)	9 (75.0)	0.157**
Haemoglobin (g/dL), mean (SD)	6.6 (2.0)	6.5 (2.0)	0.936***
Platelet count (x 10^9/L), median (range)	13.0 (7.0-16.0)	25.0 (14.0-31.0)	0.016#
LDH (U/L), median (range)	1523.0 (1099.0-2745.0)	2929.0 (647.0-3075.0)	0.517#
RDW (%), median (range)	27.5 (22.2-33.0)	20.5 (19.0-27.0)	0.022#

HIV, human immunodeficiency virus; SD, standard deviation; LDH, lactate dehydrogenase; RDW, red cell distribution width. *Chi-square. **Fischer-exact test. ***T-test. # Mann-Whitney U test. *P* value <0.05.

A comparison of presenting (clinical, laboratory and treatment) characteristics in remission and mortality groups are shown in Tables 3.8, 3.9 and 3.10 respectively. In this

cohort, neurological features were present in all TTP deaths (n=12) and impaired consciousness was associated with mortality ($P < 0.025$). In the HIV positive patients, the absolute CD4 count was lower ($P < 0.025$) in the mortality group when compared to the remission group. ICU and high care admission in the remission and mortality groups were not statistically different ($P = 0.071$). Refractory disease and the subsequent use of cryosupernatant was statistically different between the mortality and remission groups ($P < 0.001$ and $P < 0.003$ respectively). While refractory disease appeared to be more common in the mortality group, cryosupernatant was less commonly used in this group, which may be due to rapid disease progression before the plasma exchange fluid could be change to cryosupernatant. The median time to TPE initiation was not found to be statistically different in these groups ($P = 0.274$), however a delay in TPE was documented in four of seven study patients who demised after TPE initiation.

Table 3.10: A comparison of treatment characteristics at initial disease presentation in TTP remission and mortality groups

Treatment characteristics	Result in remission group (N=24)	Result in mortality group (N=12)	*P* value
ICU/high care, n (%)	7 (21.7)	8 (66.7)	0.071**
Supportive FFP, n (%)	19 (86.4)	8 (80.0)	0.637**
Time to TPE (days), mean (SD)	1.0 (1.0)	2.0 (1.0)	0.274#
Refractory disease, n (%)	14 (58.5)	8 (67.0)	0.001*
Cryosupernatant use for TPE, n (%)	5 (20.8)	1 (8.3)	0.003*
Adjunctive corticosteroids, n (%)	15 (62.5)	6 (50.0)	0.499**

ICU, intensive care unit; FFP, fresh frozen plasma, RDW; TPE, therapeutic plasma exchange; SD, standard deviation. *Chi-square. **Fischer-exact test. # Mann-Whitney U test. *P* value <0.05.

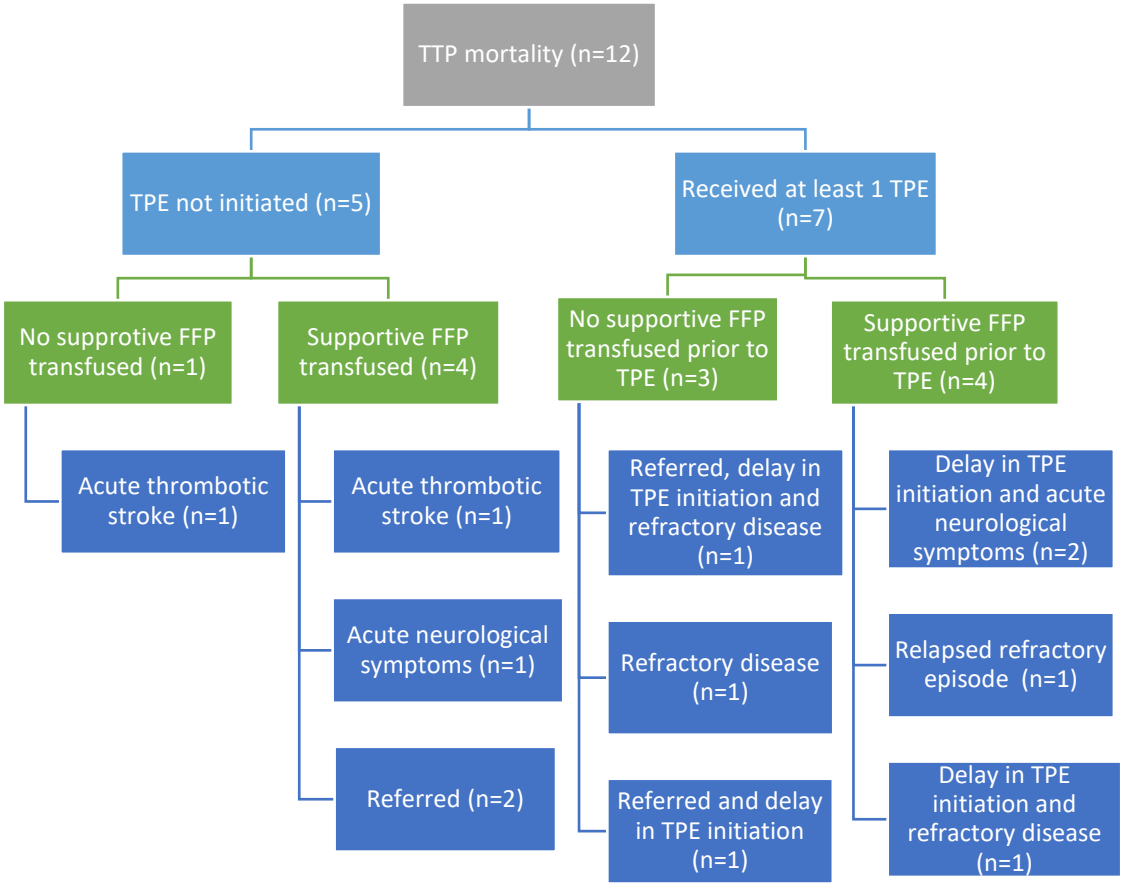

Figure 3.5: The treatment characteristics relating to plasma infusion and TPE in patients with TTP mortality

The treatment characteristics relating to plasma infusion and TPE in patients with TTP mortality are shown in Figure 3.5. Before TPE could be initiated, five of 12 (41.7%) study patients demised. Of these, four of five (80.0%) patients were infused a median (range) of three units (1.0-4.0 units) of supportive FFP. In the same group, two of five (40.0%) patients presented with acute thrombotic stroke, confirmed by computerised tomography of the brain, and this was considered the cause of death. Two of these five (40.0%) patients were referred from a peripheral/regional hospitals to the CMJAH, of which one patient also had delayed haematology consultation. Two of these, five (40.0%) patients also had acute neurological presentation and demised in ICU.

Among the patients who received TPE (n=7) prior to demise, three of seven (42.9%) did not receive supportive FFP infusion. These were associated with a median (range) delay

in TPE initiation of two days (3.0-14.0 days), of which two of three (66.7%) were attributed to delayed referral.

In this study, four of seven (57.1%) patients received both a temporary supportive FFP infusion and TPE prior to demise. Two of these four (28.6%) deaths were associated with refractory disease and two (28.6%) deaths were associated with delayed TPE initiation.

No major adverse reactions to transfused blood products were recorded and therefore no deaths were considered transfusion related. Permission to perform autopsies were not received for the demised patients to confirm TTP as the cause of death.

3.5.4.2 Deaths unrelated to thrombotic thrombocytopenic purpura

After stable remission was achieved, three of 15 (20.0%) patients demised. One patient had stage four HIV disease, was non-adherent to HAART and demised during an admission for chronic gastro-enteritis. Another patient demised of a surgical complication following insertion of a ventricular peritoneal shunt for hydrocephalus. The last patient died >30 days after completion of a second cycle of TPE and death was therefore not considered to be a TTP related mortality. The cause of death was not confirmed by autopsy.

3.5.5 Study patients lost to follow up

A total of six (14.6%) patients were lost to follow-up after discharge. After 15 days of inpatient therapy that included TPE, one (2.4%) patient was unwilling to receive further inpatient management and was discharged against advice. This patient was not yet in stable remission at that time, and never returned for follow-up. Another patient (2.4%) also signed refusal of further hospital treatment and reportedly returned to Mozambique after 10 days of TPE, although two additional days of TPE were advised to reduce the risk of relapse. After complete remission and discharge, four of 41 (9.8%) patients failed to present for outpatient follow-up.

3.5.6 Complications of therapeutic plasma exchange

Hypotension, hypocalcaemia, minor allergic reactions and problems related to central venous line patency, were common problems encountered during TPE in this study. Red cell transfusions were administered, when indicated before TPE as a measure to prevent hypotension. Intravenous calcium was administered in patients who had hypocalcaemia, secondary to anticoagulant use. Allergic reactions were primarily managed with antihistamines and steroids prior to subsequent TPE. One (2.4%) patient had documented right superior femoral vein thrombosis following a right sided femoral venous catheter insertion, which resolved on oral warfarin therapy. One (2.4%) patient was considered to have central venous line sepsis. The femoral line was subsequently removed and the patient was treated with intravenous antibiotics.

4. DISCUSSION

This is a single centre account of the treatment outcome and the clinical and laboratory prognostic factors in a series of 41 study patients diagnosed with TTP at the CMJAH over a five-year study period.

4.1 Patient characteristics

In this study, TTP was observed more commonly in the female gender with a female to male ratio of 3.5:1. The mean (SD) age was 34.3 (7.9) years with a higher incidence of TTP in patients of black ethnicity (95.1%). In other parts of the world, observations of higher TTP incidence in female gender (5, 7, 68-70) and presentation in the third and fourth decades of life (5, 7, 68, 70, 71) were also noted. Other studies of HIV-associated TTP also specifically described skewing towards female gender and black ethnicity in their cohorts (3, 34). The increased TTP frequency in black patients in this cohort may relate to higher plasma VWF levels, even in the absence of ADAMTS13 deficiency (69). However, a more plausible explanation is the predominance of black HIV positive females in this cohort.

4.2 Clinical characteristics

This study concurs with other reports (5, 7, 68, 70, 71) on the neurological presentation of TTP. Neurological signs and symptoms (such as new onset focal neurological signs, seizures, headache and/or altered consciousness) were the most common presenting features (82.9%), followed by signs of bleeding (78.1%). A third of patients presented with nausea, vomiting and/or abdominal pain, which are common gastrointestinal symptoms in general practice. A high clinical index of suspicion is required for the requisition of the appropriate laboratory diagnostic tests. Thrombotic stroke was radiologically confirmed, by computerised tomography of the brain, in 7.3% patients prior to death.

In this cohort, TTP was mostly associated with HIV infection (75.6%). Another South African TTP study also described HIV disease as the most frequent aetiological

association in TTP (6), which reflects the high prevalence of HIV in Southern Africa. In contrast, idiopathic immune TTP with high relapse rates are observed more commonly in other parts of the world (5, 68, 71-73).

4.3 Laboratory characteristics

Before TPE was recognised as the standard of care, a pentad was considered during the diagnosis of TTP. In this study, there were few presentations (9.8%) of the classic TTP pentad. In keeping with other studies (5, 7), the use of this description in the modern treatment era should be discouraged. In contrast, the diagnostic triad (thrombocytopenia, schistocytosis >1% and elevated LDH level in the absence of another identifiable cause(s) for the thrombocytopenia and schistocytosis) were identified in all TTP events in this cohort. ADAMTS13 testing is not routinely available in diagnostic laboratories in South Africa and were not measured in this study. This test is not required for the initial management of TTP. Renal dysfunction featured in 56.1% of the study population, while renal dysfunction was variably observed in other cohorts (5, 67, 68). The high frequency of renal dysfunction may be due to the high frequency of HIV in this cohort, as HIV-associated endothelial dysfunction can lead to platelet deposition in microvasculature of the kidney. HIV-associated nephropathy is common in our population, and could likely have contributed to the considerable acute renal dysfunction in this cohort. The distinction between TTP and HIV-related renal dysfunction could be aided by use of ADAMTS13 testing. As this is however not offered at the CMJAH, it limited optimal distinction between TTP and other TMA such as HUS.

At the CMJAH, monitoring of haemoglobin level, platelet counts, LDH levels and peripheral blood smear schistocyte counts are used as proxies to monitor the effectiveness of TPE therapy. At initial disease presentation and last day of follow up, haemoglobin level, platelet count, LDH level, RDW and serum creatinine showed significant differences ($P < 0.05$), indicating these are reliable markers to determine therapeutic end-points. LDH levels have not been shown to be a useful determinant of treatment duration in all studies. Studies have demonstrated that only LDH iso-enzyme one and two fractions, the principal iso-enzymes in red blood cells, are significant markers of

intravascular haemolysis which accompanies TTP (74). Several authors have also explored the utility of RDW as a monitoring tool. In these studies, the RDW was found to be an excellent monitoring tool for the presence of schistocytes (75), which is consistent with the findings of this study.

In this cohort, TTP was most frequently associated with HIV infection with a low median (range) absolute CD4 count of 137.0 x 10^6/L (59.0-194.0 x 10^6/L) and a high median (range) HIV viral load of 219 420.0 copies/ml (24782.0-558995.0 copies/ml). The reticulocyte production index was lower than expected for haemolysis in this cohort. However, in the context of advanced HIV disease presentation in this study, a blunted bone marrow response secondary to a co-existing bone marrow pathology is a plausible explanation for the lower reticulocyte production index. Given the high background prevalence of HIV in our population, it is not surprising that some patients present with a HIV diagnosis at the same time as a TTP. The causal relationship between HIV and TTP remains to be elucidated. Our results confirmed the results of another South African study, which also reported TTP in advanced HIV disease, and with TTP as the first illness associated with HIV disease in the majority of these patients (3).

4.4 Treatment protocol

While experience with the management of TTP at academic centres in Southern Africa have been published (3, 6, 66), the management of TTP may vary in the absence of standardised national guidelines. These guidelines are important to establish, especially in the light of the high prevalence of chronic infections (HIV, TB etc.) in patients diagnosed with TTP. International guidelines do not have the same pathology as that seen in sub-Saharan Africa.

4.4.1 Supportive therapy

Supportive therapy is important to prevent complications, such as neurological and renal dysfunction. In this cohort, few patients (12.2%) with renal failure received renal dialysis. Further less than half of the patients (41.5%) were managed in the intensive/high care

setting at initial disease presentation. This is a positive and encouraging finding in a resource limited setting.

Plasma infusion was administered in most TTP events as a supportive modality prior to TPE initiation. Plasma infusion alone is considered inferior to TPE (54). However, a South African study demonstrated the importance of plasma infusions when TPE is not easily accessible (3). This is a practice with high yield in resourced constrained countries. HIV positive patients were transfused with similar amounts of supportive FFP compared to HIV negative study patients at initial disease presentation (*P = 0.237*). Currently, there is insufficient evidence to support platelet transfusions in TTP (68, 76) and platelets were not transfused in any of the study patients.

4.4.2 Therapeutic plasma exchange

The introduction of TPE by Bukowski in 1961 has resulted in a decrease in the mortality of TTP to 10-20% (54). TPE is the standard of care at CMJAH. TPE using FFP at a plasma volume of 1.0 was initiated in 87.8% of initial disease presentations. In this study, a median (range) duration of 10.0 daily (7.0-15.0 daily) TPEs were administered at initial disease presentation. After achieving therapeutic end-points, TPE was continued for a further two days and then stopped. The number of treatments reported at other centres is variable: The median (range) number of TPEs were 15.5 (3.0-93.0) in a cohort of 176 study patients with predominantly (77.0%) idiopathic TTP in the United Kingdom, in which clinical and platelet count response was monitored (5). Similarly, the median (range) number of TPEs were 15.5 (1.0-25.0) in a Saudi Arabia cohort of 24 study patients with mostly (54.2%) idiopathic TTP, in which haemoglobin level, LDH level and platelet count response was measured (68). The median (range) number of TPEs were 12.0 (1.0-98.0) in an Australian cohort of 57 study patients with documented ADAMTS13 levels of <10%, in which TPE was weaned after achieving remission (7). In contrast, the median (range) number of TPEs were 3.0 (1.0-9.0) in a cohort of 60 study patients in China with predominantly (83.3%) idiopathic TTP, in which platelet count and clinical response was monitored (71). In a South African study, the median (range)

number of TPEs required for remission was 12.0 (7.0-17.0) in a cohort of 20 patients with HIV-associated TTP (6), which concurs with the findings of this study.

A delay in the initiation of TPE is considered a risk factor for mortality in TTP (77). The median (range) time to initiation of TPE at initial disease presentation was one day (1.0-2.0 days) in this study. The median time to TPE initiation was not found to be statistically different between the remission (n=24) and mortality groups (n=12) (*P = 0.274*), suggesting that this was not a significant contributor to mortality. On further analysis however, a two-day delay in TPE iniation was documented in two of 12 (16.7%) patients who demised after TPE therapy. In this study, possible contributors to a delay in TPE initiation were identified as delayed referral and/or haematology consultation as well as the availability of TPE equipment and skilled operating staff, especially at night.

4.4.3 Adjunctive therapy

Adjunctive therapies such as immunosuppressive corticosteroids are used in immune mediated TTP in view of the underlying pathogenesis. Corticosteroids were administered in the majority (61.0%) of initial TTP presentations, at the discretion of the treating physician at the time. The use of corticosteroids was not significantly associated with a response to treatment in the study patients (*P = 0.499*). The efficacy of corticosteroids in TTP remains unclear, particularly in the absence of ADAMTS13 testing (49). ADAMTS13 inhibitory assays may be beneficial in resource limited settings as a guidance measure to the use of adjunctive immunosuppressive therapies, however this has not been evaluated. Rituximab has been used in acquired autoimmune TTP to prevent relapses (56), however its efficacy is also unclear. In this cohort, no patients were managed with rituximab during initial disease presentation or relapse owing to limited resources and lack of evidence.

TTP was the initial presenting feature of HIV disease in 71.9%. Fifty percent of these HIV positive patients were initiated on HAART during in-hospital stay. Non-compliance was evident in 28.1% of patients on HAART at TTP disease presentation. This highlights the need for early and optimal HAART initiation in HIV positive patients, with continued

adherence monitoring. Furthermore, viral resistance testing may be of value in patients already on HAART, but not virologically supressed at TTP disease presentation (34). Significant changes in the South African national HAART initiation guideline (67) criteria have occurred over this five-year study period and routine fixed HAART drug combinations have more recently been made available. In addition, as of September 2016, the South African national guideline criteria for HAART initiation are no longer CD4 count based (67) and all HIV positive patients are now considered eligible for HAART, unless HAART is contraindicated. The routine use of fixed dose combinations of HAART will hopefully improve adherence and ensure continued HIV viral suppression.

4.5 Treatment response

The median (range) hospital stay during initial disease presentation was 17.0 days (12.5-25.5 days) and similarly, 19.0 days (18.0-32.0 days) at disease relapse. Another study reported a median (range) inpatient stay of 7.0 days (1.0-131.0) days (68). The high burden of refractory TTP disease in this cohort is the probable reason for the longer in-patient stay.

4.5.1 Remission group

Remission was achieved in 66.7% of the study population, with a median survival time of 516 days on Kaplan-Meier analysis. In contrast, previously published results in South Africa reported an overall response of 84.0% (3). Direct comparison is however difficult owing to different diagnostic criteria and treatment protocols. Of the patients who achieved remission, 83.3% presented for follow-up and none had features suggestive of TTP on the last follow up day of this study. Oral prednisone was continued in 24.4% of cases, with dose tapering. On follow-up, 93.3% of the HIV positive patients were virologically suppressed on HAART, suggesting viral control is important during remission. The role of maintenance therapy has not been established in TTP despite the considerable risk of relapse. Future studies will be important to determine the incidence

of HIV-associated TTP in the South African population with the implementation of new national antiretroviral guidelines.

4.5.2 Disease relapse

In this study, the relapse rate was 8.9% at a mean (SD) of 98.8 days (107.68 days) after achieving stable remission. These relapses occurred within the first year following initial disease presentation. This suggests close patient follow-up for the first year is critical to monitor for relapse. Similarly, other studies found that relapses two years after presentation were rare. In contrast, however, these studies documented higher relapse rates (5, 7, 70, 73) with TPE as the standard of care. This may be attributed to differences in aetiological associations; relapses in this study had secondary disease associations rather than primary immune mediated pathology. Relapses in this cohort were specifically associated with secondary HIV infection (50.0%) and secondary to auto-immune disease (50.0%).

Predicting which patients are at risk for relapse is a challenge. Severe ADAMTS13 deficiency has been described as a risk factor for TTP relapse (10). ADAMTS13 levels are normal to moderately reduced (10-40% of normal) in TTP with secondary associations such as HIV (39), suggesting a less pronounced role of ADAMTS13 deficiency in the South African patient population. ADAMTS13 activity was not predicative of outcome in a South-East England cohort of HIV-associated TTP (34).

4.5.3 Refractory episodes

In this study, refractory TTP was defined as failure of the platelet count to increase after three consecutive days of TPE or clinical deterioration despite adequate TPE treatment. Most of the TTP events in this study were refractory (70.7%) of which 89.7% were initial disease presentations and 10.3% were relapse presentations. This study observation is much higher than the reported frequency of 10-42% for refractory cases (4). This may be due to the high observed frequency (75.9%) of HIV infection in the refractory cases of this cohort. Another HIV-associated TTP study concluded that higher presentation HIV

viral loads were significantly associated with increased number of TPE requirements for the achievement of remission (34).

No significant statistical difference was observed during comparison of the presenting clinical, laboratory and treatment characteristics in the refractory and non-refractory groups. However, neurological and bleeding symptoms were seen at higher frequencies in the refractory group.

In this study, the TPE exchange volume was increased to 1.5 with FFP as exchange fluid, and if cases remained refractory, cryosupernatant was then used as the replacement fluid at a plasma exchange volume of 1.0. Oral prednisone (1mg/kg/day) was used in 62.1% of the refractory episodes. In this study, none of the patients received rituximab or alternative immunosuppressive agents for refractory TTP disease. The use of alternative immunosuppressive agents in refractory disease requires some consideration due to side effect profiles. At present, no clear consensus has been reached regarding the management of acute refractory TTP (4). The benefit of rituximab in HIV-associated TTP has been questioned in the absence of a causative antibody mediated pathogenesis. According to the findings of a UK multicentre study, rituximab should be reserved for cases of corticosteroid and HAART resistance, after the elimination of HAART non-adherence (34).

4.5.4 Thrombotic thrombocytopenic purpura related mortality

In this cohort, TTP mortality was 29.3%. Deaths at initial disease presentation were 91.7% and 8.3% at disease relapse. Despite the introduction of TPE, the mortality of TTP remains high. Studies have reported similar mortality rates (71) as well as lower mortality rates ranging from 8.5-16.0% (5, 68, 70, 72, 73) in other cohorts. It is difficult to compare mortality rates between studies owing to differences in diagnostic criteria, definitions of mortality, patient heterogeneity and differences in treatment protocols.

On evaluation of clinical features in this cohort, impaired consciousness at presentation was associated with increased risk of death ($P < 0.025$). Studies have reported an

association between older age, higher LDH levels and lower platelet count in those with TTP mortality (60). Another study found fever to be a predictor of mortality (78). In the mortality group of this cohort, HIV disease was a frequent (83.3%) secondary association. In contrast, a UK study found all TTP deaths to be associated with idiopathic aetiology (5). This highlights the aetiologies in different geographical sites relating to these two cohorts and possible secondary contributors to mortality. In the HIV positive patients, the absolute CD4 count was lower in the TTP mortality group when compared to the remission group ($P < 0.025$), suggesting more advanced HIV disease in those that demised.

It is interesting to note that intensive/high care admission did not influence patient outcomes ($P = 0.071$). The median time to TPE initiation was not found to be statistically different in mortality cases ($P = 0.274$). However, a delay in TPE was noted in 33.3% of the mortalities and referrals for TPE from health care facilities outside of CMJAH was evident in 58.3% of these deaths. This suggests that delayed referral and delayed TPE could have contributed at least in part to mortality.

4.5.5 Complications of plasma exchange

Low complication rates were found in this cohort with only two major complications documented, namely catheter related femoral vein thrombosis and central venous line sepsis. This may represent a reporting bias, as adverse reactions such as hypocalcaemia, minor allergic reactions, hypotension and venous catheter dysfunction occur commonly, but are corrected daily, prior to the next TPE session. There were no major adverse blood transfusion reactions in this study which could have resulted in death. Higher rates of major complications have been reported in other studies (68, 72).

5. LIMITATIONS

There are some limitations in this study which should be considered:

Firstly, the retrospective design of the study. TTP is relatively rare and therefore a retrospective method was chosen to elucidate baseline data. The clinical data of two study patients were incomplete and were therefore excluded from this study. Minor complications such as minor allergic reactions, hypotension and venous catheter dysfunction are corrected daily prior to the next TPE session and may not have always been formally documented as minor adverse reactions.

Secondly, the small sample size of the study population. The sample size was too small for statistical comparisons of study patients at risk for refractory disease, relapse or death. Larger multicentre studies are required in South Africa to determine predictors of outcome. Standardised South African national guidelines are not available for the management of TTP and this may contribute to some treatment differences between different management centres.

Thirdly, the short duration of follow-up and study patients lost to follow-up. The highest risk of relapse is in the first two years from presentation. Although registries with long-term follow-up have shown the risk of relapse is low after four years, this could not be confirmed.

Lastly, ADAMTS13 measurement was not done as part of the diagnostic work-up, while this does not limit the emergency management of TTP, it would have been useful to confirm the diagnosis of TTP. In addition, it could have aided in decisions regarding adjunctive therapies and the management refractory cases. Further, the absence of ADAMTS13 measurement limits distinction between TTP and other TMA such as HUS.

6. CONCLUSION AND RECOMMENDATIONS

TTP is a relatively rare haematological emergency but is associated with high mortality. This necessitates early and optimal specialised care, including TPE and early referral from peripheral referring centres.

The findings of this retrospective review in the era of HAART and TPE therapy may be of significance to clinicians in South Africa with a high burden of HIV infection. In this cohort, TTP was frequently secondary to HIV infection. Associated risk factors included females of black ethnicity, a low absolute CD4 count and high HIV viral load at TTP disease presentation. This contributed to the high mortality rate of 29.3%. Adjunctive HAART and adherence monitoring are essential to TTP management in South Africa. Most patients (60.0%) were referred for specialised management, which contributed to delayed TPE initiation. This emphasises the importance of early disease recognition and treatment initiation. The relapse rate was lower than reported in other studies (8.9%), however the frequency of refractory disease was higher (70.7%). Refractory disease was common in HIV-associated TTP.

The high mortality rate emphasises the importance of early diagnosis, referral and appropriate management in TTP. Identification of patients at risk for refractory disease is a diagnostic challenge. There is a need to evaluate the role of ADAMTS13 activity and inhibitory assays in a South African cohort to refine adjunctive therapy, particularly for patients at risk for refractory disease.

APPENDICES

Appendix A - Plagiarism form

University of the Witwatersrand, Johannesburg

School of Pathology

SENATE PLAGIARISM POLICY

Declaration by Students

I, Leanne Swart (Student number: 0501150H) am a student registered for Haematology in the year 3rd year. I hereby declare the following:

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 ALL the work submitted for assessment for the above course 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 in 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: _____

Date: 16/10/2017

Appendix B - Ethics clearance certificate

R14/49 Dr Leanne Swart

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M160420

NAME: (Principal Investigator)	Dr Leanne Swart
DEPARTMENT:	Molecular Medicine and Haematology Charlotte Maxeke Johannesburg Academic Hospital National Health Laboratory Service
PROJECT TITLE:	Clinical Presentation, Management and Outcome of Thrombotic Thrombocytopenic Purpura at the Charlotte Maxeke Johannesburg Academic Hospital
DATE CONSIDERED:	06/05/2016
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Dr Elise Schapkaitz and Prof JN Mahlangu
APPROVED BY:	Professor P. Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	12/05/2016

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 in Room 10004, 10th floor, Senate House/2nd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand. I/We fully understand the the conditions under which I am/we are authorised 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 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 review in April and will therefore be due in the month of April each year.

Principal Investigator Signature

Date 13/05/16

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix C - Data Collection Sheet

		Presentation:	Relapse:	Last date of follow up:
Hospital number:				
Study number				
Demographic characteristics:				
	Age	Years	Years	Years
	Gender	M F		
	Race/Ethnicity	B W C O		
	Height	m		
	Weight	kg	kg	kg
Clinical characteristics:	GIT sympt Bleeding sympt			
	Neurological dysfunction	Focal signs Intracranial bleed Seizures Impaired consciousness Headache	Focal signs Intracranial bleed Seizures Impaired consciousness Headache	Focal signs Intracranial bleed Seizures Impaired consciousness Headache
	Fever	degrees Celcius	degrees Celcius	degrees Celcius
Secondary cause identified Y/N		Auto-immune dx/Pregnancy/Malignancy/Drugs	Auto-immune dx/Pregnancy/Malignancy/Drugs	Auto-immune dx/Pregnancy/Malignancy/Drugs
Laboratory characteristics:				
FBC		Hb g/dL Plts x 10^9/L RDW %	Hb g/dL Plts x 10^9/L RDW %	Hb g/dL Plts x 10^9/L RDW %
Peripheral blood smear				
LDH		U/L	U/L	U/L
Renal function		Urea mmol/L Creatintine umol/L	Urea mmol/L Creatintine umol/L	Urea mmol/L Creatintine umol/L
Direct coombs		Neg/Pos IgG/C3	Neg/Pos IgG/C3	Neg/Pos IgG/C3
RPI				
Haptoglobin		g/L	g/L	g/L
DIC screen		D-dimer mg/L PT (INR) PTT sec	D-dimer mg/L PT (INR) PTT sec	D-dimer mg/L PT (INR) PTT sec
HIV serology		Reactive Non-reactive	Reactive Non-reactive	Reactive Non-reactive
CD4 count		x 10^6/L	x 10^6/L	x 10^6/L
Viral load		copies/mL	copies/mL	copies/mL
Treatment charactersitics:				
FFP prior to TPE initiation		Y/N Units	Y/N Units	Y/N Units
TPE		Plasma volume Duration FFP Cryosupernatant	Plasma volume Duration FFP Cryosupernatant	Plasma volume Duration FFP Cryosupernatant
Refractory		Y/N	Y/N	Y/N
Renal Dialysis		Y/N	Y/N	Y/N
ICU admission		Y/N	Y/N	Y/N
HAART		Previously Initiated	Previously Initiated	Previously Initiated
Steroids		Y/N	Y/N	Y/N
Rituximab		Y/N	Y/N	Y/N
Treatment response:				
Mortality		Y/N	Y/N	Y/N

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