Presentation and therapeutic outcomes of Thrombotic Microangiopathy in HIV positive and HIV negative patients at Helen Joseph Hospital during the period 2010-2019

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Declaration

I, Yusuf Moola, student number A0036945, hereby state that this research report, entitled: "Presentation and therapeutic outcomes of Thrombotic Microangiopathy in HIV positive and HIV negative patients at Helen Joseph Hospital during the period 2010-2019", is entirely my own work.

The submission of this research report, including the original protocol with extended literature review, is for the Degree of Master of Medicine (MMed), specialty of Internal Medicine, at the University of the Witwatersrand, Johannesburg.

This report has not been previously submitted for any other qualification at any another university.

_____ (Date: 04/05/2023)

Dr Yusuf Moola

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Abstract

Background: Thrombotic microangiopathies (TMAs) are heterogeneous disorders characterized by widespread occlusive microvascular disease, causing thrombocytopenia, microangiopathic haemolytic anaemia (MAHA) and end-organ dysfunction due to ADAMTS-13 deficiency. TMAs are broadly classified into primary and secondary, with HIV being an increasingly common secondary aetiology, specifically in HIV endemic regions, like South Africa.

Methods: 106 records of patients, diagnosed with TMA and treated with plasma exchange (PLEx) from 2010-2019, were assessed at presentation for clinical features of fever, renal and neurological involvement; laboratory parameters including haemoglobin, platelet count, and creatinine levels; treatment required such as blood product support, renal replacement therapy and use of corticosteroids and patient outcomes such as recurrence, mortality and creatinine at termination of PLEx. Further information was gleaned from the NHLS laboratory database to assess patients' response to PLEx.

Results: HIV was the most common aetiology, accounting for 82.2% of TMA. More than two-thirds of the cohort were females of Black-African ethnicity, and the median age at presentation was 36 years. Patients with HIV-associated TMA had lower haemoglobin levels, lower platelet counts, and were older at presentation compared to HIV negative patients. The diagnosis of TMA was typically associated with advanced HIV disease (median CD4 count of 147 cells/mm^3). Irrespective of the TMA aetiology, this study found that a median of 10 PLEx sessions was required to induce remission of TMA, with the crude mortality found to be 16.8% in this cohort.

Conclusion: HIV-associated TMA remains an important cause of secondary TMA in South Africa. It presents more often in Black-African females and in those with advanced stages of HIV. Compared to HIV negative individuals, HIV-infected patients present with lower

haemoglobin and lower platelet counts potentially signifying a more severe form of disease. PLEx remains an integral component in the management of TMA and is crucial to improving survival. While this modality is crucial to eliminating the pathogenic ULvWF multimers and auto-antibodies, the important role of combination antiretroviral therapy (cART) in maintaining remission and preventing relapse of TMA should not be underestimated.

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List of Abbreviations

ADAMTS-13 – A Disintegrin And Metalloprotease with Thrombospondin type-1 motif, member-13

aHUS – atypical Haemolytic uraemic syndrome

aPTT – activated Partial Thromboplastin Time

C3/5/6/7/8/9 – Complement factor 3/5/6/7/8/9

cART- combination Antiretroviral Therapy

CD-4/20 – Cluster of Differentiation-4/20

CMV – Cytomegalovirus

CNI – Calcineurin Inhibitors

CR-1 – Complement Receptor-1

D+HUS – Diarrhoea-associated Haemolytic uraemic syndrome

DAF – Decay Accelerating Factor

DIC – Disseminated Intravascular Coagulation

DL – Decilitre

ESRD – End-stage renal disease

FDA – United States Food and Drug Administration

FFP – Fresh Frozen Plasma

fL- femtolitre

HELLP Syndrome – Haemolysis, Elevated Liver enzymes, Low Platelets Syndrome

HHV-8 – Human Herpes Virus-8

HIV – Human Immunodeficiency Virus

HREC – Human Research Ethics Committee

HUS – Haemolytic uraemic syndrome

IL – Interleukin

INF-γ – Interferon Gamma

INR – International Normalised Ratio

L – Litre

LDH – Lactate dehydrogenase

MAC – Membrane Attack Complex

MAHA – Microangiopathic Haemolytic Anaemia

MCP – membrane cofactor protein

mg – milligram

mTOR – mammalian Target of Rapamycin

NHLS – National Health Laboratory Service

PLEx – Plasma exchange

SANBS – South African National Blood Service

SLE – Systemic Lupus Erythematosus

STEC – Shiga-Toxin producing Escherichia Coli

TMA – Thrombotic Microangiopathy

TNF-α/β – Tumour Necrosis Factor-Alpha/Beta

TTP – Thrombotic Thrombocytopenic Purpura

ULvWF – Ultra-large von Willebrand Factor

VEGF – Vascular Endothelial Growth Factor

vWF – von Willebrand Factor

Section I – Protocol with Extended Literature Review

1. Background

1.1 Introduction

The thrombotic microangiopathies (TMAs) are a group of heterogeneous diseases which are characterized by widespread occlusive microvascular disease, with micro-thrombus formation in arterioles and the capillary network causing endothelial swelling and damage, in turn resulting in thrombocytopenia, microangiopathic haemolytic anaemia (MAHA) and end-organ dysfunction characteristically affecting the kidneys and/or brain.[1] Based on the type of end-organ involvement encountered, TMA has traditionally been distinguished into thrombotic thrombocytopenic purpura (TTP) and haemolytic uraemic syndrome (HUS), both of which have been further characterised as primary or secondary to a variety of underlying pathological processes, the latter including such diverse diseases as malignancy, stem cell transplantation, systemic lupus erythematosus (SLE), and HIV infection.[2] These prototypical forms of TMA, i.e. TTP and HUS, are regarded as an acute haematological emergencies which without rapid intervention carries a poor prognosis. In adults, differentiating between TTP and HUS is impractical given that HUS is more commonly observed in children., and the clinical features of the acute presentation of both entities can be challenging to differentiate [3,4] With regard to the management of TMAs, plasma exchange (PLEx) in particular is known to be efficacious in both TTP and aHUS, and has been shown to improve survival rates from 10% in untreated patients up to 78% in patients receiving this therapy.[5,6] Achievement and maintenance of remission requires, in addition to PLEx, diagnosis and treatment of the underlying predisposing disease, if present.

Anecdotal evidence suggests that HIV infection is an important cause of TMA in the South African context, and recent studies by Swart et al[6] and Masoet et al[7] have helped gain some insight into this disease. However, there still remains a paucity of data concerning the entity of HIV-associated TMA and it requires further study, particularly in the local setting. This study aims to characterize and compare the presentation and therapeutic response of HIV-associated TMA in comparison with that of TMA occurring in HIV-negative patients.

1.2 Clinical Presentation of TTP/TMA

TTP, a form of TMA,[8] has classically been defined by a pentad of clinical features, namely microangiopathic haemolytic anaemia, thrombocytopenia, fever, renal dysfunction, and neurological abnormalities.[1,3,9] However, these features are not consistently observed in all patients that present with the disorder. Rogers et al reported profound thrombocytopenia being present in 70-100% of patients; microangiopathic haemolytic anaemia in 70-100%; renal dysfunction in approximately 50%; neurological abnormalities in 50-90%; and fever in only 25% of patients diagnosed with TTP.[9] It has been reported by other researchers that only 5% of patients presenting with TTP manifest with the complete pentad of clinical features.[6] As a result, the current diagnostic paradigm thus incorporates just two features: microangiopathic haemolytic anaemia and thrombocytopenia, without any other identifiable aetiology.[1,9]

1.2.1 HIV-associated TMA

TMA is well recognised and known to occur more often in the setting of HIV. It is characteristically associated with advanced infection; HIV-associated TMA is therefore considered as an AIDS-defining condition.[10] Few authors have suggested that the term HIV-associated TMA be utilised so as to differentiate it from idiopathic TTP,[4,8,11] and

this is the term we have employed in this report. HIV is thought to carry an estimated 15-40-fold increased risk of developing TMA.[12] The precise pathophysiology of HIV-associated TMA is not yet completely understood.[7] It seems to occur less often in patients who have already been initiated on combination antiretroviral therapy (cART), but remains an important cause of TMA, despite the increased access to cART in South Africa. More than 80% of TMA cases are associated with HIV in South Africa, and the incidence of HIV-associated TMA is higher than that of congenital forms of TTP.[13] In the context of HIV, TMA is seen more frequently in patients with advanced illness, lower CD4 T-lymphocyte counts, and in patients having other co-morbid illnesses or infections.[12] In another study that was conducted by Novitsky et al, presentation with TMA was the initial feature of newly diagnosed patients with HIV, and this accounted for 78% of study subjects.[14]

HIV is thought to act as a trigger of TMA, in part through the infection of cells of the vascular endothelium.[13,14] It is further postulated that the chronic inflammatory process of the virus directly leads to the production of microvascular thrombi as a consequence of direct endothelial injury and activation of the coagulation cascade, with excess thrombin generation resulting in consumption of the ADAMTS-13 (“A Disintegrin And Metalloprotease with a Thrombospondin type-1 motif, member 13”) protease.[14] The depletion of ADAMTS-13 ultimately leads to the pathological processes that unfolds in TMA. According to some authors, HIV-associated TMA is thought to be more responsive to plasma infusion regimens compared to the HIV-negative subset of TMA patients.[7,13] The absence of a highly sensitive and specific marker of HIV-associated TMA, which could assist with the prompt diagnosis and initiation of appropriate therapy

is a significant impediment, since other forms of TMA cannot be excluded with certainty.[12]

1.2.2 Haemolytic-Uraemic Syndrome

HUS is another form of TMA that is clinically defined by non-immune haemolytic anaemia, thrombocytopenia and acute kidney injury. It is a heterogenous range of haemolytic conditions within TMA which are typified by extensive destruction of endothelial cells. Toxins released by bacteria, systemic disorders, drugs, and dysregulation of the complement cascade are the main causes of HUS.[15] It is generally classified into two categories.[15,16] The first category is typical or diarrhoea-associated HUS (D+HUS), which occurs secondary to infection with "Shiga-toxin producing Escherichia coli (STEC)" in the gastrointestinal tract, and which globally accounts for the majority of cases of HUS.[15,16] The second category is "atypical HUS" (aHUS), which accounts for only 10% of cases but carries a poorer prognosis with a 25% mortality rate; and from surviving patients, approximately 50% develop end-stage renal disease (ESRD).[15] Inherited and sporadic cases of aHUS are linked to genetic aberrations or acquired quantitative or functional deficiencies of proteins in the regulation of the complement cascade.[15]

Typical HUS is the most serious and deadly consequence of STEC infection. The usual source of infection is via contaminated water and/or food from asymptomatic cattle. Secondary infection from individual-to-individual also occurs. Typical HUS is more frequently seen in infants and children, where it accounts for 5-15% of cases.[16] The risk of mortality or the development of ESRD is approximately 12%, with long-term renal consequences noted in a further 25% of survivors.[15,16] No specific treatment for typical HUS is available, and management remains largely supportive towards the

vascular, gastrointestinal, renal and haematologic symptoms.[16] The use of PLEx for typical HUS as a first-line therapy remains contentious, with randomised trials not showing great success.[15]

HUS and TTP share a mutual pathological lesion (that of TMA), but have dissimilar clinical presentations.[15] However, many authors still discuss aHUS alongside TTP. The clinical symptoms and lesions in aHUS are predominantly localised to the kidney, while the pathological lesions in TTP tend to be far more widespread.[15] aHUS is much less common than typical HUS, accounting for only 5-10% of cases, and is linked to abnormalities of the complement cascade and is associated with poorer outcomes. The disease may have an inherited or sporadic pattern, might be acquired or develop as a result of genetic aberrations.[15,17] The trigger for an episode of aHUS is commonly an infection in the form of a respiratory tract infection or gastroenteritis, rarely entero-haemorrhagic diarrhoea. The disorder may feature an insidious onset with relapse episodes being common, and disease in relatives is common[17]. In adults, the diagnosis of aHUS is made more challenging as adults may present with HUS secondary to pregnancy, drugs, or transplantation or immune-mediated TTP, which may be attributed to the presence of other autoimmune disorders.[15]

The link between aHUS and abnormalities in complement cascade regulation is well recognised. There are three pathways that are described that can trigger initiation of the complement cascade, these being the classical, alternative and lectin pathways[15,17]. In plasma, the alternative pathway is continuously active and effects trivial assaults on all substances that interact within bodily fluids, including plasma. This attack is quickly brought under control and there is no resultant cellular damage in normal conditions. Therefore, strict control and regulation of the alternative pathway is necessary so that

only foreign substances are targeted. This control is achieved by a group of regulatory proteins that are present in the plasma and attached to membranes of host cells.[15,17] The alternative pathway is kept constitutively active by the spontaneous hydrolysis of C3, which forms an anaphylatoxin C3a and fragment C3b. Via multiple steps, involving C3, C5, C6, C7, C8 and C9, the "membrane-attack complex (MAC)" is formed.[15] This MAC is ultimately behind the endothelial damage and favours the formation of thrombi. Several regulatory proteins are normally present such as "complement receptor-1 (CR1)" and "decay-accelerating factor (DAF)", which are both in competition with factor B to bind to C3b, thus preventing unnecessary complement activation and cell damage. C3 convertase formation may also be inhibited when factor I, a protein, cleaves C3b to its inactive state. In addition, factor H competes with factor B in binding to C3b, reducing its activation.[15]

However, several mutations have been found and described which interfere with the normal functioning of the above processes. Specifically, mutations have been documented in genes which have roles in the regulation of C3 convertase, such as factor H, factor I, membrane cofactor protein (MCP), and thrombomodulin.[15,17] More than one-hundred mutations of factor H have been described in adults and children diagnosed with aHUS, accounting for 20-30% of diagnosed patients. These mutations are described to result in an absolute deficit of factor H (type 1 mutation); while type-2 mutations have normal levels of factor H but aberrant functionality of the protein.[15]

1.3 Other forms of TMA

Pregnancy-associated TMA is linked to 8-18% of all cases of TMA. It may be associated with ADAMTS-13 deficiency, dysregulation of the complement cascade, or related to as

yet unknown mechanisms.[15] TMA in the setting of pregnancy occurs most often during the second and third trimesters, and the diagnosis can be challenging due to occurrence of pre-eclampsia and the HELLP (Haemolysis, Elevated Liver enzymes, Low Platelets) syndrome, which are other forms of TMA occurring at this time and which have many similarities to TTP/HUS.[15] TMAs can also occur in the post-partum period, which also confounds diagnosis. Thrombosis occurs in the placental circulation and the consequences can include intra-uterine fetal growth restriction, intra-uterine fetal death, and pre-eclampsia. The risk of relapsed TMA persists for future pregnancies.[18]
aHUS has been shown to occur in patient's post-organ (liver, kidney, heart, and bone marrow) transplantation as well. In many of these patients, immunosuppressive agents, such as calcineurin inhibitors (CNIs), are believed to trigger the disease by direct injury to endothelial cells.[15,16] mTOR inhibitor drugs, such as everolimus and sirolimus, are thought to evade this complication, but they have likewise been implicated in possible precipitation of aHUS via the inhibition of "vascular endothelial growth factor (VEGF)".[16] Antibody-mediated allograft rejection is another form of TMA that can affect the post-transplant population, and which may be difficult to distinguish from CNI-related TMA and from other TMAs in general.[4] A large number of drugs and substances have been described as having a role in the development of TMAs such as TTP/HUS, although these appear to account for <15% of cases.[18] These substances may also function as a trigger in those individuals that have underlying genetic predispositions.

The main mechanisms by which drugs result in TMA are immune-mediated damage and/or direct endothelial injury. Chemotherapeutic agents that are used in patients with malignancy have also been implicated, and some of those described include cisplatin,

mitomycin-C[15,18] and gemcitabine.[4,19] Quinine is another drug shown to cause immune-mediated HUS, with the development of auto-antibodies, especially in older women.[15] The anti-platelet agents clopidogrel and ticlopidine have also been associated with the onset of drug-induced TTP,[15] although ticlopidine is associated with more frequent occurrences than clopidogrel.[19]

Systemic infection with bacteria, viruses and/or fungi can also result in thrombocytopenia and MAHA, and hence ought to also be considered when assessing patients.[4] Infections are thought to be important causes of aHUS, especially in post-transplant patients due to their immunosuppressed state.[15] It is difficult to differentiate whether these infectious agents act as a direct cause or as a trigger on a background of increased risk in an individual.[4] Viruses such as cytomegalovirus (CMV), human herpes virus-8 (HHV-8) as well as parvovirus B19 have been described as possible infectious agents in TMA. Another virus that has been described is the H1N1 virus, which either favours co-infection with bacteria including Streptococcus pneumoniae, or it acts as an initiator of aHUS in patients with aberrations of the complement cascade.[4,15]

1.4 Pathogenesis and Pathophysiology of TMA

TMA can be conceptualised as a clinical syndrome arising from endothelial dysfunction which may be precipitated by a variety of underlying disease processes. Primary TMA presenting with TTP-spectrum features (neurological dysfunction being more prominent than renal involvement) has been shown to be due to the presence of ultra-large multimers of von Willebrand factor (ULvWF),[4] in turn due to an absolute or functional deficiency of ADAMTS-13, a protease with von Willebrand factor (vWF)-cleaving activity.[14] Failure of proteolytic cleavage of vWF facilitates the formation of these ultra large multimers, which in turn increases the binding of platelets, especially in areas of high

shear flow which includes capillaries and arterioles, where shearing forces optimise the conformation of the ULvWF to expose platelet biding sites.[14] This leads to the formation of platelet-rich thrombi in the microvasculature and hence consumptive thrombocytopenia and microangiopathic haemolysis. In a suitable clinical setting, an ADAMTS-13 activity level below 10% is highly suggestive of acquired TTP.[4,5,20]

Two major categories of ADAMTS-13 deficiency are distinguished: congenital and acquired. Acquired TTP arises from the presence of depleting or inhibitory auto-antibodies directed against the ADAMTS-13 protease; while congenital deficiency of ADAMTS-13 is much rarer, and occurs as a result of bi-allelic genetic mutations in the ADAMTS-13 gene inherited in an autosomal recessive fashion.[4,14] Acquired auto-antibodies may arise in the setting of a variety of other conditions, including autoimmune diseases (e.g., systemic lupus erythematosus, rheumatoid arthritis), haematopoietic stem cell or solid organ transplantation, drugs, malignancies, and pregnancy.[9]

The influence of acquired autoimmunity in HIV-associated TMA pathogenesis is not evident. A substantial portion of HIV-associated TMA patients do not have any measurable auto-antibodies directed against ADAMTS-13.[12] In patients with HIV-associated TMA the levels of ADAMTS-13 remain variable and may also be normal in a significant number of cases; but severe deficiency, with ADAMTS-13 levels <5%, to normal levels, have been documented in some series.[12,21] One study found that only 38% of subjects had reduced ADAMTS-13 activity, a mild reduction of ADAMTS-13 levels and increased vWF levels;[21] while the study conducted by Meiring et al[13] demonstrated significantly reduced ADAMTS-13 levels in all TMA patients. HIV infected patients not yet on cART appear to have mildly reduced ADAMTS-13 activity

compared to those already initiated on cART, although these depressed levels remain within the normal range. It is thought that decreased production of proteases, such as ADAMTS-13, in HIV infection due to micronutrient deficiencies may play a role in this observation. vWF levels have been found to be markedly raised in HIV-positive patients not yet on cART,[13] which can be as a result of the inflammatory state of HIV infection itself, or due to other co-infections. The study by Meiring et al additionally found elevated tissue factor levels in 87% of patients, a protein which is known to favour thrombus formation.[13]

Infection with HIV type-1 is associated with inflammation and a resultant increase in quantities of inflammatory cytokines, including interferon-gamma (INF-γ) and tumour necrosis factor-alpha and -beta (TNF-α, TNF-β).[13] These cytokines together with other pro-inflammatory cytokines, such as interleukin (IL)–1, 6, and 8, have significant stimulatory consequences on the ULvWF released from endothelial cells, and may inhibit the synthesis of ADAMTS-13. The resultant ADAMTS-13 deficiency and overproduction of ULvWF may facilitate a suitable milieu for the initiation of TMA.[13]

1.5 Diagnosis of TMA

The initial diagnosis of TMA should be made on the basis of history, clinical examination and laboratory analysis, including an examination of the peripheral blood film.[20] The diagnosis of TMA rests upon the demonstration of microangiopathic haemolytic anaemia as evidenced by red cell fragments (schistocytes) on blood films, elevated levels of lactate dehydrogenase (LDH) and unconjugated bilirubin, with reduced haptoglobin levels. Typically, there are no major abnormalities with regard to the clotting system, with the international normalised ratio (INR), prothrombin time, and activated partial thromboplastin time (aPTT) all being within the normal range.[9] ADAMTS-13 activity

levels are not required to make the diagnosis of TMA, in the form of TTP, but may assist in monitoring disease activity and prognostication. An ADAMTS-13 activity level of 5% seems to be specific for TTP but in itself does not recognize all patients who may relapse; levels below 10% identify patients who are at increased risk of relapse, but is also not specific for the disorder.[1] Nevertheless, Scully et al suggest that prior to the initiation of PLEx, blood samples need to be drawn for other investigations to aid in the diagnosis and include the measurement of ADAMTS-13 levels as well as for the assessment of ADAMTS-13 antibodies.[20]

TMAs are medical emergencies and require rapid initiation of treatment, so decisions regarding the utilisation of PLEx need to made using other parameters. A clinical algorithm known as the PLASMIC score was suggested by Bendapudi et al, to aid in the diagnosis of TTP with probable severe ADAMTS-13 deficiency.[22] The PLASMIC score uses seven parameters and awards 1 point to each of the following parameters: a platelet count less than 30 $x10^9$/L, a mean corpuscular volume (MCV) more than 90 fL, creatinine less than 2.0 mg/dL, INR less than 1,5, evidence of haemolysis based on reticulocyte count, haptoglobin, or unconjugated bilirubin levels, and a history of no active carcinoma or solid organ/bone marrow transplantation.[4,22] A PLASMIC score of 0-4 indicates a low risk; 5 an intermediate risk; and 6-7 a high risk of severe ADAMTS-13 deficiency, and hence TTP.[22] Another study by Coppo et al reported that patients with platelet counts <30 $x10^9$/L and creatinine concentration of <2.2 mg/dL (equivalent to ~200 µmol/L) are associated with severe ADAMTS-13 deficiency (levels <10 IU/dL).[4]

The diagnosis of TMA is a challenging one to make, as there is significant clinical overlap with autoimmune disease, scleroderma, disseminated intravascular coagulopathy (DIC), malignant hypertension, and a range of pregnancy-related conditions.[20]

However, it is a very important diagnosis to establish, as prompt treatment is necessary to prevent early mortality, which can be significantly decreased with the initiation of PLEx. TMAs can present with a diversity of clinical manifestations – including the classical pentad – but a large number of patients will not have the entire complement of features at presentation.[20]

1.6 Management of TMA/TTP

The responsiveness of TTP to plasma therapy was established by Rock et al.[5] PLEx may offer an additional benefit in reducing mortality over plasma infusion.[3,5,10] Plasmapheresis facilitates the removal of ADAMTS-13 auto-antibodies/inhibitors in circulation, while the infusion of fresh-frozen plasma (FFP) in addition replenishes the reduced ADAMTS-13 protease levels.[1,5] PLEx has thus become the mainstay of treatment of TTP,[3,7] and has been shown to induce remission in 70-90% of patients with idiopathic TTP.[5] Despite the utility of PLEx in the management of TTP, its use is not favoured in other forms of TMA apart from aHUS. PLEx should be commenced with 1.5 plasma volume exchanges, which can subsequently be reduced to 1.0 plasma volume exchange once there has been improvement to patients' clinical condition.[20] Corticosteroids may be prescribed in conjunction with daily PLEx in cases of refractory TTP or where a strong suspicion exists for the presence of underlying autoimmunity, although randomised controlled trials demonstrating their efficacy are lacking.[5] Response to treatment is generally indicated by the normalisation of the platelet count with improvement of the LDH level; while other laboratory parameters appear to be less important.[1] Daily PLEx should be sustained until a minimum of 2 days after the platelet count has normalised (>150 $x10^9$) prior to being stopped.[20]

In patients who are found to be HIV positive, viral load and CD4 counts should be measured and cART should be initiated promptly. cART has been shown to result in the normalisation of ADAMTS-13 levels.[20] However, the CD4 count and viral load in HIV do not predict the required duration of PLEx required to attain a complete remission of TMA.[12] Compliance with cART after recovery is vital, as it has been shown that there is an increased risk of relapsed TMA with discontinuation or non-adherence to cART.[20] While previous studies by Rock et al[5] demonstrated that PLEx was superior than plasma infusion in treating TMA in HIV-infected subjects, a prospective study conducted in Cape Town found that patients infected with HIV showed a markedly better response rate to high-dose plasma infusion therapy alone without necessitating PLEx.[13,14] Swart et al have reported that in the management of acute TMA, plasma infusion therapy alone was inferior to PLEx but still remains a useful therapy, especially where PLEx is not accessible due to limited resources.[6] Another South African study by Masoet et al found no difference in time to remission between subjects treated with plasma infusion alone with subjects that received plasma infusion and then subsequent PLEx.[7]
A study by Coppo et al,[23] which excluded patients with HIV infection, showed that high-dose infusions of FFP had no statistically significant difference in the recovery of platelet counts and LDH levels, or in mortality and relapse rates when compared to treatment with PLEx. However, the high-dose FFP infusion group had a higher risk of complications due in main to the excessive fluid burden placed on these patients.[23]

Between 20-50% of patients with idiopathic TTP experience a relapse, with the majority of relapses occurring within the initial two years after the index presentation.[1] However, some relapses of TTP have been reported as much as 10 to 20 years post-presentation. These relapses are always associated with a recurrence of ADAMTS-13 deficiency, and

some studies have suggested that the monitoring of ADAMTS-13 levels during remission periods might be an aid in the early detection of a reduction in activity levels and early treatment of relapses.[3]

Patients with refractory cases of acquired TMA may require other immunosuppressive therapies, in addition to plasma exchange therapy and corticosteroids.[4,14] The monoclonal antibody Rituximab, which targets the B-cell CD20 antigen, has successfully been utilised in treating both index and recurrent episodes of TMA. Rituximab interferes with the production of auto-antibodies by depleting B-cells, but its effect is delayed by up to 14 days after administration.[14] Rituximab is therefore typically used as an addition to therapy but not as a single or initial agent. Vincristine, Cyclophosphamide, Cyclosporine A, and Bortezomib have also been employed in isolated case reports of refractory TMA,[4] but are considered as third-line treatment options due to their poorly proven efficacy and severe adverse effect profile; Cyclosporine A has also been identified as a causative agent in some cases of TMA. Splenectomy has been advocated as a salvage therapy for the chronic recurrent form of TMA.[14] Eculizumab, which is a monoclonal antibody to complement factor C5, was developed to prevent complement-mediated endothelial injury in the congenital and acquired aHUS forms of TMA. This drug acts by inhibiting the C5 fraction of the complement cascade, and has been approved for use in treating TMA by the FDA in the last decade.[15]

Recombinant ADAMTS-13 is under development, and may be useful in patients with acquired forms of TMA since the inhibitor levels are often low enough to be overcome with exogenous ADAMTS-13, as noted in in-vitro studies.[3]

2. Methods

2.1 Study Objectives

The primary objective of this study was to compare TMA in HIV positive and HIV negative patients, in terms of presentation and response to PLEx at Helen Joseph Hospital during the period 1 January 2010 – 31 December 2019.

Secondary objectives for this study were:

- To determine the number of cases of TMA at Helen Joseph Hospital during the period of study.
- To characterize the clinical presentation of TMA at Helen Joseph Hospital as a whole and in consideration of aetiology (namely, HIV-associated TMA, pregnancy-induced TMA, TMA secondary to lupus nephritis, and other causes of TMA).
- To characterize the therapeutic response of TMA at Helen Joseph Hospital as a whole and in consideration of the aetiological subgroups as outlined above.

2.2 Study Design

The study comprised a retrospective analysis of a cohort of patients diagnosed with TMA and treated at Helen Joseph Hospital during the period 1 January 2010 – 31 December 2019. The study comprised both descriptive and inferential components.

2.3 Inclusion Criteria

Patients meeting the following criteria were considered for inclusion in the study:

- Age > 18 years.

- Retrospective confirmation of contemporaneous diagnosis of TTP/TMA in consideration of the “British Committee for Standards in Haematology” criteria (Annexure A).
- Documented treatment with PLEx.

2.4 Exclusion Criteria

Patients were excluded from this study where missing data was likely to affect analysis in relation to the primary objective of this study.

2.5 Data Collection

Permission has been obtained from the South African National Blood Bank Service (SANBS) to access their database of patients that had undergone PLEx in order to extract the details of cases suitable for inclusion in this study. The files of patients thus identified were retrieved from the hospital records department, relevant data as outlined below was extracted from these files and entered anonymously on individual case data collection sheets; the data collection sheet is presented below in Annexure B. Once data collection was completed, data was combined from these data collection sheets into a Microsoft Excel® database which was exported to Statistica® for analysis. Data pertaining to the following parameters was collected for analysis:

- Patient demographics (age, gender, race group).
- HIV infection status (positive and on cART, positive not yet on cART, and negative).
- Clinical features at presentation, such as fever, renal dysfunction, and/or neurological impairment.
- Aetiology of TMA (HIV-associated TMA, systemic lupus erythematosus, pregnancy, and other).

- Presenting laboratory parameters (haemoglobin concentration, platelet count, presence of schistocytes, LDH level, and creatinine concentration.
- In HIV positive patients, the CD4 count and HIV viral load.
- In newly diagnosed patients with HIV, if cART was instituted during the index admission period.
- Whether supportive therapies, in the form of haemodialysis, was required.
- Therapeutic response parameters (number of PLEx sessions required to induce remission as defined by the "British Committee for Standards in Haematology")
- Length of patients' hospital stay.
- Documented recurrence or relapse.
- Presence of residual renal dysfunction as documented by serum creatinine at termination of PLEx therapy.
- Patient survival or death.

2.6 Statistical Analysis

The total number of TMA cases diagnosed at Helen Joseph Hospital during the study period was calculated. The frequency of aetiological categories of TMA was determined and presented by means of a frequency table. The distribution of patients' demographic parameters (gender and race) and HIV infection status was computed and presented in a frequency table. Presenting laboratory parameters (haemoglobin concentration, platelet count, LDH activity, D-dimer and creatinine concentration and, where available, ADAMTS-13 activity level) and patients' ages at presentation was tested for normality of distribution using the Shapiro Wilk test. With visual inspection of the histogram plot, the central tendency and dispersion measurements was determined using the medians and interquartile ranges for non-parametric data and was calculated for the series as a whole,

for aetiological categories outlined above, and for HIV infection status categories. The frequency of neurological deficit was determined by means of a frequency table for the series as a whole and for the categories mentioned previously. The number of PLEx sessions required to induce remission was also tested for normality of distribution and described for the series as a whole, as well as the previously indicated categories as dictated by the outcome of this analysis. Similar descriptive processes were undertaken for the outcome variables serum creatinine at termination of PLEx, recurrence or relapse, and patient mortality as applicable to the continuous or categorical nature of the parameter analysed.

Comparison of the presentation of TMA as indicated by the continuous variables of haemoglobin concentration, platelet count, LDH activity level, creatinine concentration and D-dimer concentration, and ADAMTS-13 activity (where available) between HIV positive and negative patients was effected through the use of the Mann Whitney U or Student t-test, as determined by the outcome of analysis of the normality of distribution of the relevant variable; categorical variables (neurological deficit) was compared using the Fisher-Exact test. An alpha of 0.05 was considered as indicative of statistical significance.

Comparison of the therapeutic responsiveness of TMA between HIV positive and HIV negative patients was undertaken utilising the Mann Whitney U or Student t-test, as determined by the outcome of analysis of the normality of distribution of this variable. Further exploration of the validity of any observed differences regarding the response to PLEx was undertaken using linear regression analysis, in which the effect of the presenting parameters and HIV infection status had on the number of PLEx sessions needed to induce remission were analysed.

Comparison of the development of recurrent or relapse episodes of TMA between HIV positive and HIV negative groups was undertaken using the Fisher Exact test, which was also used to compare the frequency of mortality between these groups, if indicated. Finally, the creatinine concentration at termination of PLEx was compared between HIV infection groups using the Mann Whitney U or Student t-test as indicated. Further evaluation of any detected difference was made using linear regression to determine the effect of presenting parameters, HIV infection status, and number of PLEx sessions performed on the creatinine concentration.

2.7 Potential Limitations

The following were identified as potential limitations to this study:

1. TMA is a relatively rare disease so the patient cohort could be small. Approximation of sample size was anticipated to be 30-50 patients (3-5 cases per year for a 10-year duration).
2. The study, being of a retrospective nature resulted in missing data leading to exclusion of a number of patients, further reducing the size of the cohort.
3. Sampling bias may be apparent since only patients who had undergone PLEx were considered for inclusion in this study.

A small cohort increases the risk of statistical error due to sampling bias. As indicated, non-parametric testing was utilised to counteract this.

4. Ethics

Permission was granted by the SANBS to access their database of patients receiving PLEx; this was used to identify cases for inclusion in the study after review of archival notes.

Permission to conduct the study at Helen Joseph Hospital was obtained from the office of the Head of Internal Medicine, Dr Z. Bayat, and from the hospital Ethics Committee.

Ethics clearance to conduct the study was obtained from the Human Research Ethics Committee (HREC) of the University of the Witwatersrand (HREC certificate no. M2011116). As outlined above, data was collected and stored in an anonymous fashion.

5. Funding

All costs incurred during this study were privately funded by the primary investigator.

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Section II – Submissible Article

Title

Presentation and therapeutic outcomes of Thrombotic Microangiopathy in HIV positive and HIV negative patients over a 10-year period at a tertiary hospital in Johannesburg, South Africa.

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Abstract

Background: Thrombotic microangiopathies (TMAs) are heterogeneous disorders characterized by widespread occlusive microvascular disease, causing thrombocytopenia, microangiopathic haemolytic anaemia (MAHA) and end-organ dysfunction due to ADAMTS-13 deficiency. TMAs are broadly classified into primary and secondary, with HIV being an increasingly common secondary aetiology, specifically in HIV endemic regions, like South Africa.

Methods: 106 records of patients diagnosed with TMA and treated with plasma exchange (PLEx) from 2010-2019, were assessed at presentation for clinical features of fever, renal and neurological involvement; laboratory parameters including haemoglobin, platelet count, and creatinine levels; treatment required such as blood product support, renal replacement therapy and use of corticosteroids; and patient outcomes such as recurrence, mortality and creatinine at termination of PLEx. Further information was gleaned from the NHLS laboratory database to assess patients' response to PLEx.

Results: HIV was the most common aetiology, accounting for 82.2% of TMA. More than two-thirds of the cohort were females of Black-African ethnicity, and the median age at presentation was 36 years. Patients with HIV-associated TMA had lower platelet counts, and were older at presentation compared to HIV negative patients. The diagnosis of TMA was typically associated with advanced HIV disease (median CD4 count of 147 cells/mm^3). Irrespective of the TMA aetiology, this study found that a median of 10 PLEx sessions was required to induce remission of TMA, with the crude mortality found to be 16.8% in this cohort.

Conclusion: HIV-associated TMA remains an important cause of secondary TMA in South Africa. It presents more often in Black-African females and in those with advanced stages of HIV. Compared to HIV negative individuals, HIV-infected patients present with lower

platelet counts potentially signifying a more severe form of disease. PLEx remains an integral component in the management of TMA and is crucial to improving survival. While this modality is crucial to eliminating the pathogenic ULvWF multimers and auto-antibodies, the important role of combination antiretroviral therapy (cART) in maintaining remission and preventing relapse of TMA should not be underestimated.

Introduction

The thrombotic microangiopathies (TMAs) are a constellation of heterogeneous disorders which are exemplified by widespread occlusive microvascular disease, featuring micro-thrombus formation in arterioles and the capillary network which manifest in microangiopathic haemolytic anaemia (MAHA), consumptive thrombocytopenia and end-organ dysfunction, characteristically affecting the kidneys and/or brain.[1] Based on the type of end-organ involvement encountered, TMA has traditionally been distinguished into thrombotic thrombocytopenic purpura (TTP) and the haemolytic uraemic syndrome (HUS).[1,2] These prototypical forms of TMA, i.e. TTP and HUS, are regarded as acute haematological emergencies which without rapid intervention carries a poor prognosis. In adults, differentiating between TTP and HUS is impractical given that HUS is more commonly observed in children, while the clinical features in the acute presentation can be similar in both entities [3,4] Plasma exchange (PLEx) in particular is known to be efficacious in both TTP and aHUS, and has been shown to improve survival rates from 10% in untreated patients up to 78% in patients receiving PLEx.[2,5–7] The achievement and maintenance of a remission requires in addition to PLEx, the diagnosis and treatment of any underlying predisposing or precipitating condition.

The association of HIV with TMA has been well established in numerous studies.[6,8–10] HIV is postulated to cause TMA through the development of absolute or functional ADAMTS-13 deficiency,[8,11] resulting from either direct viral infection of the endothelium with a subsequent upregulation in production of ultra-large von Willebrand factor (ULvWF) multimers. This increased ULvWF overwhelms the protease effects of ADAMTS-13, and simultaneously there may be an acquisition of auto-antibodies to this protease.[10,11] As a result, the risk of TMA in HIV-positive patients has been estimated to be 15–40 times higher than that of the general population.[12]

A classic pentad of clinical features described as suggesting the diagnosis of TTP includes consumptive thrombocytopenia, microangiopathic haemolysis (MAHA), renal and neurological dysfunction, and fever.[1,13] However the complete pentad is rare and some consider obsolete; with MAHA, thrombocytopenia, and neurological phenomena being more frequently observed, while fever and renal abnormalities are being seen less often.[13]

Anecdotal evidence suggests that HIV infection is an important cause of TMA in the South African context, and recent studies by Swart et al[6], Masoet et al[14] and Louw et al[2,11] have helped gain some insight into this disease. However, there still remains a paucity of data concerning the entity of HIV-associated TMA which requires further study, particularly in the local setting. This study aims to characterize and compare the presentation and therapeutic response of HIV-associated TMA to that of TMA occurring in HIV-negative patients.

Methods

A retrospective analysis of hospital records was undertaken of patients admitted to the Helen Joseph Hospital (HJH) – a tertiary public sector academic hospital located in the western part of Johannesburg, serving an ethnically diverse population – who were diagnosed with TMA, and subsequently treated with PLEx over the period 1 January 2010 to 31 December 2019. Subjects under 18 years of age and those wherein data was not retrospectively available were excluded from analysis. Demographic, clinical, and laboratory data was collected anonymously using a Microsoft Excel® database and subsequently exported to the Statistica® software programme for statistical analysis. Statistical significance was determined by a p-value of 0.05. Since variables demonstrated non-Gaussian distribution in Shapiro Wilk testing and upon visual inspection of the histogram plot, central tendency and

dispersion measurements were represented by the median, and the range/interquartile range (IQR), respectively. The Mann-Whitney U test was utilised to compare independent continuous variables and the two-tailed Fisher exact test was similarly used for categorical variables. Linear regression modelling was used to analyse the contribution of variables to specified outcomes including the crude mortality rate, relapse of TMA within the study period and creatinine concentration at the termination of PLEx.

PLEx is typically commenced with 1.5 plasma volume exchanges, which can subsequently be reduced to 1.0 plasma volume exchange once there has been an improvement to patients' clinical condition.[15]

Ethics approval for this study was obtained via institutional board review by the University of the Witwatersrand Human Research Ethics Committee (HREC Medical reference number: M2011116) as well as from the National Health Laboratory Service (NHLS) Ethics Committee.

Results

A total of 166 patients received PLEx during the study period. From this total, 56 were excluded due to missing or incomplete data, 2 patients were excluded in view of age younger than 18 years at presentation, and a further 2 were excluded due to the aetiology being likely HELLP (Haemolysis, Elevated Liver enzymes, Low Platelets) syndrome as opposed to TMA; thus, the final number in this cohort was 106 patients.

Baseline demographics, ascribed aetiologies of TMA, and presenting laboratory and clinical parameters of this cohort are presented in Table 1. Females and patients of Black African ethnicity comprised the majority of patients in this study (68.2 and 89.7% respectively), while

people living with HIV comprised 82.2% of the cohort. Autoimmune and idiopathic categories constituted the next most frequent aetiologies at 11.2% and 6.5%, respectively. With respect to the HIV-positive group, the median CD4 count at presentation was 146.5 cells/mL. A large proportion of patients were newly diagnosed with HIV on presentation with TMA, with only 15.9% of patients having a prior history of combination antiretroviral therapy (cART) being prescribed.

As shown in table 2, HIV-positive patients manifested a lower haemoglobin level (median of 6.9g/dL; p-value = 0.233) and lower platelet count (median of $15x10^9$/L; p-value = 0.019) at presentation, compared to their HIV-negative counterparts, with neurological deficits being seen more frequently in the HIV-positive group (56.3%, p-value = 0.024) as well. Although the haemoglobin concentration was lower in HIV-positive patients, it was not statistically significant. Renal dysfunction appeared more commonly in the SLE group with creatinine concentration at presentation being significantly higher than the other aetiological categories (p-value = 0.009).

Fifteen patients from this cohort, equivalent to 16.05%, were found to have experienced a relapse of TMA during the study period, requiring further PLEx. The majority of these relapses (73.3%) were patients from the HIV-positive group. However, further analysis regarding the time frame between the index and relapse TMA episodes as well as potential causes for the relapse was not done.

The crude mortality rate for inpatients diagnosed with TMA and initiated onto PLEx in this series was 16.8%. Inpatient deaths occurred at a median of 16 days after the initiation of PLEx. A higher eGFR at presentation was associated with reduced odds of patient death (OR 0.85, 95% CI 0.72 – 1.0, p-value = 0.046). No effect was found for patient age (p-value =

0.595), sex (p-value = 0.735), presenting features – namely haemoglobin (p-value = 0.070), platelet count (p-value = 0.761), pyrexia (p-value = 0.121), neurological deficit (p-value = 0.313), or aetiological category of TMA (p-value = 0.337) on the mortality risk. No effect was also observed for CD4 count (p-value = 0.149), viral load (p-value = 0.060), or antecedent cART prescription (p-value = 0.372) on the mortality risk in patients living with HIV.

Amongst the 89 survivors of TMA, a median of 10 sessions of PLEx was required to achieve remission (IQR 8 – 14 sessions) and survivors remained hospitalized for a median of 15.5 days (IQR 13 – 28 days). The number of PLEx sessions prescribed was independent of presenting haemoglobin concentration (p-value = 0.191), platelet count (p-value = 0.500), LDH activity (p-value = 0.906), eGFR (p-value = 0.218), or the documented presence of fever (p-value = 0.696) or neurological deficit (p-value = 0.280). The median number of PLEx sessions prescribed was similar across the aetiological categories (p-value = 0.671), between ethnic groups (p-value = 0.817) and genders (p-value = 0.184).

The median creatinine at discharge in survivors was 74µmol/L (IQR 61 – 98µmol/L), equivalent to an eGFR of 99mL/min/1.73m^2 (IQR 76 – 115mL/min/1.73m^2); while 17 patients (19.1%) were discharged with an eGFR below 60mL/min/1.73m^2. A higher LDH activity at presentation was associated with an equivocally increased odds of residual renal dysfunction on discharge as defined by an eGFR of less than 60mL/min/1.73m^2 (OR 1.001, 95% CI 1.000 – 1.003, p-value = 0.020). Conversely, a better preserved eGFR at presentation was associated with a decreased risk of residual renal dysfunction on discharge (OR 0.96, 95% CI 0.939 – 0.984, p-value < 0.005). The presence of residual renal dysfunction on discharge was independent of the presenting haemoglobin (p-value = 0.932), platelet count (p-value = 0.479), or number of PLEx sessions received (p-value = 0.522).

Although the percentage of patients with residual renal dysfunction on discharge was lower in patients living with HIV (13.3%) compared to non-HIV aetiological categories (50%, p-value = 0.004), any effect for aetiological category on residual renal dysfunction was abrogated in multivariate analysis (p-value = 0.053). Nineteen patients (17.8%) required dialysis in addition to PLEx treatment; prescription of dialysis did not independently increase the risk of mortality (p-value = 0.982). The median presenting eGFR in patients requiring dialysis was 20mL/min/1.73m^2. Amongst the 11 survivors of TMA who received dialysis, 9 patients (81.8%) manifested significant residual renal dysfunction on discharge (p-value < 0.001), with the median eGFR on discharge in this group being 14mL/min/1.73m^2 (interquartile range 8 – 57mL/min/1.73m^2) and 6 patients (6.7% of survivors) having an eGFR within dialysis range (i.e., below 15mL/min/1.73m^2).

Discussion

This is the largest reported series of TMA patients reported from Africa to date. HIV is the dominant aetiological facet of TMA in the local context, and HIV-associated TMA arises more often in the background of advanced HIV infection. The use of plasma exchange (PLEx) in the management of TMA in this series was associated with reasonable mortality outcomes compared to other reported outcomes in HIV-related TMA.[6,8,14,16,17] Analysis of this cohort suggests that TMA affects predominantly younger female patients. This is consistent with the experience of the Oklahoma TTP-HUS registry (median age 40 years)[18] and with that of a previous series reported from Groote Schuur Hospital (mean age 29.5 years)[8]. A female preponderance is also observable in the present series, with similar gender distribution being reported in other series.[1,3] The predilection for TMA to present in young females of childbearing age has traditionally been explained by the higher risk of autoimmune disease in this group.[3,8,13,19] Higher rates of TMA have also been reported in

patients of Black-African ancestry, specifically females,[3,6,8,13,18] believed to reflect a genetic risk in this population. Evidence has shown this genetic risk to be related to a preponderance to develop antigenic ADAMTS-13 auto-antibodies, relating to the major histocompatibility complex (MHC)-class II genes. HLA-DRB1*04 allele is believed to exert a protective effect in preventing TMA/TTP; individuals of Black-African ancestry have been shown to have a lower prevalence of this protective allele, thus contributing to a higher susceptibility to TMA in this group.[19] The significant contribution of HIV infection as an aetiological factor in this series is representative of the burden of HIV in the local population,[9,20] a burden which is mainly borne by Black-African women of reproductive age.

Previously it has been suggested that HIV-associated TMA is associated with a more severe clinical presentation.[8,17] In the present study, haemoglobin concentration and platelet count at presentation were lower in HIV-positive patients compared to their HIV-negative counterparts. Whether this aspect reflects a more aggressive lesion in this group is not clear. The lesser haemoglobin concentration in HIV-positive patients was however not statistically significant; while the cause of lower platelet counts is multifactorial and may reflect direct effects of the virus, comorbidities with opportunistic infections/malignancies or potential drug effects. These considerations highlight the difficulties in diagnosing TMA in people living with HIV, where other conditions that may mimic TMA such as DIC, disseminated TB, and lymphoproliferative disorders may add to diagnostic confusion. This situation is exacerbated by the association of TMA with advanced and untreated HIV infection,[6,15,17] where multiple such differentials may co-exist.

Creatinine concentration at presentation was higher in patients with SLE in this study. TMA is known to occur in association with glomerular disease, reflecting endothelial injury,[16,21,22] but it is possible that the poorer renal function in the SLE group noted in this series reflects associated lupus nephritis.

In this series the diagnosis of HIV-associated TMA was associated with advanced stages of HIV infection, as manifested by low CD4 counts and elevated HIV viral load, given that the majority of cases were either cART naïve or had experienced a treatment interruption. These figures are consistent with other reported data[8,11,12,16,17] demonstrating a higher likelihood of TMA developing in the setting of advanced immune paresis.[12] Mechanisms that have been proposed to explain this include direct endothelial infection/injury and activation from the virus itself; the chronic inflammatory state in the HIV setting with augmented levels of pro-inflammatory cytokines leading to a prothrombotic state; as well as patients having a higher likelihood of developing auto-antibodies to ADAMTS-13.[2,12,17] Plasma exchange is an accepted therapy for TMA, specifically TTP, having been shown to reduce mortality in a number of studies.[1,3,5,13,15,16,23] PLEx addresses the deficiency of ADAMTS-13 by replacing the protease with substituted plasma whilst at the same time removing auto-antibodies, which if present, result in a functional or absolute ADAMTS-13 deficiency.[8] In the present study, the aetiology of TMA did not appear to affect response to PLEx, with a median of 10 PLEx sessions required to induce TMA remission. Previous work in the local context by Swart et al[6] and Gunther et al[10] reported similar findings, with a median of 10 PLEx sessions and 12 PLEx sessions needed respectively, to achieve remission in their respective series with no difference being observed between HIV-positive and HIV-negative groups.

In this series, the recovery of renal function after an episode of TMA was poorer in HIV-negative patients. It remains noteworthy that analysis of the presentation of TMA found higher creatinine in the SLE group, and that linear regression found that presenting creatinine concentration was a significant influence on creatinine at discharge. It may be that renal dysfunction in the non-HIV categories of TMA reflects concomitant lupus nephritis in this group. Given the low prevalence of renal dysfunction in TTP in general, some experts recommend investigation for additional aetiologies in scenarios when significant renal dysfunction is found.[1,21]

Fifteen patients in this cohort (16.05%) experienced a relapse of TMA requiring PLEx, with the majority of these (73.3%) being HIV-positive patients. Such relapses occurred predominantly in patients who previously were initiated on cART but subsequently experienced an interruption to HIV treatment, a known risk factor for recurrence.[6,11] The efficacy of PLEx in reducing the high mortality rates associated with TMA is well attested.[3,6,13,18,23] Difficulties in establishing an accurate diagnosis of TMA in the acute setting,[4,8,18] and delays in initiating PLEx may still result in significant mortality. Analysis of this cohort revealed a crude mortality rate of 16.8% of patients who received PLEx. With regard to the mortality rates seen in other TMA studies, a high mortality rate of 29.3% was reported by Swart et al in their analysis of patients, the majority of whom were HIV infected, who had received PLEx.[6] The study by Masoet et al reported a higher mortality rate of 44.2% of patients in their report, with the majority being from the HIV-positive cohort. However, their study analysed patients who had initially received plasma infusion therapy, and only the non-responders subsequently had been treated with PLEx.[14] Louw et al studied 21 patients (75% HIV-positive) who underwent PLEx, with only one death reported during the index presentation of TMA.[11]

Comparable mortality rates to this current study have been reported by the Oklahoma-registry study[1] and by Rock el al.[5] Of note, both of these studies had a much lower percentage of HIV-infected patients, given the different geographical regions wherein these studies were conducted.

Limitations

There are of course limitations to the present study. Sample bias, arising from the single-centre nature of this work, may have led to an over-representation of HIV as an aetiological factor for TMA. In addition, since this study only analysed patients who received PLEx as part of their management, the subset of patients who were diagnosed with TMA but did not necessitate PLEx were excluded from this cohort. This is another limitation which could have resulted in sampling bias.

Lack of the ADAMTS-13 assay and/or histological diagnosis of TMA in this series may have resulted in the inclusion of a heterogenous sample population. However, the presence of the ADAMTS-13 results would have aided in differentiating those patients with a diagnosis of TTP from other forms of TMA.

On the other hand, the single-centre nature of this study has resulted in a homogenous treatment strategy being implemented for all patients diagnosed with TMA.

Conclusion

This study comprised the largest known cohort of HIV-associated TMA patients worldwide, to date. HIV-associated TMA is an extremely frequent cause of secondary TMA in South Africa, and typically presents at advanced stages of HIV infection. It presents more commonly in Black-African females, and compared to their HIV-negative counterparts is associated with lower haemoglobin and lower platelet counts. Plasma exchange remains an

integral component to TMA management, and is critical to improving survival. Patients with advanced HIV infection show good recovery with PLEx together with the prompt initiation of cART.

Figures

Table 1. Presenting features of TMA

Age (years)	36 (32 – 43) *
Sex	Female: 72 (67.9%) Male: 34 (32.1%)
Ethnicity	Black African: 95 (89.6%) Non-black: 11 (10.4%), comprising: Mixed ethnicity: 7 (6.6%) Asian: 3 (2.8%) White: 1 (0.9%)
Haemoglobin (g/dL)	7.0 (5.9 – 8.0)
Platelet count (x10^9/dL)	16 (12 – 26)
LDH (IU/mL)	1469 (819 – 1984)
Creatinine (µmol/L)	101 (68 – 159)
CKD-EPI eGFR (mL/min/1.73m^2)	69 (44 – 102)
RBC Fragments (smear)	Numerous: 74; HIV-positive = 64 (86.5%) Moderate: 13; HIV-positive = 11 (84.6%) Scanty: 12; HIV-positive = 9 (75.0%)
Neurological deficit	33 (30.8%)
Pyrexia	7 (6.5%)
Aetiology	HIV: 88 (83.0%) ** Autoimmune disorder: 12 (11.3%), comprising: SLE: 8 (7.5%) Dermatomyositis: 2 (1.9%) Mixed connective tissue disease: 2 (1.9%) Idiopathic: 7 (6.6%)
HIV-positive patients **CD4 count (x10^6/mm^3)** **Viral load (copies/mL)** **Antecedent cART prescription**	 146.5 (79 – 212) 212000 (53000 – 693000) 17 (15.9%)

**Values are median (interquartile range)*

***Includes 1 patient with carcinoma cervix and 1 patient with non-Hodgkin lymphoma*

Table 2. Comparison of features across aetiological categories of TMA

	HIV (n = 88)	**SLE** (n = 8)	**Idiopathic** (n = 7)	**p****
Hb (g/dL)	6.9 (5.7 – 8.3) *	7.4 (6.7 – 8.2)	7.8 (7.2 – 9.1)	0.233
Platelet count (x10^9/dL)	15 (11 – 21.5)	32.5 (20 – 64.5)	26 (13 – 63)	0.019
LDH (IU/mL)	1514.5 (974 – 1984)	575.5 (338.5 – 1395.5)	1984 (1343 – 2940)	0.019
Creatinine (µmol/L)	99.5 (69.5 – 127)	260.5 (220 – 433)	101 (52 – 516)	0.009
CKD-EPI eGFR (mL/min/1.73m^2)	76.5 (48.5 – 103.5)	20 (11.5 – 32.5)	62 (9 – 124)	0.008
Neurological deficit	27 (56.3) $^+$	0	1 (20%)	0.024
Pyrexia	5 (11.6%)	1 (20%)	1 (20%)	0.780

*Values are *median (interquartile range) and $^+$number (percentage of those with recorded data)*

***Kruskal Wallis ANOVA and Pearson Chi-square test, as appropriate*

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Section III – Annexures

Annexure A: British Committee for Standards in Haematology (BSH) Guidelines on the diagnosis and management of thrombotic thrombocytopenic purpura and other thrombotic microangiopathies

Major Recommendations

Definitions for the quality of the evidence (A-C) and strength of recommendation (strong [grade 1], weak [grade 2]) are given at the end of the "Major Recommendations" field.

Diagnosis of Thrombocytopenic Purpura (TTP)

ADAMTS13 Assays

1. The diagnosis of TTP should be treated as a medical emergency (1A).
2. The initial diagnosis of TTP should be made on clinical history, examination and routine laboratory parameters of the patient, including blood film review (1A).
3. In view of the high risk of preventable, early deaths in TTP, treatment with plasma exchange (PEX) should be initiated as soon as possible, preferably within 4–8 h, regardless of the time of day at presentation, if a patient presents with a microangiopathic haemolytic anaemia (MAHA) and thrombocytopenia in the absence of any other identifiable clinical cause (1B).
4. Serological tests for human immunodeficiency virus (HIV), hepatitis B virus and hepatitis C virus, autoantibody screen and when appropriate, a pregnancy test, should be performed at presentation (1A).
5. Pre-treatment samples should be obtained to measure ADAMTS13 activity levels and to detect anti-ADAMTS13 antibodies. Measurement of ADAMTS 13 antigen levels is also useful in congenital TTP cases (1B).

Subgroups of TTP

Congenital TTP

1. Congenital TTP should be considered in neonates presenting with severe jaundice. Presentation may also occur in childhood or as an adult (1A).
2. The diagnosis of congenital TTP should be considered in children and adults with unexplained thrombocytopenia (1B).
3. The diagnosis of congenital TTP is confirmed by ADAMTS13 activity <5%, absence of antibody and confirmation of homozygous or compound heterozygous defects of the ADAMTS13 gene (1A).

Drug-Associated TTP

1. Medications associated with precipitation of TTP include quinine and oestrogen-containing medications, which should be avoided to prevent relapse in patients with a previous episode of TTP (2C).
2. Women with previous TTP should be offered non-oestrogen containing contraception (1C).

Treatment of Acute TTP

Plasma Therapy

1. PEX should be started with 1.5 plasma volume (PV) exchanges, using solvent/detergent-treated (S/D) plasma in all age groups and reassessed daily (1B).
2. The volume of exchange can be reduced to 1.0 PV when the clinical condition and laboratory test results are stabilizing (2C).
3. Intensification in frequency and or volume of PEX procedures should be considered in life-threatening cases (2B).
4. Daily PEX should continue for a minimum of 2 d after platelet count has been >150 X 109/l and then stopped (2B).

Congenital TTP

1. S/D plasma infusion or intermediate purity Factor VIII (e.g., BPL 8Y) should be used to treat congenital TTP (1C).
2. Treatment regimens for congenital TTP should be individualized according to the patient's phenotype (1A).

Treatment of TTP in Pregnancy

1. If a thrombotic microangiopathy (TMA) cannot be fully explained by a non-TTP pregnancy-related TMA, then the diagnosis of TTP must be considered and PEX should be started (2B).
2. Mothers with congenital TTP should attend a specialist centre and receive ADAMTS13 supplementation regularly throughout pregnancy and the post-partum period (1A).
3. Close liaison with an obstetrician with a special interest in feto-maternal medicine is required in mothers with TTP (1A).
4. In mothers with acquired TTP, ADAMTS13 activity should be monitored throughout pregnancy to help predict the need for adjuvant therapy and outcome (1B).
5. Pre-conceptual counselling is advised for subsequent pregnancies and women of child bearing age should be counselled about potential risks of pregnancy and combined oral contraceptive pill (COCP) (2B).

HIV-related TTP

1. If a patient with TTP is found to have HIV infection then viral load should be measured and an HIV physician should be closely involved in management (1A).
2. TTP should be considered in an HIV-positive individual with a MAHA and thrombocytopenia (1A).

3. PEX in conjunction with highly active antiretroviral therapy (HAART) (triple or quadruple therapy) should be started as soon as the diagnosis of HIV-associated TTP is made (1B).
4. HAART should be given immediately after PEX therapy to maximize time for absorption (1A).
5. HAART should be continued after remission to prevent further relapse (1B).
6. In resistant HIV-related TTP, rituximab could be considered (2B).

Malignancy-Associated Thrombotic Microangiopathy

1. PEX is not indicated in the management of malignancy and bone marrow transplant-associated TMA (1A).
2. In cancer associated TMA, further treatment for the underlying cancer should be considered (1A).

Further Treatments in Acquired TTP

Corticosteroids

1. Intravenous daily methylprednisolone (e.g., 1 g/d for three consecutive days – adult dose) or high dose oral prednisolone (e.g., 1 mg/kg/d) should be considered (1B).

Rituximab

1. In acute idiopathic TTP with neurological/cardiac pathology, which are associated with a high mortality, rituximab should be considered on admission, in conjunction with PEX and steroids (1B).
2. Patients with refractory or relapsing immune-mediated TTP should be offered rituximab (1B).

Ciclosporin A (CSA) and Tacrolimus

1. CSA may be considered as second line therapy in patients with acute or chronic relapsing acquired TTP (1C).

Splenectomy

1. Splenectomy may rarely be considered in the non-acute period of immune-mediated TTP but has limited proven benefit (2C).

Antiplatelet Agents

1. The clinical efficacy of antiplatelet agents in TTP is unproven but they are relatively safe (1B).
2. Low dose aspirin (75 mg once per day [OD]) may be given during platelet recovery (platelet count >50 X 109/l) (2B).

Supportive Therapy

1. Red cell transfusion should be administered according to clinical need especially if there is cardiac involvement (1A).
2. Folate supplementation is required during active haemolysis (1A).
3. Platelet transfusions are contraindicated in TTP unless there is life-threatening haemorrhage (1A).
4. Thromboprophylaxis with low molecular weight heparin (LMWH) is recommended once platelet count has reached >50 X 109/l (1B).

Refractory TTP

Increased frequency of PEX and addition of rituximab can be considered in refractory TTP (1B).

Relapse

1. Increased PEX and/or rituximab therapy are the agents of choice in relapsing disease (1B).
2. Patients should be counselled about symptoms, signs and risk of relapse before discharge with verbal and written information (1A).

3. In patients with a documented reduction of ADAMTS 13 activity to <5%, elective therapy with rituximab can be considered (1B).

Definitions:

Quality of Evidence

The quality of evidence is graded as high (A), moderate (B) or low (C). To put this in context it is useful to consider the uncertainty of knowledge and whether further research could change what is known or is uncertain.

(A) High Further research is very unlikely to change confidence in the estimate of effect. Current evidence derived from randomised clinical trials without important limitations.

(B) Moderate Further research may well have an important impact on confidence in the estimate of effect and may change the estimate. Current evidence derived from randomised clinical trials with important limitations (e.g., inconsistent results, imprecision - wide confidence intervals or methodological flaws - e.g., lack of blinding, large losses to follow up, failure to adhere to intention to treat analysis), or very strong evidence from observational studies or case series (e.g., large or very large and consistent estimates of the magnitude of a treatment effect or demonstration of a dose-response gradient).

(C) Low Further research is likely to have an important impact on confidence in the estimate of effect and is likely to change the estimate. Current evidence from observational studies, case series or just opinion.

Strength of Recommendations

Strong (grade 1): Strong recommendations (grade 1) are made when there is confidence that the benefits do or do not outweigh harm and burden. Grade 1 recommendations can be applied uniformly to most patients. Regard as 'recommend'.

Weak (grade 2): Where the magnitude of benefit or not is less certain a weaker grade 2 recommendation is made. Grade 2 recommendations require judicious application to individual patients. Regard as 'suggest'.

Clinical Algorithm(s)

An algorithm for the summary of treatment protocol for acute thrombotic thrombocytopenic purpura (TTP) is provided in the original guideline document.

Annexure B**:** Data Collection Sheet

Study Subject Number: _____ Ethnicity: African / Coloured / Indian / White

Gender: Male ☐ Female ☐ Pregnancy: Yes ☐ No ☐

Number of PLEx sessions received: _____ Plasma Substitute: FFP / Cryo-poor / Both

Retroviral Disease (RVD) Status: Reactive ☐ Non-reactive ☐ Unknown ☐

CD4/Viral Load (if known): _____/_____ On cART: Yes ☐ No ☐

If cART initiated during current admission: Yes ☐ No ☐

Co-morbid illnesses: _____

Clinical features: Fever ☐ Renal Impairment ☐ Neurological Impairment ☐

Blood product support: Yes ☐ No ☐ Haemodialysis: Yes ☐ No ☐

Use of Corticosteroids: Yes ☐ No ☐ Other immunosuppression: _____

Laboratory Investigations:

Date	Hb	Platelets	LDH	Creatinine	D-dimer	ADAMTS-13

Peripheral Blood Smear: _____

Other Investigations: _____

Duration of hospital stay: _____

Follow-up visit: _____

Relapse Episode of requiring PLEx: Yes ☐ No ☐ Duration after initial episode: _____

Annexure C: Human Research Ethics Committee Clearance Certificate

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 Dr Yusuf Moola

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M2011116

NAME: (**Principal Investigator**)	Dr Yusuf Moola
DEPARTMENT:	Internal Medicine Helen Joseph Hospital
PROJECT TITLE:	Presentation and Therapeutic Outcomes of Thrombotic Microangiopathy in HIV positive and HIV negative patients at Helen Joseph Hospital during the period 2010-2019
DATE CONSIDERED:	27/11/2020
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Dr Malcolm Davies and Dr Zaheera Cassimjee
APPROVED BY:	CB Penny Dr CB Penny, ~~Chairperson~~, HREC (Medical)
DATE OF APPROVAL:	15/12/2020

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

DECLARATION OF INVESTIGATORS

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

Principal Investigator Signature

Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES