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## **Thrombotic microangiopathy in the era of HIV**

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**2014**

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# Thrombotic Microangiopathy in the era of HIV

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A research report submitted to the Faculty of Health Sciences,  
University of the Witwatersrand, Johannesburg, in partial fulfilment of  
the requirements for the degree of Master of Medicine in the branch of  
Internal Medicine

Johannesburg, 2014

## **i. Declaration**

I, Malcolm Davies, do hereby declare that this research report is my own unaided work. It is being submitted for the degree of Master of Medicine in the branch of Internal Medicine. I further declare that this work has not been submitted for any other examination or degree at this or any other University.

*(signed) Malcolm Davies on this day, 24<sup>th</sup> of April, 2014*

## **ii. Publications and presentations arising from this research**

1. Oral presentation to the South African Renal Society Congress, Cape Town, 16 – 19 April 2010
  - Published abstract resulting: Davies M, Chita G. Renal dysfunction in the Thrombotic Microangiopathies. Cardiovascular J. Africa 2010(21):S4-S5
2. Poster presentation to the World Congress of Nephrology (WCN), Vancouver, 8 – 12 April 2010
  - Poster details: Davies M, Chita G, Veriawa Y, Naicker S. The Thrombotic Microangiopathies in the era of HIV – an African perspective
3. Poster presentation to the 49<sup>th</sup> Congress of the European Renal Association – European Dialysis and Transplantation Association (ERA-EDTA), Paris, 24 – 27 May 2012
  - Published abstract resulting: Davies M, Chita G, Veriawa Y, Naicker S. The Thrombotic Microangiopathies in the era of HIV - an African perspective. Nephrol. Dial. Transplantation 2012(27)(Suppl. 2):ii362-ii363

## **iii. Ethical considerations**

Permission for this retrospective study was obtained from Dr. G. Chita (Head of Department, Department of Internal Medicine, Helen Joseph Hospital), Ms M Bogoshi (Chief Executive Officer, Helen Joseph Hospital), and the Ethics Committee of the University of the Witwatersrand (clearance number M121131).

#### **iv. Abstract**

Human immunodeficiency virus (HIV) associated thrombotic microangiopathy (TMA) is thought to be a common form of the disorder in South Africa with a severe clinical presentation and poor prognosis; it remains however poorly characterized. This study was undertaken to evaluate the presentation and prognosis of HIV-associated TMA through retrospective analysis of a cohort of HIV positive and negative subjects diagnosed with and treated for TMA at a single center (Helen Joseph Hospital) from 1/1/2001 to 31/12/2009.

HIV-associated TMA was the dominant form of the disorder in this series and was associated with advanced HIV infection and a more severe presentation than TMA in HIV negative subjects. Although mortality was non-significantly more frequent, response to plasmapheresis was more rapid and recurrence less common in HIV positive subjects.

This study adds to available literature on a rarely studied disorder. Despite its aggressive nature, timely diagnosis and intervention facilitate satisfactory outcomes in HIV-associated TMA.

## **v. Acknowledgements**

The support and assistance of the following persons in the preparation of this research report is gratefully acknowledged:

- Dr. G. Chita – for assistance as supervisor to this study and review of the prepared manuscript
- Prof. Y. Veriawa – for suggesting the study under consideration
- Prof. S. Naicker – for ongoing encouragement in completion of this study
- Prof. E. Libhaber – for assistance with statistical methodology

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# **1. Background: The Thrombotic Microangiopathies**

## **1.1 Introduction**

The thrombotic microangiopathies (TMA) are a heterogeneous group of disorders with a shared pathophysiology and clinical presentation (1). Although evidence suggests that HIV infection plays a significant role in the aetiology of TMA in South Africa (2), little is known regarding the presentation and prognosis of HIV-associated TMA. The present study of a cohort of patients diagnosed with TMA at a single institution (the Helen Joseph Hospital) was undertaken in an effort to characterize the presentation, response to treatment, and prognosis of HIV-associated TMA.

## **1.2 Definitions**

The histopathological term “thrombotic microangiopathy” (TMA) was first used by Symmers in 1952 to describe a vascular lesion predominantly affecting capillaries and arterioles characterized by vessel wall thickening with swelling and detachment of endothelial cells and intraluminal platelet thrombus formation (3). This pathological lesion gives rise to a syndrome first described by Eli Moschowitz in 1925 in which microangiopathic haemolysis and consumptive thrombocytopenia are the dominant clinical findings (4). Because intraluminal conditions of high sheer force promote microvascular thrombus formation, this syndrome is associated with significant renal and / or central nervous system dysfunction (5). Following historically important case series descriptions by Singer and Gasser it has been general practice to distinguish this clinical syndrome into Thrombotic Thrombocytopenic Purpura (TTP) or the Haemolytic Uraemic Syndrome (HUS) based on the severity of central nervous system or renal dysfunction respectively (6 - 9). For reasons set out below TTP and HUS are not viewed as separate clinical entities in this study, and the term “thrombotic microangiopathy” is used to refer to the clinical disease entity.

### 1.2.1 TMA – a unified model of TTP-HUS

It has been historically accepted that TTP and HUS are clinically distinct disorders, with TTP comprising a syndrome of microangiopathic haemolysis, consumptive thrombocytopenia, neurological deficit of varying severity, pyrexia and minimal or no renal dysfunction; and HUS featuring microangiopathic haemolysis, consumptive thrombocytopenia, renal dysfunction and no pyrexia or neurological deficit (6 - 8). It was postulated that the discrepancies in organ involvement between these two disorders arose as a result of preferential sites of TMA lesion development (viz., the central nervous system in TTP and the kidneys in HUS) (10, 11).

Some evidence suggests that TTP and HUS have unique aetiologies and pathophysiologies. In 1982 Moake demonstrated the presence of prothrombotic ultralarge monomers of von Willebrand factor (ULvWF) in the sera of patients with TTP (12); later efforts by Furlan, Tsai, Levy and Gerritsen showed that this anomaly arose as a result of congenital or acquired defects in ADAMTS13 activity (13 - 19). TTP occurring in systemic lupus erythematosus (SLE), the antiphospholipid syndrome (APLS), HIV infection and with ticlopidine use has been shown to be associated with the presence of inhibitory ADAMTS13 antibodies (20 - 23).

These findings have suggested that ADAMTS13 deficiency (either acquired or congenital) is central to the development of TTP. Diminished ADAMTS13 activity allows the accumulation of ULvWF which under the high shear force conditions produced by blood flow through the microvasculature leads to the formation of ULvWF strings anchored to the endothelium by P-selectin (ADAMTS13 is responsible for the cleavage of ULvWF into smaller, less thrombotic dimers)(24, 25). The formation of these strings provides multiple sites for platelet binding and activation via the glycoprotein IbIX – factor V complex and / or platelet integrin  $\alpha_{IIb}\beta_3$ , leading to extensive platelet thrombus formation within the microvasculature, consistent with the TMA lesion (25). It has been suggested that the association of

ADAMTS13 deficiency with TTP is sufficiently robust to allow ADAMTS13 activity levels to be used as a diagnostic tool for the disorder (20, 26).

In contrast, HUS is usually (90% of cases) the result of infection with shigatoxin producing *Escherichia coli*, *Shigella dysenteriae* type 1, or *Citrobacter freundii* (9, 27). Shigatoxin has been shown to induce endothelial cell dysfunction through disruption of protein synthesis and upregulation of prothrombotic pro-inflammatory cytokines (28, 29). Acquired or congenital defects in the complement regulatory pathway involving complement factor H (CFH), complement factor I (CFI), membrane cofactor P (MCP), complement factor B (CFB), complement factor 3 (C3) or thrombomodulin have been implicated in the rare atypical (non-diarrhoeal) forms of HUS (27, 30, 31).

Although such findings appear to suggest that TTP and HUS can be viewed as separate disease entities, significant evidence has emerged to suggest that they are best interpreted as opposing extremes of a clinical syndrome caused by a single disorder of multiple aetiologies. For example, metanalysis of the available case series reports of TTP indicate that significant renal dysfunction may occur in up to 80% of patients (11). Indeed, acute renal injury severe enough to require dialysis has been reported in some patients with severely deficient ADAMTS13 activity (a finding supposedly diagnostic of TTP) (32).

Neurological deficit has been reported to occur in both atypical and diarrhoea-associated HUS (33), and autopsy series have found evidence of cerebral microcirculation TMA lesions in up to 50% of patients diagnosed with this disorder (11). Magnetic resonance imaging of the brain has also shown CNS involvement in patients diagnosed with HUS (34, 35). Analysis of a large patient cohort found that the diagnostic pentad of TTP (microangiopathic haemolysis, consumptive thrombocytopenia, pyrexia, minimal renal dysfunction, and cerebral dysfunction) did not occur in any patient in the series (n = 142) (32). Other series have also demonstrated that ADAMTS13 deficiency is not specific for TTP and may also occur in congenital HUS (36).

Thus, TTP and HUS show overlapping clinical features and share many aetiological factors. TTP and HUS therefore represent the variable clinical presentation of a number of different aetiologies which are capable of inducing TMA. Endothelial dysfunction is known to be central to the development of TMA in several disorders including atypical HUS (complement-mediated injury to the endothelium) (27), diarrhoea-associated HUS (shigatoxin-induced endothelial apoptosis) (29, 37), mitomycin and calcineurin inhibitor induced TTP (1, 9), HIV-associated TTP (38), and transplantation-associated TTP (39, 40). TMA can therefore be viewed as the pathological evidence of underlying endothelial dysfunction caused by these heterogeneous disorders. Supporting this approach is the observation that serum from patients with both idiopathic TTP and atypical HUS has been shown to directly induce endothelial cell apoptosis (41, 42) through as yet poorly defined pathways involving Fas and caspase-8 (42, 43). Animal models provide further corroboration of the central role of endothelial dysfunction in the development of the TMA lesion underlying the clinical syndromes of TTP and HUS: Vascular Endothelial Growth Factor (VEGF) knockout mice have been shown to spontaneously develop TMA lesions (44), the administration of antiendothelial cell antibodies has resulted in TMA lesions developing in a rat model (45), and an ADAMTS13 deficient murine model of TTP failed to develop TMA lesions until exposed to intravenous collagen and epinephrine, thus suggesting that ADAMTS13 deficiency itself is not sufficient to induce TMA but may require a “second hit” perhaps in the form of a prothrombotic state resulting from endothelial dysfunction (46). Significantly, endothelial dysfunction is known to result in the upregulation of ULvWF production as well as P-selectin (24, 47); under these circumstances, ADAMTS13 would rapidly become depleted. Thus, a primary defect in endothelial function may be sufficient to account for the ADAMTS13 deficiency model of TTP proposed to uniquely account for the development of this disorder.

For the above reasons it is increasingly apparent that TTP and HUS are not separate entities but rather variable clinical presentations of a single disorder characterised by endothelial dysfunction (1, 11). In the

analysis presented here the terms TTP and HUS are therefore not used, and for clarity the disorder is referred to as “TMA”.

### **1.3 Epidemiology of TMA**

TMA is estimated to have an annual incidence of 4/1 000 000 (48). Some reports have suggested that the incidence of TMA is rising although this may reflect improved disease surveillance (49, 50). A female preponderance has been reported in most case series (1, 48, 51). The disorder commonly affects individuals between the ages of 20 and 60 years (48). Persons of Black African descent are more likely to be affected with TMA than other race groups, and it has been suggested that Black African descent is a risk factor for acquired autoimmune forms of the disorder (1, 22, 51).

#### **1.3.1 Epidemiology of HIV-associated TMA**

The reported contribution of HIV infection to TMA incidence is highly variable (52) and likely reflects geographic differences in HIV prevalence. Analysis of the large TMA cohort of the Oklahoma TTP-HUS Registry found that HIV-associated TMA accounted for 1.8% of all cases of the disorder (52). This low incidence of HIV-associated TMA is consistent with the low observed incidence of TMA (0.3%) amongst the 6022 HIV positive patients enrolled in the Collaborations in HIV Outcomes / Research US study (53). In contrast, a single case series from South Africa appears to indicate that HIV-associated TMA is the most common form of the disorder in our setting (2).

TMA in HIV positive patients may occur as a complication of antiretroviral drugs (chiefly abacavir) (55), as a component of the Immune Reconstitution Inflammatory Syndrome (IRIS) (56), as a consequence of opportunistic infections such as CMV (57), or as a consequence of the HI virus itself. The central role of the HI virus in the pathogenesis of HIV-associated TMA is highlighted by case reports of this disorder responding to initiation of combination antiretroviral therapy (cART) and, in other case reports, the recurrence of TMA in patients discontinuing cART (38). HIV may induce TMA through the development

of ADAMTS13 antibodies (22, 38); however, ADAMTS13 antibodies are not universally present in patients with HIV-associated TMA (54, 58). Instead, it is likely that HIV-associated TMA occurs as a result of endothelial dysfunction. The HI virus is able to infect endothelial cells through mutations to the gp120 envelope protein (59, 60), following which the endothelial cell may undergo caspase-8 mediated apoptosis (43).

The direct role of the HI virus in causing HIV-associated TMA probably accounts for the observed tendency for HIV-associated TMA to occur in patients with low CD4 count and other features of advanced infection stage (51, 53, 54, 61). The disorder in local literature demonstrates a strong female preponderance (female to male ratio 20:1) (51); whereas in international literature case studies have shown a higher rate of male patients (up to 80% of cases) (62). This discrepancy likely reflects geographical variation in gender distribution of HIV infection. In North America and Europe HIV infection is mainly acquired through male to male sexual contact; as a result, 73% of patients living with HIV infection in the United States are male (63). In contrast, in South Africa HIV infection is acquired through heterosexual contact; as a result, women bear the brunt of HIV infection in the local setting with up to 55% of patients living with the infection being female (64).

## **1.4 TMA in the context of HIV infection**

### **1.4.1 The presentation of HIV-associated TMA**

It is generally believed that HIV-associated TMA is more severe in clinical presentation than TMA seen in HIV negative patients, although data in this regard is scarce. Local experience suggests that several parameters of TMA including markers of microangiopathic haemolysis (haemoglobin concentration and lactate dehydrogenase activity), consumptive thrombocytopaenia (platelet count), and renal dysfunction (serum creatinine) may be more severely abnormal in HIV-associated TMA (51). Furthermore, neurological deficit may be more frequent in HIV-associated TMA (51).

It remains unclear whether the severity of clinical presentation in HIV positive patients is solely a function of the TMA disease process, or whether other HIV infection-related pathophysiologies may be contributing. For example, anaemia is widely prevalent in advanced HIV infection (63 – 95% of patients) and may be due to multiple other HIV-related disease processes besides microangiopathic haemolysis, including nutritional anaemia, anaemia of chronic disease, and red cell aplasia due to antiretroviral drugs (65). Similarly, thrombocytopaenia in HIV positive patients may be exacerbated by other disease processes (for example, immune thrombocytopaenia, malnutrition, bone marrow infiltration) occurring concomitantly with the consumptive thrombocytopaenia of TMA (66). Renal dysfunction may also be a common associate of HIV infection with prevalence rates of up to 27% reported in some series (67); thus, the apparent severity of renal dysfunction in HIV-associated TMA may in part be due to underlying pre-existing renal disease.

Some evidence that the TMA disease process itself may be the most significant determinant of the severity of clinical presentation in HIV-associated TMA is to be found in reports that higher levels of D-dimer are observed in this form of the disease (2, 54). It has been suggested that this finding is indicative of a unique pathophysiology involved in HIV-associated TMA (2). D-dimers are the cleavage products of fibrin and reflect activation of the fibrinolytic system in response to thrombus formation (68); thus, a higher D-dimer level may be indicative of more extensive microthrombus formation occurring in HIV-associated TMA. It is however also known that HIV infection itself appears to increase D-dimer levels which have been shown to correlate with HIV viral load (69); this is consistent with the prothrombotic state induced by HIV infection (70). Thus, the severity of clinical presentation of HIV-associated TMA may be due to extensive microvascular thrombus formation precipitated by endothelial dysfunction caused by infection of these cells by the HI virus, aided by the prothrombotic background which is a consequence of HIV infection.

Although microangiopathic haemolysis and consumptive thrombocytopaenia are characteristic systemic features of TMA, a subset of patients may present with a renal limited form of the disorder in which these abnormalities are not readily detected (71). Renal limited HIV-associated TMA has been described (58, 72), and although the prevalence of this form of the disorder is uncertain (since diagnosis rests on renal biopsy, itself requiring a high index of suspicion on the part of the treating physician) (73), available literature suggests that 7 – 15% of HIV-associated TMA may be renal-limited (58, 72).

#### **1.4.2 Response to therapy of HIV-associated TMA**

Plasma exchange using fresh frozen plasma (FFP) or cryosupernatant plasma has become the mainstay of therapy for TMA (74). Originally described by Bukowski et al in 1977 (75), the use of plasmapheresis has improved survival rates in TMA from 10% to between 75% and 92% (74). Patients at both the HUS and TTP ends of the clinical spectrum of TMA presentation have shown response to plasma exchange (76, 77). Interestingly, plasmapheresis was also shown to be of benefit in adult patients diagnosed with diarrhoeal HUS during a 1996 outbreak in Scotland (78). Based on these and other studies the British Committee for Standards in Haematology now recommends plasmapheresis as standard first-line therapy for all cases of TMA (79, 80).

The beneficial effect of plasma exchange in TMA occurs as a result of the removal of excessively thrombogenic and anti-fibrinolytic molecules and the replacement of deficient anticoagulants and profibrinolytic molecules in order to regain the normal milieu of haemostasis (81). Thus, plasmapheresis allows for the removal of ULvWF, ADAMTS13 autoantibodies and inhibitors, interleukin 6 (known to stimulate ULvWF release from the endothelium), and thrombin; and to allow for the replenishment of ADAMTS13 (81 - 83). Since cryosupernatant plasma is ULvWF depleted, it has been thought that this may be the preferable replacement plasma for TMA patients receiving plasmapheresis (84); however, this has not been shown in prospective studies (85).

Remission of HIV-associated TMA and prevention of relapse is dependent on improving the immune status of the patient; for this reason, the British Committee for Standards in Haematology recommends urgent initiation of cART in this disorder in addition to plasma exchange (80).

Despite such recommendations, controversy exists as to the responsiveness of HIV-associated TMA to therapeutic intervention, mainly as a result of the paucity of data available in this regard. Tamkus et al, Abraham et al and Tostivint et al have reported mortality rates higher than those seen in HIV negative patients despite the use of standardized therapy (86 - 88), suggesting poorer responsiveness. However, Miller et al have reported a similar therapeutic response in terms of survival rates in HIV-associated TMA compared to that seen in HIV negative patients (38), and Novitzky et al have shown lower mortality rates in HIV positive patients compared to HIV negative patients (51). This latter study also provides the only interrogation of the rate of response as a function of time of HIV-associated TMA compared to TMA in HIV negative patients, demonstrating a more rapid induction of remission in the former group (51).

### **1.4.3 Prognosis of HIV-associated TMA**

The prognosis of HIV-associated TMA must be interpreted in the context of 3 main outcomes of therapy, viz. mortality rates, residual target organ deficit (neurological and renal), and recurrence rates.

#### **1.4.3.1 Mortality**

As previously noted, controversy exists regarding the mortality rates observed in HIV-associated TMA, with mortality rates variously reported as higher, equal to, and lower than those seen in HIV negative patients (38, 51, 86 - 88). Data from other series have additionally suggested that the occurrence of TMA in an HIV positive patient predicts mortality even if the TMA episode is successfully treated, with patient death ensuing within 1 – 2 years of diagnosis of TMA (61, 89), an observation which may reflect the association of the disorder with advanced HIV infection (51, 53, 54, 61, 89). Mortality in patients with HIV-associated TMA has been suggested to be associated with neurological signs (86) and high LDH

activity at presentation (90). In view of the paucity of data concerning mortality in HIV-associated TMA, consideration may be given to that available from analysis of TMA occurring in HIV negative patients in order to extrapolate risk factors for death from TMA to HIV positive patients. In so doing, however, it should be stated that the accuracy of such data may itself be limited by the retrospective nature of many of the case series from which this data is derived, which results in bias due to the inclusion of cases of TMA from a variety of causes (which may have differing prognoses) as well as the inclusion of multiple confounding factors (90). Neurological deficit, older age (>40 years), lower haemoglobin (< 6.5g/dl), lower platelet count (<  $15 \times 10^9/l$ ), pyrexia (> 38.5°C), and renal dysfunction (creatinine > 175µmol/l) have been described in various series to be associated with mortality (91 - 93).

#### **1.4.3.2 Residual target organ dysfunction**

Neurological deficit in TMA is thought to arise through two main mechanisms; microinfarction as a result of microthrombus formation, and endothelial dysfunction leading to fluid transudation and petechial haemorrhage through the resultant disrupted blood-brain barrier (94). Case series using imaging with computerised tomography (CT) or magnetic resonance imaging (MRI) indicate the latter to be the more common pathophysiology (94). This results in a reversible posterior leukencephalopathy syndrome, which most commonly presents with confusion and/or lethargy but may include hemiparesis (94). The response of serious neurological symptoms such as hemiparesis and altered levels of consciousness to plasma exchange is usually rapid and permanent clinically obvious neurological deficits are rare (74, 94), but subtle abnormalities of cognitive function including concentration and memory may be an underappreciated and frequent sequelae (74). The latter consideration, and the observation that neurological dysfunction in TMA may be multifactorial in origin (including for example, electrolyte disturbances caused by renal dysfunction) (35), the use of data from retrospective studies (in which the severity of neurological dysfunction is unclear and may be over-emphasised) (90), and the prevalence of neurological abnormality in HIV positive patients (40-60% of patients with advanced HIV infection may

have some degree of neurological deficit) (95) combine to make analysis of the response of neurological dysfunction in HIV-associated TMA difficult.

The response of renal dysfunction caused TMA in general to standard therapy remains controversial. Tostivint has found that plasma-based therapies result in 73.3% of patients with TMA recovering normal renal function (89). Hollenbeck has reported that the use of plasma exchange led to an 81.8% reduction in the relative risk of developing end-stage renal disease (96). In contrast, Schieppati et al have reported that 70% of patients with TMA have some degree of on-going renal dysfunction despite therapy, with 26% developing end-stage renal disease requiring dialysis (97). In this series, a poor renal outcome was associated with a higher creatinine at presentation, lower presenting haemoglobin, and a higher presenting platelet count; this suggests that established or background renal dysfunction at presentation determines the likelihood and severity of residual renal dysfunction rather than the severity of presentation of TMA (97). This is consistent with Tostivint et al's report indicating that the presence of an underlying renal disease and requirement for dialysis during hospitalization are independent risk factors for chronic renal insufficiency, whereas a high LDH at presentation (indicative of TMA severity) was associated with better renal outcomes (89).

The response of HIV-associated TMA to standard therapy remains uncertain, mainly because of the limited data available in this regard. Gervasoni et al have reported that the use of plasma exchange is associated with resolution of TMA lesions in the renal biopsy specimens of patients with HIV-associated TMA (98); however, large patient cohort studies confirming this finding are not available. Analysis of the response of renal dysfunction in HIV-associated TMA is further complicated by the prevalence of background renal dysfunction in patients living with HIV (67) and the probability that renal dysfunction in the disorder may be due to other concomitant factors, such as dehydration due to altered levels of consciousness or diarrhoea secondary to opportunistic infections.

### 1.4.3.3 Recurrence

Available literature suggests a wide disparity in the rate of recurrence of TMA dependent on the aetiological factors involved. Whilst relapse of diarrhoeal HUS is extremely rare (9), the rate of recurrence is higher in patients with congenital defects in complement regulation or ADAMTS13 activity (27). TMA secondary to autoimmune disorders may also be associated with a higher rate of recurrence (99). In acquired forms of TMA associated with ADAMTS13 deficiency the risk of recurrence may depend on the severity of ADAMTS13 deficiency present when in remission (27, 99). Overall, 15 – 30% of patients with TMA experience a recurrence of the disorder (27, 100). Whilst Tuncer et al have suggested that a lower platelet count at presentation may identify patients at risk of recurrence (91), in general, clinical parameters at presentation do not predict recurrence (101, 102).

Recurrence of HIV-associated TMA is rare in patients commenced on antiretroviral therapy (51); significantly, however, recurrence may be precipitated by patients discontinuing cART (38). Isolated case series have also suggested that deficiency of ADAMTS13 detected in HIV positive patients in TMA remission may also predict risk of recurrence (103).

Recurrent TMA appears to respond well to plasma exchange (104, 105), and the risk of mortality in patients experiencing TMA recurrence appears to be very low (90). It is likely that the better mortality outcomes observed in recurrent TMA reflects earlier diagnosis and therapeutic intervention as a result of improved symptom recognition on the part of patients or frequent symptom and laboratory parameter surveillance on the part of attending medical staff (90).

## 1.5 Summary

The Thrombotic Microangiopathies represent a group of heterogeneous disorders which act through the shared pathophysiological mechanism of endothelial dysfunction to produce a syndrome characterised by microangiopathic haemolysis and consumptive thrombocytopenia with varying degrees of target organ injury chiefly affecting the central nervous and renal systems. A single case series suggests that HIV may be the dominant cause of TMA in the local setting (2). HIV-associated TMA has been poorly studied, and the presentation of the disorder, response to treatment (plasma exchange), and prognosis (including mortality rates and likelihood of residual target organ dysfunction) are poorly characterized and therefore remain areas of controversy. The study presented here was undertaken with the aim of better characterizing HIV-associated TMA.

## **2. Study design: a retrospective analysis of TMA in the era of HIV**

### **2.1 Study objectives**

This study was undertaken with the aim of better characterizing the presentation, response to a standardized therapy using plasma exchange, and the prognosis of HIV-associated TMA. The presentation of HIV-associated TMA was analysed in respect of the following:

1. The epidemiology and demographics of patients presenting with TMA
2. Clinical and laboratory parameters measured at TMA presentation.

The response to plasma exchange was analysed in respect of the following:

1. The number of plasma exchange sessions required to induce remission of TMA
2. The improvement of clinical and laboratory parameters in response to plasma exchange
3. Complications of plasma exchange

The effect of the type of plasma replacement used (FFP or cryosupernatant plasma) on response to therapy was also characterized in respect of 1. and 2. above.

The prognosis of the disorder was analysed in respect of the following:

1. Mortality in subjects diagnosed with TMA
2. Residual renal dysfunction following treatment with plasma exchange
3. The recurrence of TMA following plasma exchange

In order to determine whether the presentation, therapeutic response, and prognosis of HIV-associated TMA differ from that seen in other forms of the disorder, results of the above analyses were also compared to data derived from HIV negative subjects diagnosed with and treated for TMA during the period of the study.

## **2.2 Patients and methods**

### **2.2.1 Study population**

Data used in this analysis was obtained from those subjects diagnosed with TMA at the Helen Joseph Hospital (HJH) between 1/1/2001 and 31/12/2009. HJH is a secondary level hospital which serves the western areas of Johannesburg. The patient population resident in these areas is heterogeneous and includes patients of African, Asian, Indian, Caucasoid and Coloured (mixed race) ancestry. During the time period of this study a total of 40 subjects were diagnosed with TMA, comprising 35 HIV positive subjects, 4 HIV negative subjects, and 1 subject who demised before HIV serological testing could be undertaken. Seven subjects subsequently suffered a recurrence of TMA following standard therapy, so that there were a total of 47 separately treated instances of TMA during the course of this study.

### **2.2.2 Data collection**

Subjects potentially suitable for inclusion in this study were identified from a registry of patients known with TMA maintained by the Department of Haematology at HJH. Since all patients with suspected TMA at the hospital are referred to this department for management this approach identified all known cases. Case files for all patients diagnosed with TMA between 1/1/2001 and 31/12/2009 were obtained and retrospectively reviewed. The closing date for this series reflects the period of protocol submission for this study. In cases where data was incomplete resort was made to the electronic records of the National Health Laboratory Service (NHLS) at the hospital.

### 2.2.3 Inclusion criteria

Patient data was included in this series where:

1. Retrospective review confirmed the original diagnosis of TMA using criteria accepted by the British Committee for Standards in Haematology and by the authors of the large Oklahoma TTP-HUS Registry (1, 79, 80), specifically:
  - a. Evidence of microangiopathic haemolysis (anaemia, red cell fragments on peripheral smear, and elevated serum LDH activity), and
  - b. Evidence of consumptive thrombocytopenia
  - c. No other cause for the above apparent

Because data from the Oklahoma TTP-HUS Registry indicates that the full pentad of TTP rarely occurs (1), the presence of features other than 1a and 1b above (viz., pyrexia, neurological abnormality, and renal dysfunction) were not used as diagnostic criteria. Since histological evidence of TMA (for example, from renal biopsy) is not required for the diagnosis of the disorder by either the British Committee for Standards in Haematology or the Oklahoma TTP-HUS Registry (1, 79, 80), inclusion in this study did not require this criteria to be met.

2. In view of data suggesting that TTP and HUS are variable clinical manifestations of the same underlying disorder (TMA) no attempt at distinction was made in this series between these two clinical syndromes. In so doing this analysis follows the convention of the Oklahoma TTP-HUS Registry (1).

Both HIV positive and HIV negative patients were included in this study in order to characterize HIV-associated TMA against TMA seen in HIV negative patients.

## 2.2.4 Exclusion criteria

Patients were excluded from enrollment in this series where:

1. Retrospective review indicated an incompleteness of data and / or follow up which might otherwise affect statistical analysis
2. Retrospective review indicated another possible aetiology for the clinical and laboratory data recorded, namely:
  - a) Disseminated Intravascular Coagulopathy (DIC)
  - b) Malignant hypertension
  - c) Pre-eclampsia / eclampsia
  - d) Scleroderma renal crises.

2a) DIC was retrospectively diagnosed if patients had evidence of an elevated International Normalized Ratio (INR) and other recorded data consistent with known precipitant of DIC.

2b) Malignant hypertension was retrospectively diagnosed if case files demonstrated the presence of elevated blood pressure (systolic BP > 140mmHg or diastolic BP > 90mmHg) on two occasions with evidence of renal dysfunction (abnormal blood urea, electrolyte and creatinine tests with confirmed proteinuria and / or dysmorphic erythrocytes on urinalysis) and recorded evidence of a grade 3 or 4 hypertensive retinopathy (haemorrhages and exudates or papilloedema) in addition to microangiopathic haemolysis and consumptive thrombocytopenia (106).

2c) Pre-eclampsia / eclampsia was diagnosed if the patient had recently been pregnant (< 6 months post-delivery), was hypertensive (systolic BP > 140mmHg and / or diastolic BP > 90mmHg), and had

proteinuria, with or without evidence of encephalopathy or seizure, in addition to the features of malignant hypertension discussed above (107).

2d) Patients were retrospectively considered to have evidence of scleroderma renal crisis where there was evidence of renal dysfunction with active urinary sediment (proteinuria and / or dysmorphic erythrocytes on urine microscopy) with seropositivity for scleroderma (anticentromere or antitopoisomerase antibodies positive) (108).

### **2.2.5 Definitions of clinical disease categories associated with TMA in this series**

Patients included in this series and diagnosed as being HIV positive or admitted with this diagnosis already established were retrospectively validated as HIV positive if review of laboratory results satisfied established World Health Organization criteria (109), namely:

1. Positive HIV antibody testing (either rapid or laboratory-based enzyme immunoassay) confirmed by a second HIV antibody test (either rapid or laboratory-based enzyme assay) relying on different antigens or different operating characteristics; or
2. Positive virological test for HIV or its components (HIV-RNA or HIV-DNA or ultrasensitive HIV p24 antigen) confirmed by a second virological test obtained from a separate determination.

Patients included in this series who were diagnosed with Systemic Lupus Erythematosus (SLE) or who were admitted with the diagnosis of TMA on a background of previously diagnosed SLE were retrospectively validated as having SLE if case review documented the presence of at least 4 of the 11 criteria proposed by the American College of Rheumatology within a 6 month period (110).

### 2.2.6 Statistical analysis

Analysis of data in this series was performed using Statistica™ version 9 (series 231.14) statistical software (Statsoft®). Statistical significance was determined by a  $p < 0.05$ .

Assessment of the normality of distribution of continuous variables was performed using the Shapiro-Wilk W test and in consideration of the Central Limit Theorem. Because most variables analysed were not shown to be normally distributed, the distribution of continuous variables analysed in this series is presented with central tendency being represented by the median, and the dispersion measurement being given by the range and interquartile range (IQR).

Testing of data sets was performed using the Mann-Whitney U test for non-parametric independent continuous variables. Analysis of association was undertaken using the Spearman rank order method. The predictive role of variables analysed for certain outcomes in this series was performed using the ordinary least squares method of linear regression. Comparative analysis of categorical variables was made using a two-tailed Fisher exact test.

### 3. Results

#### 3.1 The Epidemiology and Demographics of HIV-associated TMA

Thirty-nine of the 40 subjects included in this series were tested for HIV infection on presentation with TMA (97.5%); one subject died before testing could be performed. Of those tested, 35 subjects (89.74%) were found to be HIV positive and 4 (10.26%) were found to be HIV negative, thus giving a ratio of HIV positive to HIV negative subjects of 8.75:1.

Amongst the HIV positive subjects, 23 were newly diagnosed with the infection (65.71%) and 12 were known to be HIV positive before presentation with TMA (34.29%). Three of the subjects who were previously known to be HIV positive had already been initiated onto combination antiretroviral therapy (cART) before presentation with TMA (25% of subjects known to be HIV positive before presentation with TMA, and 8.57% of all HIV positive subjects in this series). The range of duration of cART in these patients was 3 – 7 days (median 6 days). None of these patients received a drug regimen containing abacavir, and it is unlikely that cART was an aetiological factor in their subsequent presentation with TMA. The median age amongst HIV positive subjects was 33 years (IQR 28 - 40 years and range 23 – 62 years). Twenty-six subjects were female (74.3%) and 9 were male (25.71%); the female to male ratio was 2.89:1. Thirty-four subjects were of Black African descent; 1 subject was of Mixed Race (Coloured) origin and there were no Indian or Caucasian subjects in this subgroup; the Black African to non-black ratio was thus 34:1.

Amongst the HIV negative subjects who presented with TMA during the course of this series, 3 fulfilled American College of Rheumatology criteria for the diagnosis of Systemic Lupus Erythematosus (SLE) (75% of HIV negative subjects and 7.5% of all patients in this series) (110). In one subject no definitive underlying aetiology could be determined despite intensive investigation; this subject was labeled as a case of idiopathic TMA (25% of all HIV negative subjects in this series and 2.5% of all subjects in this

series. The median age of HIV negative subjects was 40.5 years (IQR 28 – 44.5 years, range 17 – 67 years). All subjects (100%) were female. 2 subjects were of Mixed Race (Coloured) origin (50%), and one subject each was of Black African and Indian descent (25% each). The Black African to non-black ratio was thus 1:3.

Thus, a preponderance of female subjects overall was noticed in this series. Two-tailed Fisher Exact testing did not reveal a statistically significantly higher frequency of female subjects in the HIV negative subject group ( $p = 0.556$ ). In contrast, the preponderance of subjects of Black African descent with HIV-associated TMA was shown to be statistically significant ( $p = 0.002$ ) (table 3.1).

*Table 3.1 1 Epidemiology and demographics of TMA at Helen Joseph Hospital, 2001 - 2009*

|               | <i>HIV positive subjects</i><br>N (% of total) / median (IQR)                                              | <i>HIV negative subjects</i><br>N (% of total) / median (IQR)                                         | p      |
|---------------|------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------|--------|
| HIV infection | N = 35 (89.7%)                                                                                             | N = 4 (10.26%)                                                                                        |        |
| Age (years)   | 33 (23 – 62)                                                                                               | 40.5 (28 – 44.5)                                                                                      |        |
| Sex           | Female: N = 26 (74.3%)<br>Male: N = 9 (25.71%)                                                             | Female: N = 4 (100%)<br>Male: N = 0 (0%)                                                              | 0.556* |
| Race          | Black African: N = 34 (97.14%)<br>Mixed Race: N = 1 (2.86%)<br>Caucasian: N = 0 (0%)<br>Indian: N = 0 (0%) | Black African: N = 1 (25%)<br>Mixed Race: N = 2 (50%)<br>Caucasian: N = 0 (0%)<br>Indian: N = 1 (25%) | 0.002* |

*\*Fisher Exact test*

Three episodes of TMA in this series occurred following pregnancy. Two of these presentations occurred in two subjects who were subsequently diagnosed with SLE. Both of these subjects experienced subsequent episodes of TMA exacerbation which were not associated with pregnancy, which suggests that in both of these cases SLE was the dominant aetiological factor of TMA. In both subjects there was no evidence of secondary antiphospholipid syndrome. One case of peripartum TMA occurred in an HIV positive subject. This subject had no other episodes of TMA or further pregnancies during the course of this series, and it is difficult to establish which of these two possible aetiologies was dominant.

### 3.2 Presenting features of HIV-associated TMA

The following presenting features were analysed in this study: pyrexia, neurological abnormality, serum haemoglobin concentration, LDH activity, presence of red cell fragments, reticulocyte production index (RPI), platelet count, D-dimer concentration, urea concentration, and creatinine concentration (table 3.2.1). All cases of TMA included in this series had systemic manifestations of microangiopathic haemolysis (anaemia, raised LDH activity and red cell fragments detected on peripheral blood smear) and consumptive thrombocytopenia (platelet count below laboratory normal). No cases of renal limited disease were identified during the course of this series.

*Table 3.2 1 Presenting features of TMA in HIV positive subjects*

|                                                     | Valid N<br>(%)* | N<br>(%)** | Median | IQR         | Range       |
|-----------------------------------------------------|-----------------|------------|--------|-------------|-------------|
| Oral temperature (°C)                               | 17 (44.7)       | 7 (41.2)   | 37.0   | 36.7 – 37.5 | 36.0 – 38.0 |
| LDH activity (IU/l)                                 | 38 (100)        | 38 (100)   | 1549   | 1208 – 2289 | 465 – 4554  |
| Fragments observed                                  | 38 (100)        | 38 (100)   |        |             |             |
| +                                                   |                 | 6 (15.8)#  |        |             |             |
| ++                                                  |                 | 5 (13.2)#  |        |             |             |
| +++                                                 |                 | 27 (71)#   |        |             |             |
| Platelet count (x10 <sup>9</sup> /mm <sup>3</sup> ) | 38 (100)        | 38 (100)   | 17     | 13 – 48     | 7 – 127     |
| Haemoglobin (g/dl)                                  | 38 (100)        | 38 (100)   | 6.35   | 5.0 – 7.1   | 2.8 – 9.3   |
| RPI                                                 | 29 (76.3)       | 21 (72.4)  | 1.9    | 0.9 – 3.1   | 0.03 – 9.2  |
| D-dimer (mg/l)                                      | 23 (60.5)       | 23 (100)   | 3.53   | 2.4 – 5.4   | 0.3 – 20.0  |
| Urea (mmol/l)                                       | 38 (100)        | 27 (71.1)  | 10.3   | 5.7 – 14.6  | 1.7 – 36.9  |
| Creatinine (µmol/l)                                 | 38 (100)        | 28 (73.4)  | 127    | 97 – 252    | 50 – 651    |
| Neurological deficit                                |                 | 25 (65.8)  |        |             |             |
| Confusion                                           |                 | 15 (60)##  |        |             |             |
| Headache / dizziness                                |                 | 5 (20)##   |        |             |             |
| Generalized seizure                                 |                 | 3 (12)##   |        |             |             |
| Focal deficit                                       |                 | 2 (8)##    |        |             |             |
| CD4 count (x10 <sup>6</sup> /mm <sup>3</sup> )      | 37 (97.4)       | 36 (94.7)  | 115    | 42 – 250    | 6 – 744     |

\* Valid N = subjects for whom data is available, % = % of series as a whole, \*\*N = subjects with abnormal values, % = % of subjects with abnormal values of those subjects with available data, #% of all subjects with fragments ##% of all subjects with neurological deficit

41.2% of subjects presenting with HIV-associated TMA in whom oral temperature was documented on admission were pyrexial, and 65.8% had neurological abnormality. The presence of pyrexia or

neurological deficit was not more frequent in HIV positive subjects when compared to HIV negative subjects in this series (two-tailed Fisher Exact test  $p = 0.624$  and  $p = 0.657$  respectively). Measured oral temperature was not significantly different in HIV positive subjects at presentation ( $p = 0.505$  in two-tailed Mann Whitney U test) (table 3.2.2).

*Table 3.2.2 Comparison of presenting features by HIV infection status*

|                                              | <i>HIV positive subjects</i> | <i>HIV negative subjects</i> | <i>p</i> |
|----------------------------------------------|------------------------------|------------------------------|----------|
| Pyrexia                                      | 7 (41.2)*                    | 5 (62.8)*                    | 0.624#   |
| Neurological abnormality                     | 25 (65.8)*                   | 7 (87.5)*                    | 0.657#   |
| Oral temperature (°C)                        | 37.0 (36.7 – 37.5)**         | 37.5 (37 – 37.8)**           | 0.505##  |
| LDH activity (IU/l)                          | 1549 (1208 – 2289)**         | 1184 (1000 – 1791)**         | 0.374##  |
| Platelet count ( $\times 10^9/\text{mm}^3$ ) | 17 (13 – 48)**               | 24 (11 – 55)**               | 0.898##  |
| Haemoglobin (g/dl)                           | 6.35 (5.0 – 7.1)**           | 6.7 (5.0 – 8.6)**            | 0.416##  |
| RPI                                          | 1.9 (0.9 – 3.1)**            | 3.7 (2.3 – 5.4)**            | 0.049##  |
| D-dimer (mg/l)                               | 3.53 (2.4 – 5.4)**           | 1.1 (0.58 – 1.4)**           | 0.018##  |
| Urea (mmol/l)                                | 10.3 (5.7 – 14.6)**          | 7.9 (4.9 – 11.5)**           | 0.234##  |
| Creatinine ( $\mu\text{mol/l}$ )             | 127 (97 – 252)**             | 92 (79 – 111)**              | 0.019##  |

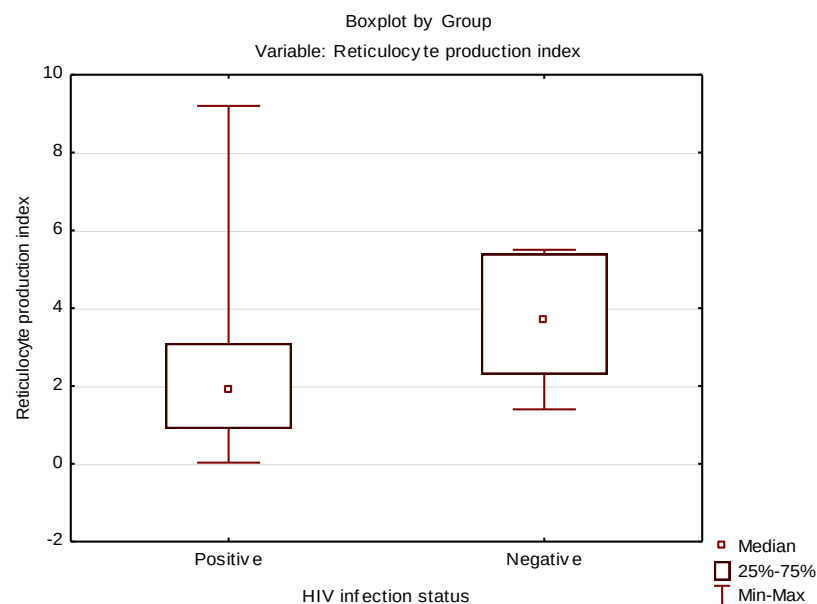
\* *N* (% of total), \*\*median (IQR), #Fisher Exact test, ##Mann Whitney U test

The most commonly recorded form of neurological deficit in subjects presenting with HIV-associated TMA was confusion of varying severity ( $n = 15$  cases, 60% of all recorded neurological deficit in the HIV positive subgroup, and occurring in 39.47% of all TMA presentations in HIV positive subjects). In one of these recorded cases of confusion subsequent investigation revealed associated cryptococcal meningitis. Generalized tonic-clonic seizures were recorded in three presentations of this subgroup (12% of neurological abnormality in this subgroup, occurring in 7.90% of all TMA presentations in HIV positive subjects). Two subjects developed focal neurological deficit, in both cases this took the form of hemiplegia (8% of all neurological deficit recorded in this subgroup during the course of this series, and 5.26% of all presentations of HIV-associated TMA). Both of these subjects underwent CT scanning of the brain which revealed no obvious abnormality, subsequent lumbar puncture was also normal, and in both cases neurological deficit resolved with successful treatment of TMA. In five presentations neurological deficit was evidenced by headache and dizziness (representing 20% of all documented

neurological abnormality in the HIV positive subgroup, and 13.16% of all presentations of HIV-associated TMA). In contrast, in HIV negative subjects, neurological abnormality was mild involving only headache and / or dizziness; confusion, seizure and focal deficit were not observed.

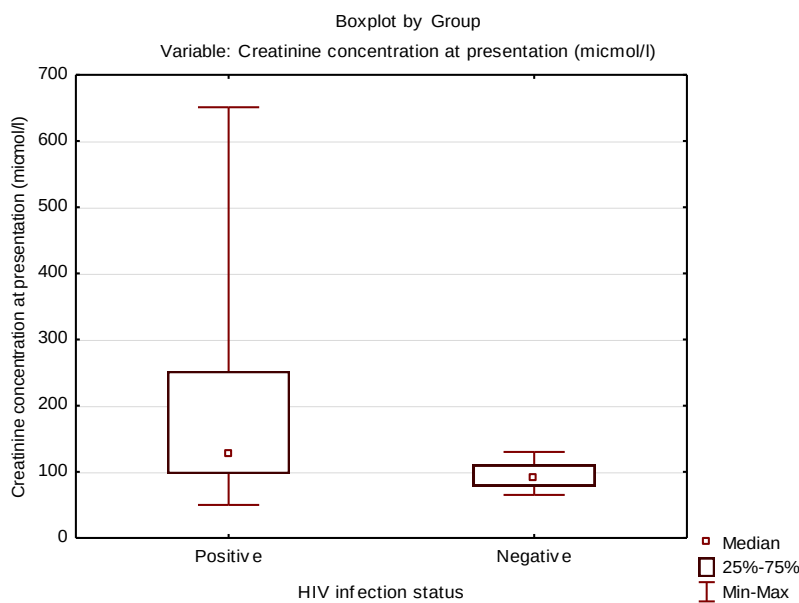
HIV-associated TMA in this series was not shown statistically to be associated with more severe microangiopathic haemolysis or consumptive coagulopathy as evidenced by haemoglobin concentration, LDH and platelet count (two-tailed Mann Whitney U test  $p = 0.416$ ,  $p = 0.374$ ,  $p = 0.989$  respectively) (table 3.2.2). Interestingly, RPI was significantly lower in subjects with HIV-associated TMA ( $p = 0.049$ ) (table 3.2.2, figure 3.2.1).

*Figure 3.2 1 Box and whisker plot, comparison of RPI by HIV infection status*



Renal dysfunction as evidenced by serum creatinine was significantly more severe in HIV-associated TMA (two-tailed Mann Whitney U test  $p = 0.019$ ), and in 28 presentations (73.4%) presenting serum creatinine concentration exceeded the laboratory normal of  $100\mu\text{mol/l}$  (table 3.2.2, figure 3.2.2).

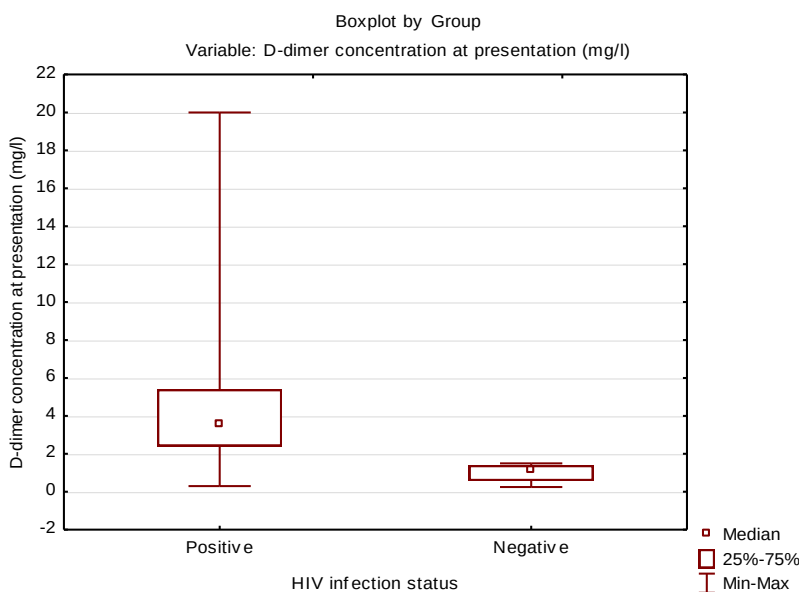
Figure 3.2 2 Box and whisker plot, comparison of presenting creatinine concentration by HIV infection status



Consistent with previous reports, D-dimer concentration was higher in HIV-associated TMA ( $p = 0.018$ )

(2) (table 3.2.2, figure 3.2.3).

Figure 3.2 3 Box and whisker plot, comparison of presenting D-dimer concentration by HIV infection status



The median CD4 count in subjects presenting with HIV-associated TMA was  $115 \times 10^6/\text{mm}^3$  (IQR 42 –  $250 \times 10^6/\text{mm}^3$  and range 6 –  $744 \times 10^6/\text{mm}^3$ ). 91.89% of all subjects presenting with HIV-associated TMA met the WHO definition of advanced HIV infection (CD4 count below  $350 \times 10^6 / \text{mm}^3$ ) (109), and 64.86% of these subjects met the Centers for Disease Control definition of AIDS (CD4 count below  $200 \times 10^6 / \text{mm}^3$ ) (111). Analysis of a potential association between severity of TMA at presentation (as indicated by haemoglobin concentration, LDH activity, platelet count, D-dimer level, and urea and creatinine concentration) and severity of HIV infection (as determined by CD4 count) was made using the Spearman Rank Order Correlation test. No significant association was shown for any of these analysed parameters (for haemoglobin,  $p = 0.481$ , for LDH,  $p = 0.241$ , for platelet count,  $p = 0.093$ , for D-dimer,  $p = 0.849$ , for urea,  $p = 0.247$ , for creatinine,  $p = 0.178$ ) (table 3.2.3).

*Table 3.2 3 Correlation of CD4 count with presenting parameters, HIV positive subjects*

|                                              | N  | R      | p     |
|----------------------------------------------|----|--------|-------|
| LDH activity (IU/l)                          | 37 | 0.197  | 0.241 |
| Platelet count ( $\times 10^9/\text{mm}^3$ ) | 37 | -0.280 | 0.093 |
| Haemoglobin (g/dl)                           | 37 | -0.119 | 0.481 |
| RPI                                          | 28 | 0.228  | 0.242 |
| D-dimer (mg/l)                               | 22 | 0.043  | 0.849 |
| Urea (mmol/l)                                | 37 | -0.195 | 0.247 |
| Creatinine ( $\mu\text{mol/l}$ )             | 37 | -0.226 | 0.178 |

*Spearman Rank Order method*

Further analysis of the possibility of an effect for severity of HIV infection as indicated by CD4 count in determining the severity of TMA presentation was undertaken by comparing presenting parameters between subjects grouped by CD4 count into those with and without advanced HIV infection (World Health Organization definition, CD4 count  $< 350 \times 10^6 / \text{mm}^3$ ) (106) and those with and without a CD4 count indicative of AIDS (Centers for Disease Control definition, CD4 count  $< 200 \times 10^6 / \text{mm}^3$ ) (111). In the former analysis the small number of patients with a CD4 count  $> 350 \times 10^6 / \text{mm}^3$  ( $n = 3$ ) precluded meaningful statistical analysis; nevertheless, analysed parameters showed striking similarity between the two groups (table 3.2.4). In the latter analysis, two-tailed Mann Whitney U testing failed to show

statistically significant differences in oral temperature, haemoglobin concentration, LDH activity, RPI, platelet count, D-dimer level, urea concentration or creatinine concentration ( $p = 0.290$ ,  $p = 0.962$ ,  $p = 0.291$ ,  $p = 0.781$ ,  $p = 0.519$ ,  $p = 0.821$ ,  $p = 0.766$ , and  $p = 0.545$  respectively) (table 3.2.4).

*Table 3.2 4 Comparison of presenting parameters in HIV positive subjects according to CDC and WHO definitions of advanced infection*

|                                              | CDC criteria          |                      |      | WHO criteria         |                     |
|----------------------------------------------|-----------------------|----------------------|------|----------------------|---------------------|
|                                              | CD4 < 200*            | CD4 > 200*           | p    | CD4 < 350*           | CD4 > 350*          |
| LDH activity (IU/l)                          | 1489<br>(1063–2287)   | 1900<br>(1337–2451)  | 0.29 | 1574<br>(1101–2289)  | 2509<br>(1524–3493) |
| Platelet count ( $\times 10^9/\text{mm}^3$ ) | 19<br>(12 – 62)       | 17<br>(13 – 26)      | 0.51 | 18<br>(13 – 54)      | 12<br>(7 – 17)      |
| Haemoglobin (g/dl)                           | 6.4<br>(4.6 – 7.4)    | 6.5<br>(5.2 – 7.0)   | 0.96 | 6.4<br>(5.0 – 7.2)   | 5.35<br>(3.8 – 6.9) |
| RPI                                          | 1.8<br>(1.1 – 3.1)    | 2.1<br>(0.6 – 3.6)   | 0.78 | 1.95<br>(0.9 – 3.1)  | 2.4<br>(0.6 – 4.2)  |
| D-dimer (mg/l)                               | 2.8<br>(1.65 – 11.95) | 4.15<br>(2.4 – 5.4)  | 0.82 | 3.53<br>(2.4 – 5.4)  | 1.3<br>(n = 1)      |
| Urea (mmol/l)                                | 10.1<br>(5.4 – 14.5)  | 10.1<br>(6.9 – 14.4) | 0.77 | 10.1<br>(5.7 – 14.6) | 8.1<br>(2.8 – 13.4) |
| Creatinine ( $\mu\text{mol/l}$ )             | 132<br>(101 – 265)    | 125<br>(91 – 198)    | 0.54 | 128<br>(97 – 252)    | 88<br>(59 – 116)    |

\*CD4  $\times 10^6/\text{mm}^3$ . Data is expressed as median (IQR)

### 3.2.1 D-dimer level and HIV-associated TMA

It has previously been suggested that higher D-dimer levels observed in cases of HIV-associated TMA may be indicative of an underlying unique pathophysiology of this disorder (2). Since D-dimer levels are representative of microthrombus formation (68) and since HIV-associated TMA may be more severe in presentation (51), the association of D-dimer levels with presenting parameters and with target organ injury in this series was tested; however, no statistically significant association was shown for D-dimer levels at presentation and presenting haemoglobin concentration ( $p = 0.203$ ), LDH activity ( $p = 0.084$ ), platelet count ( $p = 0.715$ ), urea concentration ( $p = 0.242$ ) or creatinine ( $p = 0.364$ ) in subjects diagnosed with HIV-associated TMA (Spearman Rank Order Correlation test).

No statistically significant difference was found in D-dimer levels between subjects with neurological deficit (median D-dimer 2.85mg/l, IQR 2.4– 6.8mg/l) and those with no neurological deficit (median D-dimer 5.4mg/l, IQR 3.9 – 5.4mg/l) using the Mann Whitney U test ( $p = 0.526$ ).

Although D-dimer levels were not shown to be correlated with presenting serum creatinine, additional analysis of the possible association between this potential marker of microthrombus formation and target organ injury was performed by testing for statistically significant difference in D-dimer levels between those subjects with HIV-associated TMA and renal dysfunction as evidenced by serum creatinine above the laboratory normal of 100 $\mu$ mol/l and those without evidence of renal dysfunction as evidenced by serum creatinine below laboratory normal of 100 $\mu$ mol/l. Although D-dimer levels were higher in those subjects with serum creatinine above 100 $\mu$ mol/l (median 3.9mg/l vs. 1.1mg/l), this difference was not statistically significant ( $p = 0.132$ ).

### **3.2.2 The role of infection in the severity of presentation of HIV-associated TMA**

HIV infection increases the risk of opportunistic infection (112), and such infection may exert a significant effect on several parameters associated with presentation with TMA. The reported prevalence of anaemia amongst hospitalized subjects ranges between 55% in those with mild infection to 70 – 90% in those with severe sepsis (113, 114) resulting from a complex underlying pathophysiology involving a combination of haemodilution, blood sampling, impaired erythropoiesis, altered iron metabolism, and (critically for this study) haemolysis (114, 115). LDH is often significantly elevated in the serum of HIV positive subjects with *Pneumocystis jiroveci* pneumonia (116) but levels may also rise in other lower respiratory tract infections (117), and murine models indicate that systemic infection with bacteria including *Salmonella* species produces dramatic increases in serum LDH (118).

Thrombocytopenia is a not uncommon finding in infection occurring in 20 – 40% of septic subjects

(114, 119). D-dimer levels may also be increased by infection (120), and sepsis is a common cause of acute kidney injury in HIV positive subjects, accounting for up to 84% of all such cases (121).

In order to investigate whether concomitant infection may have contributed to the apparent severity of presentation of HIV-associated TMA, comparison was made of the presenting features of TMA in those subjects with evidence of infection versus those without such evidence (table 3.2.2.1).

*Table 3.2.2 1 Comparison of presenting parameters, HIV positive subjects with and without concomitant infection*

|                                                     | <i>Subjects with<br/>concomitant infection</i> | <i>Subjects without<br/>concomitant infection</i> | <i>p*</i> |
|-----------------------------------------------------|------------------------------------------------|---------------------------------------------------|-----------|
| Oral temperature (°C)                               | 37.0 (36 – 37.8)                               | 37.0 (37 – 37.5)                                  | 0.721     |
| LDH activity (IU/l)                                 | 1763 (1335 – 2451)                             | 1498 (1208 – 2285)                                | 0.305     |
| Platelet count (x10 <sup>9</sup> /mm <sup>3</sup> ) | 17 (14 – 48)                                   | 15 (13 – 32)                                      | 0.657     |
| Haemoglobin (g/dl)                                  | 6.4 (5.2 – 7.4)                                | 5.8 (4.5 – 7.0)                                   | 0.526     |
| RPI                                                 | 1.1 (0.6 – 3.6)                                | 2.1 (1.7 – 3.0)                                   | 0.347     |
| D-dimer (mg/l)                                      | 3.92 (2.5 – 5.4)                               | 2.4 (0.9 – 4.0)                                   | 0.284     |
| Urea (mmol/l)                                       | 10.8 (7.2 – 15)                                | 9.7 (5.0 – 13.9)                                  | 0.465     |
| Creatinine (μmol/l)                                 | 125 (101 – 239)                                | 121 (89 – 375)                                    | 0.950     |
| CD4 count (x10 <sup>6</sup> /mm <sup>3</sup> )      | 229 (31 – 285)                                 | 82 (55 – 170)                                     | 0.359     |

*\*Mann Whitney U test. Data is expressed as median (IQR).*

22 presentations of HIV-associated TMA were associated with evidence of concomitant infection (57.89% of all presentations of HIV-associated TMA).

The following lists the concomitant infectious diseases diagnosed in subjects presenting with TMA during the course of this series (in descending order of frequency):

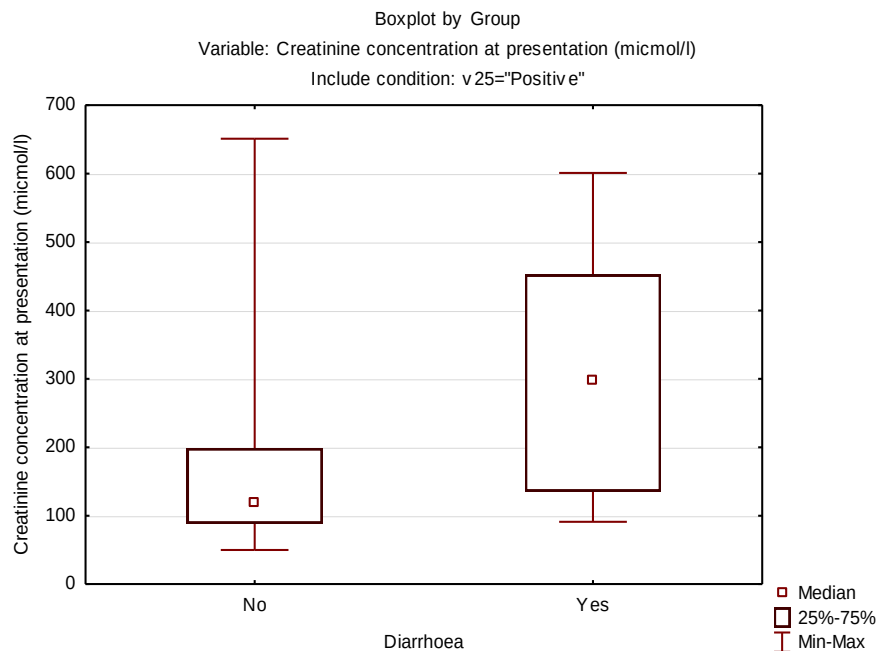
1. Disseminated / extrapulmonary tuberculosis (n = 7, 30.43% of all concurrent infectious diseases)
2. Gastroenteritis (n = 6, 26.08%)
3. Pulmonary tuberculosis (n = 2, 8.70%)
4. Urinary tract infection (n = 2, 8.70%)
5. *Pneumocystis jiroveci* pneumonia (n = 2, 8.70%)

6. Community acquired pneumonia (n = 2, 8.70%)
7. Bacterial meningitis (n = 1, 4.35%)
8. *Cryptococcus neoformans* meningitis (n = 1, 4.35%)

Presenting serum LDH activity and D-dimer level were higher in HIV positive subjects with evidence of infection, although these differences did not reach statistical significance ( $p = 0.305$  and  $p = 0.284$  respectively). Statistically significant difference was similarly not shown for presenting oral temperature (in frequency or severity) haemoglobin concentration, platelet count, urea concentration, creatinine concentration, and frequency of neurological deficit between these two groups. Surprisingly, CD4 count was higher in those subjects with evidence of infection, although this difference too did not reach statistical significance.

In view of the prevalence of gastroenteritis as a superadded infection in this series and the potential for dehydration due to gastro-intestinal fluid loss to contribute to renal dysfunction, further subanalysis was performed to evaluate severity of renal dysfunction as evidenced by creatinine in HIV positive subjects admitted with concomitant diarrhoea compared to HIV positive subjects without concomitant diarrhoea. The median presenting creatinine in subjects with diarrhoea was  $297\mu\text{mol/l}$  (range 91 – 601 and IQR 136 – 452 $\mu\text{mol/l}$ ) compared to a median creatinine in subjects without diarrhoea of  $119\mu\text{mol/l}$  (range 50 – 651, IQR 89 – 198 $\mu\text{mol/l}$ ). Although suggestive that concomitant diarrhoea may have played a role in determining the severity of renal dysfunction in the presentation of HIV-associated TMA it should be noted that this difference did not reach statistical significance ( $p = 0.057$ ) (figure 3.2.2.1).

*Figure 3.2.2 1 Box and whisker plot, comparison of presenting creatinine concentration in HIV positive subjects with and without concomitant diarrhoea*



When subjects who presented with concomitant diarrhoea were excluded from analysis presenting serum creatinine remained significantly higher in those with HIV-associated TMA compared to those HIV negative subjects presenting with the disorder (for HIV positive subjects without diarrhoea median creatinine was 125 $\mu$ mol/l with IQR 89 - 198 $\mu$ mol/l and range 50 – 651 $\mu$ mol/l, for HIV negative subjects without diarrhoea median creatinine was 91.5 $\mu$ mol/l with IQR 78.5 – 110.5 $\mu$ mol/l and range 63 – 130 $\mu$ mol/l;  $p = 0.028$ ). This suggests that whilst infectious diarrhoea may have played a role in affecting the severity of renal dysfunction in some cases of HIV-associated TMA, the disorder itself plays an important role in the apparently more severe renal dysfunction seen in HIV positive subjects.

In conclusion, although opportunistic infection was a frequent concomitant diagnosis in subjects with HIV-associated TMA, the presence of infection does not appear to have significantly contributed to the abnormality of any of the parameters used in assessing the severity of the disorder.

### 3.3 Response to treatment of HIV-associated TMA

The standard therapeutic protocol for HIV-associated TMA used at the Helen Joseph Hospital consists of plasma exchange using FFP as replacement fluid; plasma infusion as sole therapy is not used.

Cryosupernatant plasma is substituted for FFP as plasma exchange replacement fluid in patients demonstrating a poor response to this initial treatment. In addition to plasmapheresis, all patients are initiated onto cART. Oral prednisone (1mg/kg per day) was routinely prescribed for the treatment of HIV-associated TMA until 2008, following which prednisone was used only in refractory cases of the disorder. Additional therapies such as other immunosuppressive drugs or splenectomy were not utilized for patients with HIV-associated TMA during the course of this series.

Thirty-two separate courses of plasma exchange were performed on 29 subjects presenting with HIV-associated TMA (including episodes of recurrent disease) during the course of the study period; six subjects demised before therapy could be instituted. Thus, 84.21% of all cases of HIV-associated TMA received plasma exchange therapy. Three subjects demised despite receiving plasma exchange (10.34% of all HIV positive subjects receiving plasma exchange, and 9.38% of plasma exchange therapy courses). In two of these subjects cause of death was ascribed to sepsis; one subject demised as a consequence of suspected pulmonary thromboembolism. Excluding these mortalities there were thus a total of 26 survivors of HIV-associated TMA undergoing 29 separate courses of plasma exchange available for analysis of therapeutic response (table 3.3.1).

Induction of remission as indicated by normalization of both platelet count and LDH activity to laboratory reference range for 2 consecutive days (as defined by the British Committee for Standards in Haematology) (79, 80) was attained in survivors of HIV-associated TMA after a median of 11 exchanges (range, 7 – 24 and IQR 8 - 15). Platelet count responded with greater rapidity than LDH activity (median

number of exchanges to normalize platelet count = 6 vs. median number of exchanges to normalize LDH activity = 11).

Haemoglobin concentration rose by a median of 3.7 g/dl to a median of 10.2g/dl in response to therapy involving plasma exchange; it is likely that this improvement was facilitated by transfusion in many cases.

Renal dysfunction similarly demonstrated improvement in response to plasma exchange with creatinine decreasing by a median of 37.0 $\mu$ mol/l to a median of 79 $\mu$ mol/l.

*Table 3.3 1 Therapeutic response of TMA to plasmapheresis in HIV positive subjects*

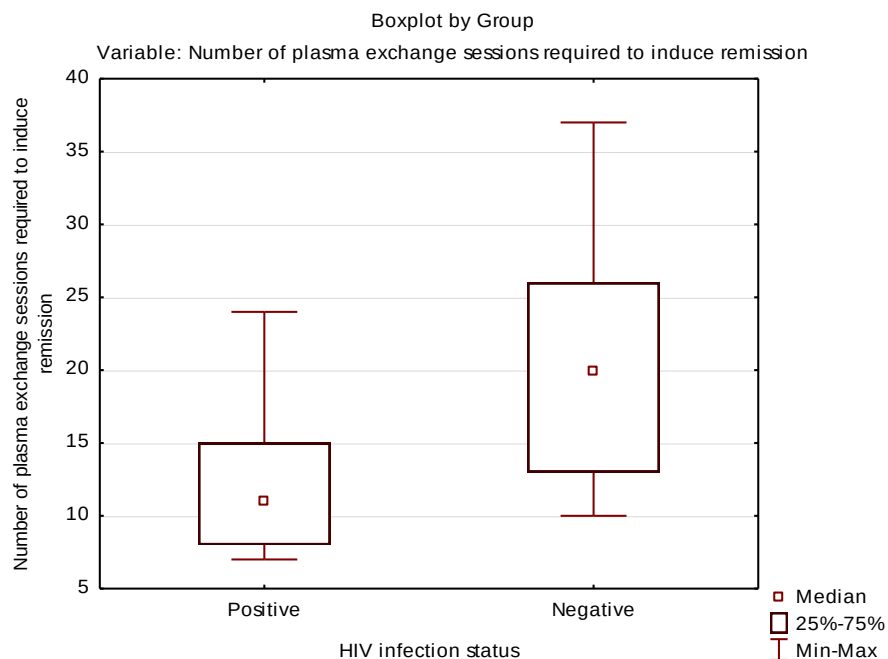
|                                                                                                    | Valid N<br>(% of total) | Median | IQR        | Range      |
|----------------------------------------------------------------------------------------------------|-------------------------|--------|------------|------------|
| Number of plasma exchanges required to induce remission                                            | 29 (100)                | 11     | 8 - 15     | 7 - 24     |
| Number of plasma exchanges required to normalize platelets                                         | 29 (100)                | 6      | 5 - 10     | 0 - 24     |
| Number of plasma exchanges required to normalize LDH                                               | 29 (100)                | 11     | 8 - 15     | 7 - 24     |
| Haemoglobin concentration following plasma exchange (g/dl)                                         | 29 (100)                | 10.2   | 8.9 - 11.1 | 7.1 - 17.1 |
| Haemoglobin concentration change from presentation in response to plasma exchange                  | 29 (100)                | 3.7    | 2.5 - 6.1  | 1.4 - 10.1 |
| Creatinine concentration following plasma exchange ( $\mu$ mol/l)                                  | 29 (100)                | 79     | 71 - 93    | 42 - 371   |
| Change in creatinine concentration from presentation in response to plasma exchange ( $\mu$ mol/l) | 29 (100)                | 37     | 8 - 74     | -180 -311* |

*\*5 subjects (17.2% of subjects receiving plasmapheresis) demonstrated a deterioration in creatinine following therapy, the median increase in creatinine in these patients from presentation was 15 $\mu$ mol/l with IQR 6 - 177 $\mu$ mol/l)*

The response of neurological dysfunction to plasma exchange is difficult to retrospectively analyze; nevertheless, it is significant that both cases of focal neurological deficit in this series appear to have disappeared in response to plasma exchange.

In order to further evaluate the responsiveness of HIV-associated TMA to plasma exchange, data from HIV positive survivors of TMA was compared to that from HIV negative subjects receiving plasma exchange at the Helen Joseph Hospital during the same time period ( $n = 7$ ). HIV-associated TMA was found to respond more rapidly than TMA in HIV negative subjects ( $n = 7$ ), requiring fewer plasma exchange sessions to induce remission (median number of plasma exchanges to induce remission in HIV-associated TMA = 11 vs. median number of plasma exchanges to induce remission in HIV negative subjects with TMA = 20,  $p = 0.013$ ) (figure 3.3.1).

*Figure 3.3 1 Box and whisker plot, comparison of number of plasmapheresis sessions required to induce remission by HIV infection status*



As further evidence of the responsive nature of HIV-associated TMA to plasma exchange, it is noted that no statistically significant difference remained in creatinine concentration following plasma exchange therapy (as compared to a significantly higher creatinine in subjects with HIV-associated TMA at presentation) ( $p = 0.873$  (figure 3.3.2)), and that the improvement in creatinine from presentation baseline was significantly larger in subjects with HIV-associated TMA ( $p = 0.027$ ) (figure 3.3.3).

Figure 3.3 2 Box and whisker plot, comparison of creatinine concentration following induction of remission by plasmapheresis between HIV infection status groups

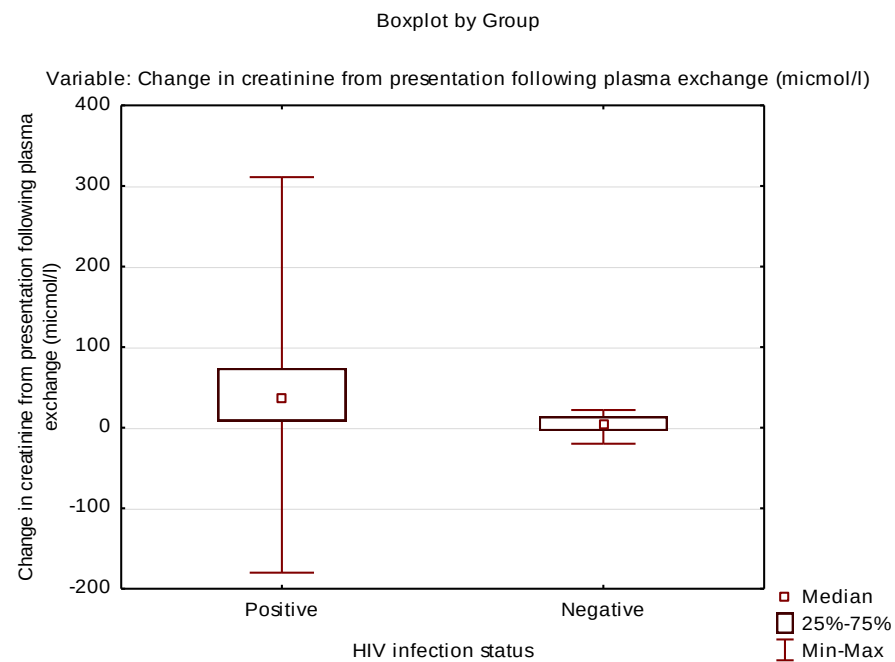
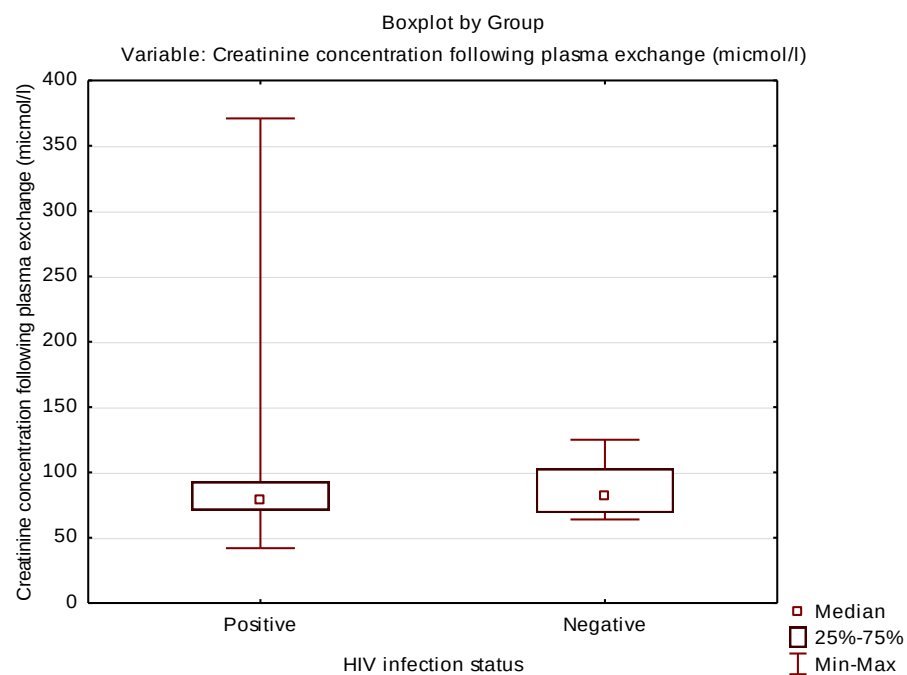


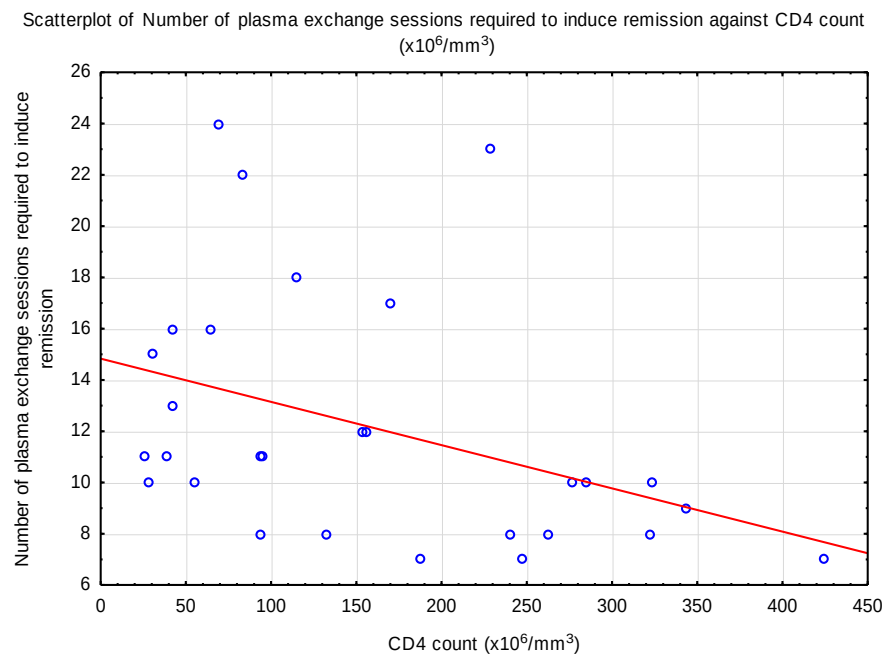
Figure 3.3 3 Box and whisker plot, comparison of change in creatinine concentration from baseline following induction of remission by plasmapheresis between HIV infection status subgroups



It is possible that response to plasma exchange may be affected by 3 main factors; viz.: severity of TMA at presentation (before initiation of plasma exchange), severity of HIV infection, and choice of plasma infusant used. To investigate these hypotheses, association was tested between presenting parameters and CD4 count and the number of plasma exchanges required to induce remission (analysis of the effect of plasma infusant is presented separately below).

Whilst presenting parameters (haemoglobin concentration, LDH activity, platelet count, D-dimer concentration, creatinine concentration) did not show statistically significant correlation ( $p = 0.586$ ,  $p = 0.475$ ,  $p = 0.574$ ,  $p = 0.763$ , and  $p = 0.477$  respectively), inverse correlation was found for severity of HIV infection as determined by CD4 count and the number of plasma exchanges required to induce remission ( $R = -0.496$ ,  $p = 0.006$ ) (figure 3.3.4).

*Figure 3.3 4 Correlation plot, number of plasma exchange sessions required to induce remission and CD4 count at presentation*



In further univariate regression analysis using the least squares method, CD4 count at presentation was also shown to be a weak predictor of the number of plasma exchanges required to induce remission ( $R = 0.396$ ,  $R^2 = 0.157$ ,  $F = 5.01$ ,  $p = 0.034$ ).

### **3.3.1 Effect of plasma infusant choice on therapeutic response**

In view of controversy regarding the potential for choice of plasma infusant to affect therapeutic response (84, 85), subanalysis was performed of the response of HIV-associated TMA to plasma exchange using FFP compared to cryosupernatant as plasma infusant. In 6 of the 29 separate courses of successful plasma exchange therapy described above, initial FFP was substituted by cryosupernatant plasma. These therapeutic courses were excluded from the following analysis, giving a total of 23 courses of plasma exchange were performed using only FFP ( $n = 17$ ) or only cryosupernatant plasma ( $n = 6$ ).

In HIV-associated TMA, the use of cryosupernatant plasma did not result in a significant difference in the number of exchanges required to induce remission (median number of plasma exchanges in subjects receiving FFP = 10 with IQR 10 – 12, median number of plasma exchanges in subjects receiving cryosupernatant plasma = 10 with IQR 10 – 11;  $p = 0.944$ ). Although haemoglobin concentration following plasma exchange was slightly higher in subjects receiving cryosupernatant plasma (median haemoglobin 11.2g/dl vs. 10.4 g/dl) and creatinine following plasma exchange slightly lower (median creatinine 62mmol/l vs. 79mmol/l), these differences were not statistically significant ( $p = 0.806$  and  $p = 0.175$  respectively).

### **3.3.2 Complications of plasma exchange**

Of 32 separate courses of plasma exchange prescribed for HIV-associated TMA during the course of this study, 29 were associated with a complication thereof (90.63%). Many subjects experienced multiple adverse events during the same course of plasma exchange.

Complications of plasma exchange retrospectively identified in these series categorized as either major (imminently life-threatening) or minor (not imminently life-threatening) are listed below and presented graphically in figure 3.3.2.1.

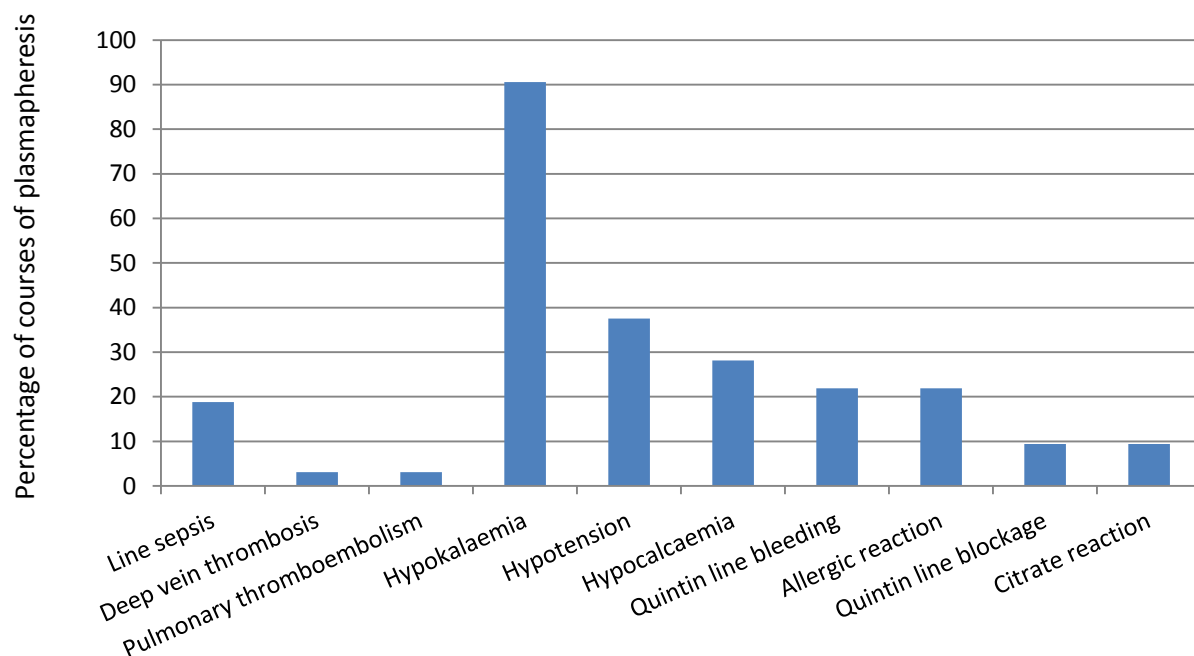
1. Major complications:

- a. Line sepsis (occurring in 6 courses, 18.8% of plasmapheresis courses)
- b. Deep vein thrombosis (1 course, 3.1%)
- c. Pulmonary thromboembolism (1 course, 3.1%)

2. Minor complications:

- a. Hypokalaemia (29 courses, 90.6%)
- b. Hypotension (12 courses, 37.5%)
- c. Hypocalcaemia (9 courses, 28.1%)
- d. Quintin line bleeding (7 courses, 21.9%)
- e. Allergic reaction (7 courses, 21.9%)
- f. Quintin line blockage necessitating line change (3 courses, 9.4%)
- g. Citrate reaction (3 courses, 9.4%)

Figure 3.3 5 Bar chart, complications of plasmapheresis in HIV-associated TMA



Line sepsis was defined for the purposes of this analysis as laboratory documented microbiological colonization of a Quintin line. Of a total of 6 episodes of proven line sepsis, in 3 cases a *Staphylococcus aureus* was cultured, whilst in one case each an *Enterococcus faecalis*, Coagulase negative *Staphylococcus*, and micrococcus species were cultured. All such episodes responded to Quintin line removal or replacement and appropriate antibiotic use.

One subject was found to have demised during a session of plasma exchange. Retrospective review of this event appears consistent with sudden cardiac death. No other biochemical abnormality was found which may have contributed to this subject death, and at contemporaneous Mortality and Morbidity meeting the likely cause of death was considered to have been pulmonary embolism.

The complications of hypokalaemia and hypocalcaemia were defined as the presence of a potassium or calcium serum level less than or equal to that of laboratory normal (for potassium, 3.5 mmol/l; for calcium, 2.15 mmol/l) occurring during the course of plasma exchange. The median hypokalaemic

reading observed was 3.0 mmol/l (range 2.2 – 3.3 mmol/l). The median hypocalcaemic reading observed was 1.98 mmol/l (range 1.58 – 2.04 mmol/l).

For the purposes of this analysis, hypotension was defined retrospectively as a blood pressure less than 90mmHg (systolic) and 60mmHg (diastolic) occurring during the course of a session of plasma exchange. The median hypotensive blood pressure recorded under this definition was 77/53mmHg. All such episodes responded to colloid volume challenge, and in none of these episodes was inotropic support required. No evidence could be found to suggest that any of these episodes contributed to patient death.

Bleeding occurred in 7 placements of Quintin lines for plasma exchange. In all cases these occurred immediately following line placement. In 3 of these episodes bleeding was limited to haematoma formation, and in the remaining four episodes this bleeding took the form of oozing from the entry site. No evidence could be found that these episodes contributed to hypotension, nor was evidence found to indicate that packed red cell transfusion was required as a direct result.

Quintin line blockage was retrospectively defined as episodes in which the notes of the plasma exchange nurse suggested this to be his / her explanation for plasma exchange machine dysfunction, and which led to line replacement. Three such episodes were encountered during the course of this series. A deep vein thrombosis (the diagnosis being retrospectively validated through review of contemporaneous femoral vessel Doppler) occurred in one patient as a result of Quintin line insertion.

Reactions to citrate, plasma infusant, and whole blood infusion were retrospectively analysed using contemporaneous plasma exchange nurse diagnoses. Plasma infusant reaction was most commonly diagnosed when subjects complained of a pruritic sensation during a session of plasma exchange, and was retrospectively deemed to have been of clinical significance when the nurse was shown to have considered the administration of an antihistamine or steroid to be necessary. The diagnosis of citrate

reaction was made when subjects reported the presence of paraesthesias most commonly described as “cramping” and usually affecting the digits during a session of plasma exchange. None of these episodes was shown to have been associated with patient death, and no record was found of any of these reactions having been serious enough to require intubation, inotrope use or ICU admission. No record was found to suggest a transfusion reaction during the course of this series.

### **3.4 The prognosis of HIV-associated TMA**

#### **3.4.1 Mortality**

Nine subject deaths were recorded during the period of this study; the mortality rate for HIV-associated TMA in this series was therefore 25.71%. This mortality subgroup comprised six female subjects and three male subjects, giving a female to male ratio of 2:1. All subjects were of Black African descent.

The median time to death was 1 day (range, 1 – 60 days); five subjects (55.56%) demised within 24 hours of presentation with HIV-associated TMA. Three subjects (33.33%) demised despite receiving plasma exchange, the remaining six subjects demised before plasma exchange could be instituted. Post mortem examination was not performed on any subject who demised during the course of this series, and cause of death is therefore here reported as recorded by the attending medical unit.

Sepsis was the leading ascribed cause of subject death occurring in 4 subjects (44.44%), followed by TMA occurring in 3 subjects (33.33%), whilst 1 subject (1.11%) each demised as a result of hyperkalaemia (caused by microangiopathic haemolysis on a background of renal dysfunction) and cardiac arrest precipitated by suspected pulmonary embolism. Amongst subjects who demised despite receiving plasma exchange, two cases were ascribed to sepsis and one as a consequence of suspected pulmonary thromboembolism complicating Quintin line use. In both cases of death due to sepsis the subject was admitted with signs of infection; it is therefore unclear whether these deaths were a result

of this pre-existing infection or due to acquired Quintin line sepsis or immunosuppression arising as a result of plasmapheresis.

Analysis of presenting features of HIV-associated TMA by mortality outcome is presented in table 3.4.1.

*Table 3.4 1 Comparison of presenting features of HIV-associated TMA by mortality outcome*

|                                                     | Survivor subgroup |                        | Mortality subgroup |                        | p         |
|-----------------------------------------------------|-------------------|------------------------|--------------------|------------------------|-----------|
|                                                     | Median            | IQR                    | Median             | IQR                    |           |
| Age (years)                                         | 32.5              | 27 - 38                | 41                 | 31 - 49                | 0.077*    |
| Oral temperature (°C)                               | 37.0              | 36.7 - 37.8            | 37.5               | 37.0 - 37.5            | 0.598*    |
| LDH activity (IU/l)                                 | 1563              | 1222 - 2245            | 1574               | 1475 - 3493            | 0.230*    |
| Platelet count (x10 <sup>9</sup> /mm <sup>3</sup> ) | 15                | 13 - 27                | 48                 | 17 - 75                | 0.070*    |
| Haemoglobin (g/dl)                                  | 6.5               | 5.2 - 7.1              | 5.6                | 4.5 - 6.3              | 0.410*    |
| RPI                                                 | 2.1               | 1.1 - 3.1              | 1.2                | 0.6 - 2.5              | 0.435*    |
| D-dimer (mg/l)                                      | 3.30              | 2.4 - 5.4              | 3.53               | 3.1 - 3.9              | 0.970*    |
| Urea (mmol/l)                                       | 9.4               | 4.8 - 13.4             | 22.5               | 14.4 - 31.2            | 0.0049*   |
| Creatinine (μmol/l)                                 | 117               | 89 - 175               | 298                | 239 - 518              | 0.0005*   |
| CD4 count (x10 <sup>6</sup> /mm <sup>3</sup> )      | 145               | 64 - 263               | 42.5               | 9.5 - 278              | 0.231*    |
|                                                     | N                 | % of total             | N                  | % of total             | p         |
| Sex                                                 | F = 20<br>M = 9   | F = 68.97<br>M = 31.03 | F = 6<br>M = 3     | F = 66.67<br>M = 33.33 | 1.000#    |
| Concomitant infection                               | 14                | 51.85                  | 7                  | 77.78                  | 0.252#    |
| Neurological deficit                                | 19                | 73.08                  | 6                  | 66.67                  | 0.694#    |
| Received plasma exchange                            | 29                | 100                    | 3                  | 33.33                  | < 0.0001# |

\*Mann Whitney U test, # Fisher Exact test

The median age in subjects who demised was higher than that in survivors of HIV-associated TMA (41 vs. 33 years), although this finding did not reach statistical significance (p = 0.077). Median CD4 count was lower in the mortality subgroup (42.5 x 10<sup>6</sup>/mm<sup>3</sup> vs. 133 x 10<sup>6</sup>/mm<sup>3</sup>); this too did not reach statistical significance (p = 0.231). No difference was observed in sex ratios between these two groups (p = 1.00). No statistically significant difference was observed in presenting oral temperature (p = 0.598), haemoglobin concentration (p = 0.410), LDH activity (p = 0.230), platelet count (p = 0.070) or D-dimer level (p = 0.970). Although a clinical suspicion for concomitant opportunistic infection was more frequent in the mortality subgroup (66.67% vs. 51.85% of cases), this difference was not statistically significant (p = 0.252).

Target organ injury in the form of neurological deficit was less frequent in the mortality subgroup (66.67% vs. 73.08% of cases). Neurological deficit in subjects who demised most commonly took the form of confusion (55.56% of cases). Two episodes of seizure were recorded. The observed difference in the frequency of neurological deficit between subjects who demised and survivors of HIV-associated TMA was not found to be statistically significant ( $p = 0.694$ ). In contrast, target organ injury in the form of renal dysfunction was significantly more severe in the mortality subgroup, as evidenced by a higher presenting median urea concentration (22.5mmol/l vs. 9.30mmol/l,  $p = 0.0049$ ) and a higher presenting median creatinine concentration (298 $\mu$ mol/l vs. 117 $\mu$ mol/l,  $p = 0.0005$ ).

Plasma exchange was employed with greater frequency in survivors of HIV-associated TMA than in those HIV positive subjects diagnosed with the disorder who demised during the period of this review (100% of survivors received exchange compared to 33.33% of mortalities); this difference was statistically significant ( $p = 0.00003$ ).

### **3.4.1 Residual target organ dysfunction**

Analysis of the frequency and severity of residual neurological system disturbance in this series is limited by the retrospective nature thereof since objective data in this regard is in general poorly recorded; however, no indication was found in review of clinical case notes to indicate the persistence of neurological deficit in subjects included in this series. Analysis of objectively verifiable residual target organ dysfunction in HIV-associated TMA is therefore restricted to renal dysfunction. This is further considered appropriate given that renal injury appears significantly more severe in HIV-associated TMA than in TMA occurring in HIV negative subjects in this series, whereas the frequency of recorded neurological deficit was not different in these two forms of the disorder (thus suggesting that the kidney is an important site of injury in HIV-associated TMA). Analysis of residual renal dysfunction is, however, limited by the short duration of follow-up time from cessation of plasma exchange to the last recorded

serum creatinine (median 3 days, range 0 – 32 days) and relatively low number of subjects ( $n = 18$ ), as a result of the high rate of subjects defaulting outpatient follow-up immediately following discharge from hospital, and routine renal function measurement not being used as a standard screening test for TMA recurrence in the outpatient department, as well as the exclusion of data from subjects with recurrent episodes of HIV-associated TMA from this analysis.

The median last recorded creatinine following cessation of plasmapheresis in in-patient HIV-positive survivors of TMA was  $79\mu\text{mol/l}$  (IQR  $66.5 - 113.5\mu\text{mol/l}$  and range  $49 - 316\mu\text{mol/l}$ ). Last recorded serum creatinine of greater than the laboratory normal of  $100\mu\text{mol/l}$  in in-patient survivors was noted in five cases (27.78% of the analysed subgroup); in this subgroup the median last recorded creatinine was  $169\mu\text{mol/l}$  (IQR  $118 - 285\mu\text{mol/l}$ , range  $109 - 316\mu\text{mol/l}$ ). Deterioration in renal function as evidenced by a higher serum creatinine at last recorded outpatient follow-up compared to presenting serum creatinine was observed in two subjects (increase in creatinine  $34\mu\text{mol/l}$  and  $150\mu\text{mol/l}$  respectively). No subject in this series required the initiation of long term renal replacement therapy.

Severity of HIV-associated TMA at presentation as evidenced by oral temperature, haemoglobin concentration, LDH activity and platelet count did not show correlation with last measured serum creatinine ( $p = 0.559$ ,  $p = 0.342$ ,  $p = 0.687$ , and  $p = 0.766$  respectively). In contrast, presenting serum creatinine was found to have significant positive correlation with last recorded creatinine ( $R = 0.485$ ,  $p = 0.041$ ) (figure 3.4.2.1), and D-dimer was found to have significant inverse correlation with this parameter ( $R = -0.740$ ,  $p = 0.0025$ ) (figure 3.4.2.2). No correlation was shown between either total number of plasma exchanges and number of plasma exchanges required to induce remission of TMA and severity of residual renal dysfunction as indicated by last recorded serum creatinine ( $p = 0.337$  and  $p = 0.217$  respectively).

Figure 3.4.2 1 Correlation plot, presenting creatinine concentration with last recorded creatinine concentration following induction of remission with plasmapheresis in HIV positive survivors of TMA

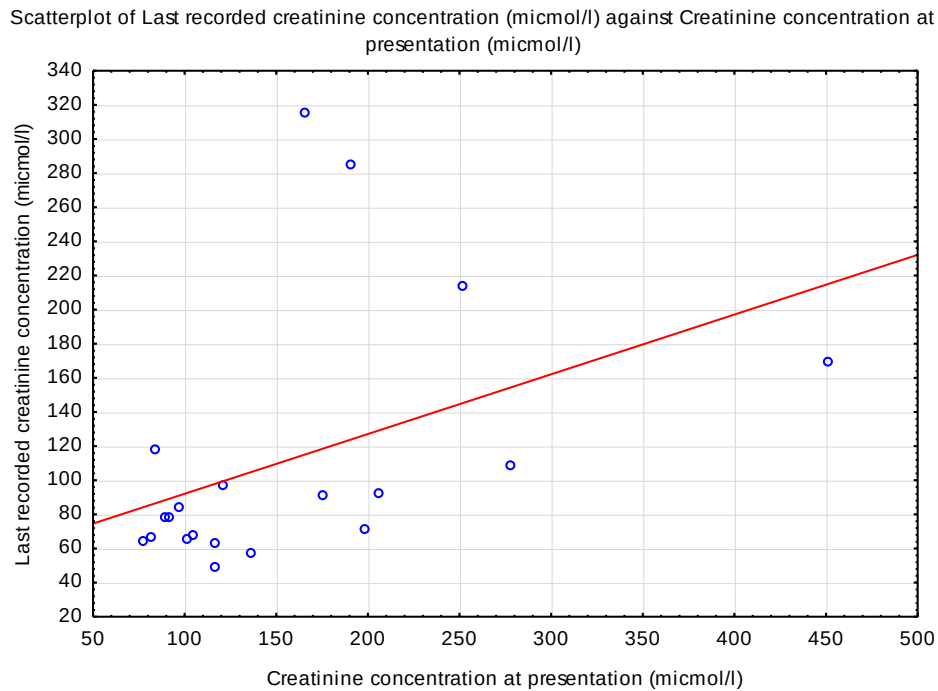
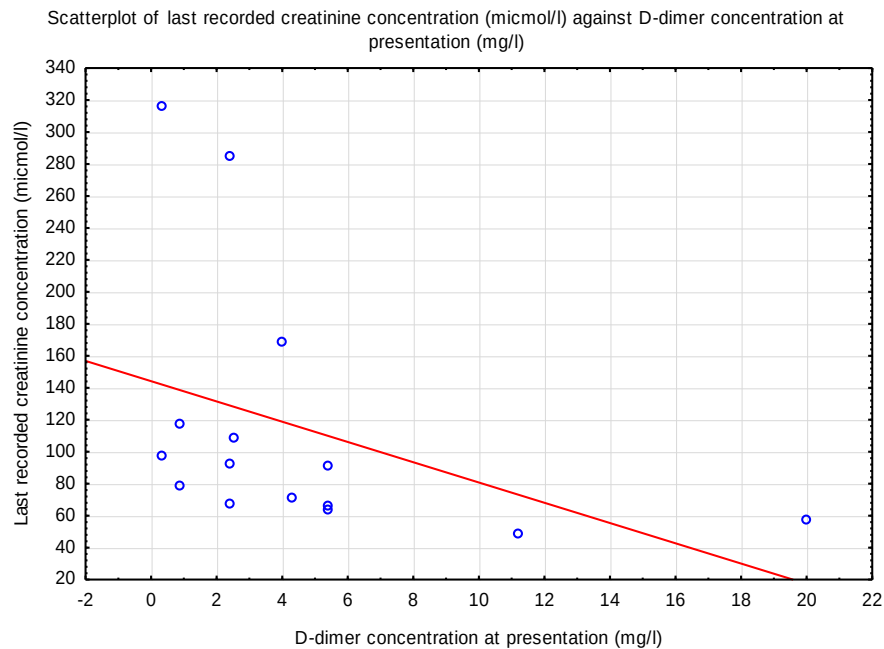


Figure 3.4.2 2 Correlation plot, presenting D-dimer concentration with last recorded creatinine concentration following induction of remission with plasmapheresis in HIV positive survivors of TMA



### 3.4.2 TMA Recurrence

International consensus classifies TMA recurrence as either exacerbation (recurrence occurring within 30 days of induction of remission) or relapse (recurrence occurring more than 30 days after induction of remission) (1, 79, 80). During the period of study, there were 2 cases of exacerbation and 1 case of relapse of HIV-associated TMA documented in 3 subjects (11.54% of HIV positive subjects in this series). In contrast, there were 4 episodes of TMA recurrence in 3 HIV negative subjects (75% of all HIV negative subjects) during the same study period; this difference was statistically significant ( $p = 0.018$  in two-tailed Fisher Exact testing).

Presentation with recurrent HIV-associated TMA was associated with a higher haemoglobin concentration (median 6.9g/dl with range 3.9 – 8.6g/dl) compared to index presentation in the same subjects (median 6.1g/dl with range 4.1 – 9.3g/dl). LDH activity was also lower at recurrence (median 1101IU/l, range 465 – 3410IU/l vs. median 2221IU/l, range 1208 – 2285IU/l at index presentation). Platelet count was higher at recurrence (median  $18 \times 10^9/\text{mm}^3$ , range  $10 - 127 \times 10^9/\text{mm}^3$  vs.  $11 \times 10^9/\text{mm}^3$ , range  $10 - 31 \times 10^9/\text{mm}^3$ ), and creatinine was lower at recurrence (median 128 $\mu\text{mol/l}$ , range 113 – 252 $\mu\text{mol/l}$  vs. median 191 $\mu\text{mol/l}$ , range 97 – 375 $\mu\text{mol/l}$ ). In view of the small numbers of subjects ( $n = 3$ ), meaningful statistical analysis of the significance of these observed differences was however not possible.

All patients presenting with recurrent HIV-associated TMA were treated with plasmapheresis using cryosupernatant plasma as replacement fluid. Two patients (66.67% of patients with recurrence) received in addition oral prednisone.

## 4. Discussion

### 4.1 The Epidemiology and Demographics of HIV-associated TMA

Analysis of this series indicates that HIV infection was the most common aetiological factor involved in the development of TMA diagnosed at Helen Joseph Hospital between 2001 and 2009, with 89.74% of subjects presenting with the disorder being HIV positive. This finding is consistent with Gunther et al's report that HIV was the dominant cause of TMA in the local context (54). In most cases, TMA was the presenting feature of HIV infection with 65.71% of subjects being diagnosed at presentation with HIV.

Interrogation of data in this series indicates that HIV-associated TMA demonstrates a predilection to affect young Black women. It is likely however that the epidemiological patterns of HIV infection in South Africa have contributed substantially to this observed pattern. South Africans of Black African descent, and women in particular, are known to have the highest prevalence rates of HIV infection of all population groups (64, 122) and Black African women of reproductive age have been determined to have the highest seroprevalence rate of HIV infection of any race or age group in South Africa at 32.7% (123). Thus, demographic patterns of HIV-associated TMA in this single center series parallels those of HIV infection in South Africa.

Such considerations are likely to account for the similarity this study shares with the only other reported series of HIV-associated TMA from South Africa (that of Novitzky et al) which also found a predominance of young Black African women affected with the disorder (51). In contrast, HIV-associated TMA as generally reported in the international literature appears to show a predilection for male patients (62). This apparent discrepancy is also explained by the demographics of HIV infection; in North America and Europe the infection is mainly acquired through male to male sex so that men carry the highest seroprevalence rates (with up to 73% of individuals living with HIV / AIDS in the United States being male) (63).

## 4.2 Presenting features of HIV-associated TMA

Pyrexia appears to be a not infrequent presenting feature of HIV-associated TMA in this series, occurring in 41.2% of cases. The prevalence of pyrexia in HIV-associated TMA in the available literature is variable (Malak et al: 50% of cases, Rarick et al: 75% of cases) (103, 124); Novitzky et al reported a prevalence of pyrexia of 100%, although this reflects the use in this series of the Amorosi and Ultmann diagnostic criteria for TTP which require the presence of pyrexia (51). Analysis of the large Oklahoma TTP-HUS Registry found a prevalence of pyrexia in HIV-negative patients with TMA of 23%, considerably lower than that reported in case series of HIV-associated TMA (71, 51, 103, 124). This may indicate a tendency for pyrexia to be more frequent in HIV-associated TMA; however, analysis of this series does not show a significant difference in either the frequency of pyrexia or severity (as indicated by oral temperature) between HIV positive and negative patients with TMA.

It should also be considered that pyrexia in HIV positive patients with TMA may arise from other concomitant disorders such as opportunistic infection; however, analysis did not indicate a significant difference in either the frequency or severity of pyrexia in those patients with evidence of infection compared to those with no evidence of infection.

Neurological deficit has been reported in various case series to occur in between 61 – 69% of cases of HIV-associated TMA (51, 103, 124), the prevalence of neurological deficit in this series of 65.8% of cases is neatly consistent with these reports. Novitzky et al have reported that the most commonly encountered neurological abnormality in HIV-associated TMA is seizure (9 of 21 cases or 42.9%), followed by confusion (6 of 21 cases, 28.57%) (51); in contrast, in this series confusion was more common than seizure. It is likely that the retrospective nature of this series has led to an over-emphasis of confusion as a presenting feature of TMA since multiple confounding factors and the lack of an objective assessment of individual patient confusion (for example, using a mini-mental state

examination) may have resulted in observer bias. Focal neurological deficit is less common in this series than that reported by Novitzky et al (7.9% of cases vs. 17.4% of cases); it is possible that the presence of focal neurological deficit is under-reported in this series due to difficulties encountered by the examining doctor in accurately assessing focal deficit in the context of a confused and non-compliant subject; additionally, the ability to elicit subtle focal deficit is dependent on the clinical skill of the examining doctor and such factors are difficult to control for in retrospective review.

The severity of presentation of HIV-associated TMA has been poorly studied in comparison to the disorder in HIV negative patients. Novitzky et al have reported that HIV-associated TMA is associated with more severe derangement of markers of microangiopathic haemolysis (lower haemoglobin concentration and higher LDH activity) and lower platelet count (51), findings that suggest that HIV-associated TMA may be associated with a more severe microangiopathy. Analysis of the HJH data shows similar trends, although statistical significance was not reached in this series, possible due to the relatively small number of HIV negative subjects diagnosed with TMA during the period of study.

The possibility that HIV-associated TMA results in more severe microangiopathy may explain the significantly disparate levels of D-dimer observed in this series consistent with Gunther and Dhlamini's previous report (2). Since D-dimers reflect recent thrombus formation (68), high D-dimer levels may be indicative of the extent of recent microthrombus, and hence may be expected to show correlation with other parameters related to microthrombus load such as haemoglobin and LDH (microangiopathic haemolysis caused by circulating erythrocytes being forced through the lumina of vessels obstructed by thrombus) and platelet count (consumptive thrombocytopenia resulting from the formation of platelet rich microthrombi). However, analysis of data from the HJH series did not show statistically significant correlation between these variables; nor were D-dimer levels shown to have meaningful statistical difference with regards to target organ dysfunction (specifically, neurological deficit and renal dysfunction).

It is possible that the apparent severity of HIV-associated TMA over the disorder as encountered in HIV negative subjects may result from confounding factors related to the pathophysiology of HIV infection itself. For example, D-dimer levels are increased in HIV infection (68, 70) and are known to show significant positive correlation with the severity of HIV infection as indicated by HIV viral load (125); this association is thought to arise as a result of increased monocyte expression of tissue factor caused either directly by HIV RNA or indirectly as a result of opportunistic infection (69). Similarly, anaemia is not infrequently observed and becomes more common with advancing HIV infection (65). Analysis of the HJH data shows that RPI is significantly lower in subjects with HIV-associated TMA as compared to HIV negative patients presenting with the disorder; this suggests that suppression of erythropoiesis by a variety of factors related to HIV infection plays a role in the tendency for haemoglobin concentration to be lower at presentation in HIV-associated TMA.

It is probable that such considerations underlie the observed significant disparity in severity of renal dysfunction in this series. Previous reports indicate that renal target organ injury is more severe in HIV-associated TMA (51) consistent with the findings of this series; however, analysis suggests that in some cases concomitant diarrhoea is likely to have contributed to the severity of renal dysfunction observed (as indicated by more severe renal dysfunction in those subjects with diarrhoea compared to those without at a level approaching statistical significance), although the finding that renal dysfunction as indicated by presenting serum creatinine remained significantly more severe in subjects with HIV-associated TMA when those with concomitant diarrhoea were excluded from comparison with HIV negative subjects with TMA suggests that the disorder itself is an important determinant of renal injury in HIV positive subjects. However, the influence other confounding factors such as the prevalence of underlying HIV-associated renal disease in an HIV positive cohort (67) cannot be excluded due to the retrospective nature of this study (in particular, the lack of urinalysis and renal biopsy makes assessment of this confounding factor impossible).

Analysis of the HJH series supports other case reports indicating that TMA is a feature of advanced HIV infection (51, 53, 54, 61) with a median CD4 count at presentation of  $115 \times 10^6 / \text{mm}^3$ . The WHO has defined a CD4 count below  $350 \times 10^6 / \text{mm}^3$  as evidence of advanced HIV infection (109), whilst the Centers for Disease Control regards a CD4 count below  $200 \times 10^6 / \text{mm}^3$  as indicative of the acquired immunodeficiency syndrome (AIDS) (108); the median CD4 count of subjects in this series falls well within both of these definitions, and using WHO criteria 92.11% of subjects in this series can be considered to have advanced HIV infection. This observation accounts for the significant number of subjects in this series who presented with evidence of concomitant opportunistic infection (57.89% of presentations). Although concomitant opportunistic infection may have been expected to add to the severity of presentation of HIV-associated TMA (113 - 121), statistical analysis of this series does not support a significant role for opportunistic infection in determining presenting parameter aberration. CD4 count at presentation was not shown to correlate with presenting parameters; however, lack of significant linear association does not necessarily indicate that severity of TMA presentation is not affected by severity of HIV infection. CD4 count is susceptible to significant variation: normal diurnal variation can produce up to a 50% change in an individual patient (126), and physiological stressors (including exercise, diet, smoking, psychological stress, menstruation, and intercurrent infection) are known to alter CD4 count (127, 128). The retrospective nature of this study makes analysis of the effect of these confounding factors on individual subject CD4 count difficult, and they may account for the lack of correlation reported. An attempt was made to correct for potential individual subject CD4 count variation by analyzing the difference in severity of presenting parameter aberration according to severity of HIV infection determined by CD4 count as defined by the World Health Organization (109) and the Centers for Disease Control (111). Although no significant difference was found in either of these analyses, it should be considered that the majority of subjects in this series met CD4 count definitions of advanced HIV infection or AIDS as proposed by these bodies (109, 111); thus,

determination of statistical difference in accordance with these definitions is likely compromised by the relatively small numbers of subjects in this series who do not meet these definitions of severe HIV infection.

### **4.3 Response to treatment of HIV-associated TMA**

Available literature detailing the response of HIV-associated TMA to plasma exchange is limited to isolated small case series reports in which therapeutic response is analysed only in terms of patient survival (38, 86 - 88). Patient survival in response to plasma exchange appears lower in HIV-associated TMA in some series in comparison to that generally reported for HIV negative patients (86 - 88); Novitzky et al have reported both better survival rates and a shorter duration of therapy using plasma infusion in a prospective cohort study comparing HIV positive and negative patients with TMA (51). Thus, although evidence suggests that plasma exchange may be a superior therapy to plasma infusion (77), this treatment remains poorly studied in HIV-associated TMA.

Analysis of the HJH series indicates that HIV-associated TMA treated with plasmapheresis has a similar survival rate (90.6%) to that previously reported for HIV negative subjects (75 – 92%) (77). In comparison to a contemporaneous cohort of HIV negative subjects, HIV positive subjects in the HJH series required a significantly shorter duration of therapy to induce remission, consistent with Novitzky et al's previously reported findings using plasma infusion as therapy (51). Additional evidence of the responsive nature of HIV-associated TMA to plasmapheresis may be found in the improvement in target organ injury (specifically, renal dysfunction) observed in this series.

Interestingly, induction of remission using plasmapheresis in the HJH series was more rapid than that reported by Novitzky et al using plasma infusion (median duration of plasma exchange: 11 days vs. median duration of plasma infusion: 21 days) (51), suggesting a possible superiority for plasma exchange over plasma infusion in HIV-associated TMA as previously reported for HIV negative patients (77).

However, the possible superiority of plasmapheresis over plasma infusion needs to be weighed against the cost and availability of the former therapy in a resource poor environment such as South Africa.

Plasmapheresis requires expensive equipment operated by skilled technicians, and is not readily available outside of advanced care level institutions (51). As such, plasma infusion which has been shown by Novitzky et al to be effective in HIV-associated TMA may be an acceptable first line therapy for the disorder (51). The choice of plasma replacement used in plasma exchange does not appear to affect therapeutic response of HIV-associated TMA, consistent with the findings of a prospective study reported by Raife et al in HIV negative patients (85).

CD4 count at presentation appears to correlate inversely with and predict (albeit weakly) the duration of plasma exchange required to induce remission of HIV-associated TMA. This suggests that the severity of HIV infection affects therapeutic responsiveness; however, this observation is at odds with other analyses of the HJH data which show no correlation between CD4 count and severity of disease presentation (assuming that the severity of disease presentation determines therapeutic response) or between presenting parameters and duration of therapy required to induce remission. A possible explanation for these discrepancies is that presenting parameters may have been affected by multiple factors in addition to HIV infection and TMA severity resulting in the loss of linear correlation.

Plasma exchange is not without complications, and the risk of complication may be higher in HIV positive patients. 2.4% of (HIV negative) patients in the Oklahoma TTP-HUS Registry died as a result of complications directly attributable to plasma exchange and an additional 40% of patients in that series experienced non-fatal side effects related to this therapeutic modality (129). Although the incidence of fatal complications of plasma exchange in the HJH series compares favourably with this data (at 3.1% of cases), the incidence of non-fatal complications is dramatically higher than that reported by the Oklahoma TTP-HUS Registry (90.63% of cases vs. 25.72% of cases) (129) and more than double that reported by the Canadian Apheresis Study Group (which includes plasmapheresis performed for

indications other than TMA) in which 40% of patients experienced at least one complication related to the procedure (130). This disparity may however reflect the retrospective nature of this study. Whilst some adverse events such as hypokalaemia, hypocalcaemia, hypotension, line sepsis and deep vein thrombosis are readily verifiable in retrospective review, other events such as Quintin line blockage due to thrombosis, Quintin line bleeding; and allergic, citrate and transfusion reactions are less amenable to retrospective analysis. It is likely that the inability of this retrospective review to independently validate the accuracy of the diagnosis of adverse events in this series may have biased this analysis toward a view of plasma exchange therapy being associated with a higher frequency of adverse events than might be expected from the work of the Canadian Apheresis Study Group and the Oklahoma TTP-HUS Registry.

## **4.4 The prognosis of HIV-associated TMA**

### **4.4.1 Mortality**

Analysis of this series suggests that HIV-associated TMA responds well to plasmapheresis and that survival rates in those subjects receiving this therapeutic modality are similar to those previously reported for HIV negative patients in local and international series (51, 77). However, since a significant number of HIV positive subjects demised before plasma exchange could be instituted, it could be argued that HIV-associated TMA is a more severe form of the disorder resulting in rapid deterioration and death, as reported in other series (86 - 88).

Clarity in this regard was sought through interrogation of the ascribed causes of death in this series; sepsis arising from opportunistic infection was identified as the dominant cause (44.44% of mortalities), followed by TMA (33.33% of mortalities), suggesting that complications arising from HIV infection other than TMA may play an important role in determining patient outcome. Opportunistic infections are known to be an important predictor of outcome in HIV infected patients in general, having been reported as being the most frequent cause of death in seropositive individuals (131). Importantly, sepsis

is known to act as a trigger and exacerbating factor for TMA in susceptible individuals (132). Thus HIV positive patients in an advanced stage of viral infection presenting with TMA which may itself carry a high mortality (86 – 88), face additive mortality risk arising directly from opportunistic infection (112, 131) as well as from resultant opportunistic infection-related exacerbation of TMA (132). It is therefore likely that the discrepancy in the number of mortalities between the HIV positive and HIV negative patient groups of this series (although failing to reach statistical significance as a result of the paucity of HIV negative patients) and the rapidity of demise in a significant portion of this HIV positive mortality cohort (55.56% dying within 24 hours of presentation) reflects a “perfect storm” engendered by advanced HIV infection in which the resultant pathophysiological processes of opportunistic infection and TMA contribute together to mortality outcomes.

In comparison to literature pertaining to mortality in TMA in HIV negative patients, analysis of the HJH series does not demonstrate significant differences between mortality and survivor subgroups of HIV-associated TMA in terms of age (92), oral temperature (92), haemoglobin concentration (92, 93), or platelet count (93).

Although neurological deficit has previously been shown to be more frequent in patients who do not survive TMA (91), in this series no significant such difference was shown between mortality and survivor subgroups, consistent with Wyllie et al’s observations (92). Wyllie et al’s methodology in reaching this conclusion has been subject to the criticism that this study included patients with mild presentations of neurological deficit in analysis resulting in biased outcome (90); similar considerations occasioned by the retrospective nature of this study (which prevents accurate determination of the severity of neurological abnormality such as confusion) may explain the apparent (albeit non-significant) higher frequency of neurological deficit in survivors of HIV-associated TMA. This finding is counter-intuitive as it is expected that target organ injury should be more frequent and more severe in those subjects who do not survive HIV-associated TMA. In comparison, renal dysfunction is readily analysed through review of documented

serum creatinine and urea. In this series renal dysfunction was significantly more severe in those subjects with HIV-associated TMA who demised, consistent with data previously reported by Ferrari et al (93). Interpretation of the nature of renal dysfunction in this series appears to indicate a predominant role for acute kidney injury occurring as a direct consequence of TMA. Thus, severity of renal dysfunction may be considered a marker for endothelial injury. Since patients in this series did not develop uraemia severe enough to require initiation of dialysis it is likely that the association between mortality and severity of renal dysfunction reflects instead the significance of underlying endothelial injury.

#### **4.4.2 Residual target organ dysfunction**

Although limited by small subject numbers and lack of long term follow-up data, analysis of the HJH series demonstrates a good response of HIV-associated TMA to therapeutic intervention, with only 25.78% of subjects having some degree of residual renal dysfunction (as evidenced by a serum creatinine in excess of the laboratory normal of 100 $\mu$ mol/l) following therapy compared to 83.33% of this cohort of subjects at presentation, and the general improvement in creatinine. Whilst other series have suggested that plasma exchange reduces the risk of end stage kidney disease by up to 81% (96) and results in the recovery of normal renal function in up to 73% (89) of cases of TMA in HIV negative patients, the possibility that other interventions (especially fluid resuscitation, given the possible role played by concomitant diarrhoea in determining the severity of renal dysfunction in this series) may have contributed substantially to plasmapheresis in the recovery of renal function in the HJH data cannot be fully excluded. Furthermore, reliance on creatinine only as a marker of residual renal deficit has important limitations. Schieppatti et al have shown that when the definition of renal deficit is extended to include the range of possible manifestations thereof (from hypertension to ongoing need for dialysis) up to 70% of patients with TMA may manifest some form of residual dysfunction (97).

The lack of presenting parameters in general to show correlation with renal function following therapy is consistent with the report of Hollenbeck (97). The inverse correlation between presenting D-dimer and last recorded creatinine is interesting and has not previously been investigated or reported. This suggests that the severity of microthrombus load at presentation may be associated with improvement in renal dysfunction with therapy. It has been proposed that the significantly elevated levels of D-dimer reported in HIV-associated TMA reflect the prothrombotic milieu of HIV infection with superimposition of TMA-related active microthrombus formation (2, 69, 70). D-dimer half-life is approximately 8 hours (133); the inverse association observed may therefore be explained by the hypothesis that the quantitative recovery of renal function is likely to be more significant in those subjects with more recent microthrombus formation, whilst those with longer standing microthrombus (with lower D-dimer levels) are likely to experience less recovery due to the development of irreversible renal injury.

The positive correlation between presenting creatinine and creatinine at last follow-up following plasma exchange is consistent with Schieppatti et al's observations (97). Schieppatti et al also reported the association of a poorer renal outcome with a lower haemoglobin concentration and higher platelet count at presentation; this disconnect in renal prognosis between haemoglobin concentration and platelet count was interpreted as evidence that the lower haemoglobin concentration at presentation in patients with poor renal outcomes was due to non-TMA causes, specifically (in the context of the association of poor renal outcomes with presenting creatinine) underlying but undiagnosed renal dysfunction (97). The lack of renal biopsies undertaken in the HJH series preclude definitive assessment of this possibility; nevertheless, the prevalence of renal disease in HIV positive subjects (67) makes this possibility worthy of consideration. In this regard the lack of association of residual renal dysfunction with haemoglobin concentration at presentation in the HJH series is not in keeping with Schieppatti et al's report and may indicate that renal dysfunction in this series is mainly a result of acute kidney injury

caused by TMA. In this hypothesis, the severity of established acute renal injury at presentation is likely to determine the severity of persistent renal dysfunction following therapy.

#### **4.4.3 TMA Recurrence**

The frequency of TMA recurrence in this series was significantly lower in HIV positive subjects compared to HIV negative subjects. In general, TMA can be expected to recur in 15 – 30% of cases (93, 100, 134), although the probability of recurrence of individual cases is strongly determined by underlying aetiology (27); HIV-associated TMA may recur less frequently but may be precipitated by cART non-compliance (38, 51). It is significant that most (67%) of recurrences of HIV-associated TMA occurred early as exacerbations, whilst only one patient experienced a relapse of TMA (i.e., recurrence more than 30 days after induction of remission). Since cART was initiated in all subjects with HIV-associated TMA in this series this suggests that this therapy is successful in preventing relapse, presumably through the mechanism of reducing HIV viral load.

HIV-associated TMA appears to be less severe in presentation at recurrence compared to index presentation. It is likely that this reflects earlier diagnosis of the disorder due to improved subject and clinician surveillance; however, the potential effect of cART in ameliorating clinical severity cannot be fully excluded.

## 5 Conclusions

HIV infection is the dominant aetiological factor in TMA in the community served by the Helen Joseph Hospital. HIV-associated TMA occurs in the context of advanced HIV infection. The disorder appears to show a predilection for young woman of Black African descent reflecting local demographic patterns of HIV infection.

Renal target organ injury was more severe in HIV-associated TMA than that seen in HIV negative subjects with the disorder. It is possible that HIV-related diarrhoeal illnesses may have contributed to this finding. The observation that RPI was significantly lower in subjects with HIV-associated TMA similarly suggests that other disease processes related to HIV infection may play an important role in determining the severity of presentation of HIV-associated TMA. Opportunistic infection may be expected to contribute significantly to the severity of presentation (especially in a subject cohort in which advanced HIV infection is common such as the HJH series); however, this data does not support this hypothesis.

Analysis of this series confirms that HIV-associated TMA is associated with higher D-dimer levels than TMA occurring in HIV negative subjects, as previously reported (2). The significance of this association remains unclear, since D-dimer did not appear to correlate with other presenting parameters, was not higher in subjects with more severe target organ damage, did not correlate with therapeutic response, and was not higher in subjects who demised during the course of this study. D-dimer levels are known to be elevated in HIV infection and may merely represent a pre-existing prothrombotic state. An inverse association was observed between severity of residual renal deficit and D-dimer at presentation; this may indicate that D-dimer levels reflect recent microthrombus formation which is potentially reversible with therapy.

HIV-associated TMA responds well to plasma exchange, and induction of remission appears to require a shorter duration of therapy than is the case in HIV negative subjects. Target organ injury (neurological deficit and renal dysfunction) improves with plasmapheresis, although in the case of the latter adjuvant therapy with fluid replacement may have played an important role. The type of plasma infusant used (FFP or cryosupernatant plasma) does not appear to affect therapeutic response. Presenting parameters do not appear to affect the duration of plasmapheresis; however, subjects with more advanced HIV infection (as indicated by lower CD4 count) require a longer duration of therapy to achieve remission. Side effects of plasma exchange are not uncommon but are usually mild, and plasmapheresis is efficacious in reducing mortality.

The mortality rate of HIV positive subjects with TMA in this series is similar to that reported for HIV negative patients in other series. Severe renal dysfunction at presentation may identify subjects likely to demise. Timely intervention with plasma exchange is required to avert death.

Residual target organ dysfunction following plasma exchange is rare in this series. Presenting D-dimer level appears to show inverse association with creatinine at follow-up through possible mechanisms outlined above. Presenting creatinine shows positive correlation with creatinine at follow-up, indicating that the severity of established renal acute injury (or chronic underlying disease) plays a role in determining the degree of recovery possible in cases of HIV-associated TMA.

HIV-associated TMA rarely recurs, but may be precipitated by non-compliance with cART. In the event of recurrence, TMA appears to be milder as a result of earlier detection.

In summary, HIV infection is a common cause of TMA in the local setting. HIV-associated TMA in this series is characterized by marked renal dysfunction. The disorder responds rapidly to plasma exchange and initiation of cART, interventions which effectively prevent death and improve target organ

dysfunction. Although HIV-associated TMA is rare, the efficacy of plasmapheresis mandates that clinicians should have a high index of suspicion for the disorder in HIV infected patients.

## 6 Limitations

The following limitations are identified in respect of this study:

1. *The retrospective nature of this study:* TMA is comparatively rare, with an estimated annual incidence of 4 cases / 1 000 000 people (48), which necessitated the retrospective method employed. Although this approach has generated a large number of presentations for analysis compared to other reports available in the international literature, retrospective analysis carries with it inherent weaknesses which may result in incomplete or inaccurate results. Although these effects are mitigated in this study through reliance on objective laboratory data, it is possible that these weaknesses have compromised the analysis of clinical parameters.
2. *The small sample size analysed:* although this series is comparatively large in comparison to other series of HIV-associated TMA reported in the literature, total sample size remains small. This resulted in non-normal distribution for most parameters analysed in this series as evidenced by the Shapiro-Wilk W test and in consideration of the Central Limit Theorem. This was compensated for in statistical analysis using non-parametric methods of significance testing; however, it should be considered that small sample size carries with it the inherent risks of bias.
3. *The disparity in sample size between HIV positive and HIV negative subject groups:* The small total number of subjects in this series found particular expression in the HIV negative cohort. As a result, the HIV positive and negative cohort had a significant discrepancy in size. Non-parametric testing using the Mann Whitney U test and the Fisher Exact test were employed in an attempt to compensate for these differences. Nevertheless it should be considered that the small number of HIV negative subjects exposes the analysis of this series to potential bias.

4. *The short duration of follow-up:* Analysis of long-term outcomes of patients included in this series has been hampered by a relatively short duration of individual subject follow-up. This lack of long-term follow-up has made a full and accurate analysis of TMA recurrence and residual renal dysfunction (which might account for considerable morbidity) difficult.
5. *The lack of histopathological material:* The lack of renal biopsies in this series may have resulted in a failure to include cases of renal-limited HIV-associated TMA in this analysis. This may have resulted in a bias of the interpretation of the presentation of HIV-associated TMA toward a more severe systemic presentation of HIV-associated TMA form of the disorder. However, available data suggests that renal limited HIV-associated TMA is comparatively rare accounting for only 7 – 15% of presentations (58, 72) so that bias if so engendered is likely to be relatively mild.

## 7 Appendix

*Table 7 A. Laboratory normal values, Helen Joseph Hospital*

| Parameter                                      | Laboratory normal range*                                           |
|------------------------------------------------|--------------------------------------------------------------------|
| LDH activity (IU/litre)                        | 100 – 200                                                          |
| Platelet count ( $\times 10^9/\text{mm}^3$ )   | 137 – 373                                                          |
| Haemoglobin concentration (g/dl)               | 14.3 – 18.3                                                        |
| Reticulocyte production index (RPI)            | < 1: inadequate marrow response<br>1 – 2: adequate marrow response |
| D-dimer concentration (mg/l)                   | 0.00 – 0.25                                                        |
| Urea concentration (mmol/l)                    | 2.1 – 7.1                                                          |
| Creatinine concentration ( $\mu\text{mol/l}$ ) | 64 – 100                                                           |
| Calcium concentration (mmol/l)                 | 2.15 – 2.50                                                        |
| Potassium concentration (mmol/l)               | 3.5 – 5.1                                                          |
| CD4 count ( $\times 10^6/\text{mm}^3$ )        | 500 – 2010                                                         |

*\*As defined by the National Health Laboratory Service on-site laboratory, Helen Joseph Hospital*

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