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Thrombotic thrombocytopenic purpura in patients with retroviral infection is highly responsive to plasma infusion therapy

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Summary

We prospectively studied presentation biological differences and the response to therapy in patients with thrombotic thrombocytopenic purpura (TTP) associated with, or unrelated to human immunodeficiency virus (HIV) infection. TTP patients underwent standard evaluations and were treated with prednisone 1 mg/kg in addition to infusions of fresh frozen plasma (FFP; 30 ml/kg/d) until normalization of the platelet count. Unresponsive patients were referred for plasma exchange. Compared with HIV− TTP patients ($n = 23$), in HIV+ subjects ($n = 21$) microangiopathy was dominant among Black females, who had lower presentation Hb (median 5·8 g/dl; $P = 0{\cdot}03$), platelet count (13×10^9/l; $P = 0{\cdot}05$) and a CD4 count of $0{\cdot}096 \times 10^9$/l. HIV+ individuals responded to FFP faster than HIV− patients and none of them required apheresis. Ten HIV− TTP patients required apheresis ($P = 0{\cdot}03$) and four died. Responses in the HIV+ and HIV− groups occurred after treatment with a median of 33 and 55 units (one unit = 320 ml) of FFP ($P = 0{\cdot}004$) respectively. Response to this protocol was seen in 84% (95% response in HIV+ patients). Regression analysis showed that survival was associated with younger age ($P = 0{\cdot}001$), rapid platelet ($P = 0{\cdot}001$) and Hb ($P = 0{\cdot}0009$) recovery, and fewer FFP units to normal lactate dehydrogenase levels ($P = 0{\cdot}006$). We conclude that in HIV+ individuals, microangiopathy is highly responsive to plasma infusions. This observation is important particularly when apheresis is not available.

Keywords: thrombotic thrombocytopenic purpura, immunodeficiency syndrome, apheresis.

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Thrombotic thrombocytopenic purpura (TTP) is a relatively uncommon disorder characterized by the formation of platelet-rich thrombi in the arterioles and capillaries (Moschcowitz, 1924). A pentad of signs and symptoms has been associated with TTP: thrombocytopenia, microangiopathic haemolytic anaemia, neurological abnormalities, renal failure, and fever. In actual practice, however, the triad of thrombocytopenia, schistocytosis, and elevated lactate dehydrogenase (LDH) levels is often sufficient to suggest the disorder (Moake, 2002). If severe renal failure is the predominant feature at presentation, then the disorder is often considered to be haemolytic–uraemic syndrome, although the clinical distinction is not always clear-cut (Kaplan *et al*, 1998).

The management of TTP remains a focus of debate. Without treatment, the disease is associated with a mortality rate of greater than 90%. When treated with plasma infusion or plasma exchange, 60–90% of the patients survive the acute episodes (Bell *et al*, 1991; Rock *et al*, 1991: Moake *et al*, 1994). Removal of a neutralizing antibody against von Willebrand factor (VWF) cleaving proteinase and of the larger VWF molecules has been proposed to benefit patients undergoing apheresis (Zheng *et al*, 2004). Replacement of the decreased enzyme by infusions of fresh frozen plasma (FFP) may also be effective. While the Canadian apheresis trial concluded that plasma exchange was superior to infusion schedules, the volume of plasma employed in patients undergoing this procedure was larger (Rock *et al*, 1991). When the volumes of plasma infused were similar to those prescribed in the apheresis schedules, in a smaller study, no significant difference in the outcome was noted (Novitzky *et al*, 1994).

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TTP has been associated with advanced forms of human immunodeficiency (virus) infections (HIV) (Chu *et al*, 1995; Sood *et al*, 1996; Bell *et al*, 1997; Hymes & Karpatkin, 1997; Sutor *et al*, 1999; Ahmed *et al*, 2002), and seems less frequent in patients who are treated with highly active anti-retroviral agents (HAART) (Gervasoni *et al*, 2002). While in those infected with HIV, some features on presentation were comparable with the classical form (HIV−), a more heterogenous clinical picture was also depicted (Gadallah *et al*, 1996). However, these descriptions derived from case reports and small series. There has not been a comparative trial studying prospectively the initial physical and laboratory parameters and outcome of patients with TTP who are HIV(+) and HIV−. We present the admission clinical findings and outcomes of a cohort of patients with 'classic TTP' or HIV infection-related TTP who were treated with a similar therapeutic protocol at a single hospital. From this study, we concluded that, in South Africa, HIV-associated TTP is typically seen in African females with advanced disease. Of interest is that this presentation is highly responsive to plasma infusion schedules. This is relevant as it enables effective therapy with little delay at institutions where apheresis is not readily available, particularly in isolated communities and in developing countries.

Patients and methods

Between 1996 and 2003, 44 patients fulfilled the diagnostic criteria and consented to participate in this study, at Groote Schuur Hospital, a teaching hospital in the Western Cape region of South Africa. Patients were counselled of the nature of the disease and requested permission for testing for infection with HIV. When patients were unable to provide consent, permission for the investigations was given by the medical superintendent. The objective of the study was to determine any differences in the clinical and laboratory parameters at presentation, as well as response to therapy in patients with idiopathic TTP or who were infected with the immunodeficiency virus. We also looked at the response to plasma infusion therapy as previously described (Novitzky *et al*, 1994) and focused on the time to normal platelet count, to normal serum LDH level, the volumes of plasma administered until response, the duration of the treatment, proportion achieving remission, as well as relapse and mortality rates. We have also reviewed the treatment-related complications of this strategy on the two patient groups. Anti-retroviral therapy was not available at state hospitals in South Africa over the study period. Therefore, none of these patients had been treated with HAART before therapy for TTP.

Diagnostic investigations

The diagnosis was established using previously described criteria (Amorosi & Ultmann, 1966), however a minimum of red cell fragmentation (microangiopathic anaemia), elevated LDH (normal range 80–400 IU/l) and thrombocytopenia ($<100 \times 10^9$/l) with no other identifiable cause, was required (Moake, 2002). In each case, the diagnosis was confirmed by a haemato-pathologist. Coagulation studies were performed on all patients at entry, as well as serum chemistry, including LDH. To exclude other mechanisms of fragmentation, patients were required to have normal international normalized ratio, partial thromboplastin time and serum fibrinogen levels. Samples were also referred for testing for anti-nuclear factor (ANF), anti DNA and Rheumatoid factor. HELLP syndrome (haemolysis, elevated liver enzyme and low platelets) was distinguished from TTP by the clinical context of often elevated arterial blood pressure, liver dysfunction, substantial proteinuria, oedema, variable confusion and reversal of all manifestations after delivery.

Definition of the retroviral status

Testing for HIV was by enzyme-linked immunosorbent assay. Positive results were confirmed by Western blot. Blood was subsequently taken for the determination of $CD4^+$ cells by standard flowcytometry. Efforts were made to exclude the presence of opportunistic infections or HIV-associated malignancies by physical examination, serology, imaging of lymph nodes or masses in the chest or abdomen and, as appropriate, histological or microbiological assessment of abnormal tissues.

Therapy

Patients fulfilling the criteria for TTP were immediately started with prednisone 1 mg/kg and cryoprecipitate-poor FFP at a dose of 30 mL/kg/d (Novitzky *et al*, 1994). This fluid load was given in three divided doses. Whenever clinically indicated, diuresis with furosemide was induced. At our hospital FFP is formulated in acid citrate dextrose (ACD) to a final volume of 320 mL (50 mL ACD). Blood investigations were repeated daily until response was achieved. Response was defined as normalization of the haemoglobin and platelet counts, with recovery of the serum LDH level. Responding patients were continued on the same schedule until the platelet count recovered. Patients who were unable to receive this fluid load due to cardiac or renal decompensation, or failed to show a clinical improvement after 48 h (worsening of the mental state, new neurological manifestations, progressive purpura, oliguria) or who had no improvement in the serum LDH, haemoglobin blood level and platelet count, were referred for plasmapheresis. Evaluation of response included the number of FFP units to normal platelet counts and serum LDH values, as well as units processed during apheresis until response.

Plasma exchange was performed with a Cobe Spectra continuous flow blood fraction separator according to manufacturer's specifications. During each procedure, the volume of patient plasma exchanged with FFP was not less than 1·5 times the calculated plasma volume over a period of 4 h. Anticoagulation was with ACD, infused at a concentration rate to FFP of 1:15. Apheresis took place on alternate days until normalization

of the platelet count. On the days when plasma exchange was not performed, patients received infusions of FFP at a dose of 30 ml/kg. Thereafter, FFP infusions were given at the standard treatment schedule, unless patients were intolerant to fluids when apheresis was continued daily. In all responding patients the infused FFP volume was slowly reduced by 320 ml every third day. Corticosteroids were tapered off over 2 months once the FFP infusion therapy had been concluded. No other medication, such as vincristine or cyclosporine, or splenectomy was prescribed for these patients.

Statistical analysis

Student's *t*-test was used to evaluate significance between pairs of groups. Both the Kolmogorov–Smirnov normality test, the Mann–Whitney rank sum test was used as appropriate. Survival of the two groups was calculated according to the Kaplan and Meier method. Statistical significance was defined as $P < 0{\cdot}05$. All data analyses were performed using the STATISTICA (StatSoft Inc. Tulsa, OK, USA) computer software package.

Results

Forty-four consecutive patients were prospectively enrolled into this study. No patient was excluded. Their presentation clinical and laboratory values are shown in Tables I and II. All five clinical manifestations of the disease (Bukowski, 1982; Byrnes & Moake, 1986) were present in 75% of patients. All patients were pyrexial on admission. TTP occurred during pregnancy in five instances. No patient had elevated liver enzymes, hypertension or cholestatic jaundice. Confusion, stupor or focal neurological findings were present in 35 individuals. In this cohort, there was no association between the presence of neurological abnormalities and severity of the disease as shown by creatinine levels, LDH, Hb or platelet count and response to therapy. In four instances, the Medical Superintendent on duty gave permission for the investigations. The admission serum creatinine was elevated in 10 patients, but this was not related to response to therapy or survival. TTP was linked to pregnancy in four individuals, and five subjects who were HIV− had a reactive ANF test but no patient had clinical manifestations of systemic lupus erythematosus. None of those infected with HIV showed reactivity for ANF ($P = 0{\cdot}04$).

Thirty-four patients responded to the therapeutic protocol of FFP infusion and required a median of 36 (30–186) units to reach a normal platelet count (Table III). Normalization of platelet count and serum LDH levels occurred at a median of 6 (5–21) and 22 (10–42) d from starting therapy respectively.

In this group, 23 were HIV− and 21 were HIV+. Patients infected with HIV were all African Black and mostly females (Table I). On presentation, they had a significantly lower Hb and platelet count (Table II). Their median CD4 count was $0{\cdot}096 \times 10^9$/l, suggesting that TTP in this context is seen mainly in patients with advanced disease. However, in 19 individuals, TTP was the first illness associated with this infection. Neurological manifestations were evident in 21 of 23 HIV− patients, ranging from confusion (5/21) focal deficits (4/23), convulsions (8/23) and coma (4/23). The corresponding figures for HIV+ patients were six of 21, five of 21, nine of 21 and zero of 21 (coma: $P = 0{\cdot}06$) respectively. However, between these two groups there was no difference in the presentation serum LDH, bilirubin and creatinine values.

HIV negative group

Twenty-three patients were HIV−. Ten of 23 required plasmapheresis. Apheresis was prescribed in six patients as they could not tolerate the required volume of FFP (cardiac, renal or multiorgan failure). Four patients did not meet the

Table I. Clinical characteristics of the study population on presentation.

	ALL ($n = 44$)	HIV− ($n = 23$)	HIV+ ($n = 21$)
Age (years); median (range)	29·5 (14–80)	30 (14–80)	28 (24–51)
Gender M/F	6/38	5/18	1/20
Racial group*			
Caucasian	2	2	0
Mixed race	12	12	0
African Black	30	9	21 ($P = 0{\cdot}004$)
Associated medical condition			
Pregnancy	5	4	1
ANF+	6	5	1
Cardiac failure	2	2	
Pancreatitis	2	2	
Neurology present/absent	35/9	16/7	18/3 ($P = 0{\cdot}02$)

*The figures published by the Central Statistical Service in a table of population by geographical areas shows that in the Western Cape province of South Africa the population distribution includes 23·8% Caucasians, 58·2% Mixed race and 17·6% Black Africans.

Table II. Presentation laboratory parameters of the study population.

	ALL ($n = 44$)	HIV− ($n = 23$)	HIV+ ($n = 21$)
Blood parameters			
HB (g/dl); median (range)	6·4 (3·5–11·9)	7·2 (3·5–11·9)	5·8 (3·9–9·8) ($P = 0·03$)
Leucocyte count ($\times 10^9$/l); median (range)	10·2 (3·7–26)	11·9 (4·5–26)	7·8 (3·7–26) ($P = 0·17$)
Platelet count ($\times 10^9$/l); median (range)	15 (7–86)	20 (7–86)	13 (7–49) ($P = 0·05$)
CD4 ($\times 10^9$/l); median (range)	–	–	0·096 (0·001–0·22)
Serum chemistry			
LDH (IU/l); median (range)	3235 (671–11290)	3133 (1346–11290)	3242 (671–7100) ($P = 0·3$)
Bilirubin (μmol/l); median (range)	49 (17–431)	65 (30–431)	30 (17–111) ($P = 0·25$)
Creatinine (μmol/l); median (range)	103 (49–386)	107 (54–330)	97 (49–386) ($P = 0·5$)

Table III. Response to therapy according to HIV infection status.

	ALL ($n = 44$)	HIV− ($n = 23$)	HIV+ ($n = 21$)
All patients			
Complete response (%)	39 (89)	19 (78)	20 (95)
Alive (%)	33 (75)	18 (78)	15 (71)
Died of TTP (%)	6/44 (16)	5 (21)	1 (5)
Overall number of FFP Units* used; median (range)	36 (30–186)	55 (30–186)	33 (30–93); ($P = 0·0046$)
Days to platelets $>150 \times 10^9$/l; median (range)	6 (5–21)	7·5 (5–21)	6 (5–16); ($P = 0·03$)
Days to normal LDH; median (range)	22 (10–42)	24·5 (21–42)	21 (10–42); ($P = 0·01$)
FFP Units* used to normalize platelet count; median (range)	36 (30–186)	54 (30–186)	33 (30–93); $P = 0·0046$)
Patients undergoing Apheresis ($n = 10$)			
Median (range) number of procedures to response		5 (3–9)	
FFP units* used to normalize platelet count, median (range); apheresis		96 (70–186) ($P = 0·004$)	
Number of patients responding		6/10	
Patients not undergoing apheresis (responded to FFP infusion; $n = 34$)			
FFP units* used to normalize platelet count, median (range); FFP infusion only		41 (30–74) ($P = 0·1$)	33 (30–93)

*The volume of a unit of FFP is 320 ml.

response criteria at 48 h. Of these 10 individuals, six patients had good response to apheresis, reaching normalization of platelet count after a median of five aphereses. Four patients were mechanically ventilated at the time of diagnosis and all failed to respond to daily apheresis and died at a median of 14 (range 10–17) d. Cause of death was multiorgan failure in two and neurological deterioration (computed tomography of the brain showed diffuse brain oedema) in another two, despite receiving seven (range 6–9) aphereses. Response to plasma exchange, demonstrated by the normalization in the platelet count and serum LDH values, was seen at a median of 7·5 (6–21) and 35·5 (21–42) d respectively (Table III). All the remaining patients ($n = 13$) responded to plasma infusions and their platelet count and serum LDH normalized at a median of 6·5 (range 5–14 $P = 0·1$) d and 22·5 (range 21–32; $P = 0·0043$) respectively. Three patients relapsed at a median of 4 months from presentation. Two responded well to a second course of FFP infusions and splenectomy but the third, who was pregnant, failed to respond to FFP followed by plasma exchange and died of multiorgan failure.

HIV positive group

Twenty-one patients were infected with HIV. One patient, who was transferred from another hospital, died within 48 h of diagnosis from nosocomial Gram(−) septicaemia. Except for this subject, all responded to FFP infusions and their platelet count and serum LDH levels normalized significantly faster than the HIV− group, at a median of 6 (5–16; $P = 0·03$) and 21 (10–42; $P = 0·01$) d respectively. To normalize the platelet count, HIV+ patients required a median of 21 units of FFP and in total, they were infused a median of 33 (range 30–93) units of plasma, which was significantly less than the HIV− group (Table III; $P = 0·004$). One patient relapsed 9 months from presentation and responded to a further course of FFP infusion. However, another five died at a median of 5 months

from response, of opportunistic infections and deterioration due to acquired immunodeficiency syndrome (AIDS).

Factors associated with outcome

Overall, 84% responded to this protocol, which was particularly favourable in patients with HIV infection (95% response). Response in the HIV− and HIV+ groups was at a median of 96 and 41 units of FFP ($P = 0{\cdot}004$) respectively. However, if those requiring plasmapheresis were excluded, no difference in the volume of infused FFP was noted between the HIV+ and HIV− groups.

To determine whether associations such as pregnancy ($n = 5$) or reactivity for ANFr ($n = 6$) could have affected the response to therapy, a new analysis was undertaken, where 11 patients with these additional factors were excluded. No differences in the results of the parameters tested were found.

Multiple regression analysis showed that the significant favourable factors for response were HIV+ status ($P = 0{\cdot}002$), age younger than median ($P = 0{\cdot}0001$) and no need for apheresis ($P = 0{\cdot}001$). Factors associated with improved survival were younger age ($P = 0{\cdot}001$), rapid platelet ($P = 0{\cdot}001$) and Hb ($P = 0{\cdot}0009$) recovery, and lower number of FFP units to normalize LDH ($P = 0{\cdot}006$). Cox proportional hazards analysis for response confirmed that younger age ($P = 0{\cdot}001$), female gender ($P = 0{\cdot}01$), LDH on presentation ($P = 0{\cdot}001$) and no need for apheresis ($P = 0{\cdot}004$) were significant factors for response. The negative interactions for survival were HIV+ status ($P = 0{\cdot}003$), pregnancy ($P = 0{\cdot}03$) and greater number of FFP units to normal LDH ($P = 0{\cdot}05$).

Toxicity

Three patients developed allergic reactions while receiving FFP infusion, which responded to conventional treatment. Fluid overload was seen in four of 34 individuals despite the use of loop diuretics with the FFP therapy. Hypokalaemia was reported in 13 of 34 and required vigorous replacement. In the apheresis group, haematomas at the site of the catheter placement was seen in five of 10 patients. Catheter-related septicaemia was seen in four of 10, which became a problematic complication in those requiring ventilation.

Discussion

TTP remains a challenging disease to treat due to the heterogeneity in its clinical presentation, the lack of a simple and clear-cut diagnostic test, variability in the response to therapy and frequent relapses.

In patients with TTP, the systemic clumping of platelets mediated by unusually large multimers of VWF often results in profound thrombocytopenia (Moake & McPherson, 1989). This leads to 'shearing' of red cells by mechanical damage and the formation of the characteristic schistocytes. Recent studies have demonstrated that a severe deficiency (<0·1 U/ml) of the protease (ADAMTS 13) that cleaves VWF multimers is associated with the development of TTP, suggesting that assays of the enzyme may distinguish TTP from other types of thrombotic microangiopathies (Furlan *et al*, 1997). Autoimmune inhibitors of ADAMTS13 are detected in patients with the acquired form of TTP (including HIV-infected patients), while genetic mutations of ADAMTS13 are found in patients with the hereditary form of the disease (Furlan & Lammle, 2001; Sahud *et al*, 2002). However, while assays of the ADAMTS 13 protein were found to be of diagnostic value, they are mainly available only at research laboratories. Of further interest is that direct toxicity to endothelial cells by circulating soluble factors has also been reported, explaining the heterogenous nature of the disease (Laurence *et al*, 1996).

Both plasma infusion and exchange correct the dramatically reduced metallo-proteinase levels and have been effective in this disease. While a prospective controlled study concluded that apheresis was superior therapy, the volume of plasma prescribed in the infusion arm was substantially lower (Rock *et al*, 1991; Rock, 2001). Nevertheless, three studies have suggested that FFP infusions may have similar effectiveness, provided that patients can tolerate the volume load (Ruggenenti *et al*, 1993; Novitzky *et al*, 1994; Coppo *et al*, 2003). Without doubt, plasmapheresis is more tolerable in patients who have developed fluid overload or oliguria. Nonetheless, apheresis requires skilled operators, expensive equipment and is associated with complications from the invasive nature of the procedure (Rizvi *et al*, 2000). Moreover, apheresis equipment may not be immediately available in all hospitals, particularly in developing countries and thus plasma infusion may be used with care in selected patients with this disease.

This series of consecutive patients is, to our knowledge, the first study that has prospectively compared patients with HIV infection to the standard type of TTP with regards to presentation clinical parameters and response to a single therapeutic strategy. It has confirmed that there may be some distinctions between HIV− patients who develop TTP or in those following infection with HIV. Specifically, in the HIV− cohort, the distribution between the dominant 3 racial groups and the two genders was relatively similar while, in a proportion of cases, fragmentation was associated with positive ANF. Despite the fact that the main racial group in the Western Cape and attending our hospital are Cape Coloureds (Table I; du Toit *et al*, 1988), patients with TTP associated with HIV infection were exclusively of the African Black race and mainly female (20/21). They also had lower presentation haemoglobin and platelet count. These features may represent more aggressive TTP or alternatively, a more advanced form of the disease, perhaps because of greater difficulties in the access to medical care, rather than a real biological difference. Their low CD4 counts confirmed previous observations that this complication was a sign of advanced HIV infection and indeed in patients responding to therapy, the salient cause of death has remained opportunistic infections. Of interest, in 18 of 21 individuals, TTP was the first manifestation of AIDS in an

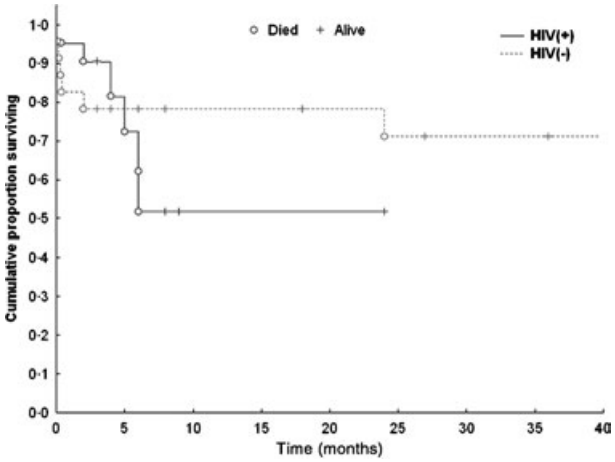

Fig 1. Survival of patients with TTP according to HIV status. The two curves represent patients who were HIV+ (solid line) and HIV− (broken line) at the time of presentation.

otherwise asymptomatic population and in each case, patients had a CD4 count of $<0{\cdot}2 \times 10^9$/l. For this reason, the prevalence of TTP is likely to remain high in communities with elevated rates of HIV infection.

Notably, we observed that with this protocol, patients with HIV-related TTP had a significantly higher response rate to FFP infusions and none required progression to plasmapheresis. Table III shows that patients suffering from HIV infection had a significantly shorter time to normal platelet count ($P = 0{\cdot}03$), to normal serum LDH values ($P = 0{\cdot}01$) and required fewer units of FFP ($P = 0{\cdot}004$). The single death in the HIV+ group was related to a severe nosocomial infection contracted before presentation at our unit. However, HIV− patients responsive to FFP behaved similarly to HIV+ patients regarding the rate of recovery and number of FFP units required for response, supporting the observation of variable clinical expression (severity) of this disease. This led to 84% of all patients responding to plasma infusion (but only 56% to FFP in the HIV− group), which was particularly effective in HIV+ patients (95% response). Nonetheless, the survival curve (Fig 1) showed that, despite rapid normalization of the blood parameters, five individuals infected with the retrovirus died later on from HIV progression, usually because of opportunistic infections as (except for one) they were unable to procure effective antiretroviral therapy. However, HAART therapy is increasingly becoming more accessible to all patients in South Africa and, as already described (Gervasoni *et al*, 2002), we can expect changes in the rate of presentation, incidence of opportunistic infection and cause of death. On the contrary, patients who were HIV negative and responded to plasma therapy did particularly well as only three had recurrent disease, which was effectively treated in two patients.

We conclude that, in patients infected with HIV, the presentation disease parameters were of a more advanced disease stage than in the HIV− cohort, while their response to plasma infusion therapy was significantly better and faster. This is of substantial relevance for patients who present to regional hospitals that may not have apheresis facilities, as this therapy must be started without delay. Nevertheless, urgent referral of these patients to tertiary centres is still recommended as these individuals often have complex associated problems. Future prospective controlled trials should determine whether apheresis is superior to the infusion of adequate volumes of FFP in those individuals with TTP related to HIV infection.

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