HIV-associated Thrombotic Thrombocytopenic Purpura – What We Know So Far

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Abstract
Thrombotic thrombocytopenic purpura (TTP) is a life-threatening disease characterised by microvascular platelet deposition and thrombus formation in selected organs, resulting in microangiopathic haemolytic anaemia, thrombocytopenia, neurological symptoms and renal failure. Typically a very rare disorder, TTP is being seen with increased frequency in patients infected with the human immunodeficiency virus (HIV). Deficiency of the von Willebrand factor (VWF) and the production of anti-ADAMTS13 antibodies are implicated as the cause of HIV-associated TTP. This is not always the case. However, the pathophysiology of HIV-associated TTP and the thrombotic potential in these patients are not known. This article provides not only an overview of the literature regarding HIV-associated TTP, but also presents new data on this disease. We propose a mechanism for the initial onset of HIV-associated TTP that includes the release of extreme amounts of von Willebrand factor and the production of autoantibodies to ADAMTS13.

Keywords
Thrombotic thrombocytopenic purpura, HIV, ADAMTS13, anti-ADAMTS13 antibodies, von Willebrand factor

Thrombotic thrombocytopenic purpura (TTP) is an acute prothrombotic disorder resulting from a deficiency of the von Willebrand factor cleavage protease ADAMTS13. The enzyme ADAMTS13 (a disintegrin and metalloprotease with thrombospondin type 1 repeats) cleaves the peptide bond between Tyr-842 and Met-843 of the mature subunit of von Willebrand factor (VWF) and prevents the interactions of the largest VWF multimers with platelets. In the plasma of patients with TTP, ultra large VWF multimers (ULVWF) have been observed that provoke widespread microvascular thrombosis. TTP is characterised by microvascular platelet aggregation and thrombus formation resulting in thrombocytopenia, microangiopathic haemolytic anaemia, variable renal and neurologic dysfunction and fever. Infection with the human immunodeficiency virus (HIV) is postulated as a direct precipitant of TTP, predominantly through infection of vascular endothelial cells resulting in dysfunction, localised thrombin generation and consumption of ADAMTS13. HIV-associated TTP was first described in 1987 by Jokela et al. Since then, several case studies have been reported. The occurrence of TTP in association with HIV infection is now well recognised.

Prevalence of HIV-associated Thrombotic Thrombocytopenic Purpura
Although congenital TTP is a very rare disease, the incidence of HIV-associated TTP is much higher. A group in South Africa estimated the incidence of TTP in HIV-infected individuals to be 15–40 times that in non-infected individuals. Also, more than 80 % of TTP cases in South Africa are found to be HIV-related and it is expected that the incidence of HIV-associated TTP will continue to rise. HIV-associated TTP cases were also described in other countries, such as the UK and Italy.

Features and Treatment of HIV-associated Thrombotic Thrombocytopenic Purpura
HIV-associated TTP is a haematologic disorder observed in patients infected with HIV. As with congenital non-HIV-associated TTP, this syndrome is characterised by microangiopathic haemolytic anaemia, thrombocytopenia, renal dysfunction, fluctuating neurological abnormalities and fever. Treatment regimens include highly active antiretroviral therapy (HAART), infusion of fresh frozen plasma, plasma exchange, steroids and immunomodulatory agents. One case study stressed the importance of CD4 count and viral loads in HIV-associated TTP. The patient presented with a normal CD4 count, a high viral load and a suboptimal response to plasma exchange while remission was obtained with combined antiretroviral therapy (cART). Other case studies confirmed that there may be a causative link between TTP and advanced HIV infection, since all patients presented with extremely low CD4 and CD8 counts. This is not always the case in our experience (unpublished data). The treatment strategy in the case described by Miller et al. included fresh plasma and a corticosteroid, but only after plasma exchange did the platelet count start to rise and the steroid was tapered; cART treatment was started afterwards. Another case study described a patient with myocardial injury in HIV-associated TTP who responded poorly to plasma infusion and steroid therapy, but recovered fully following plasma exchange.
Original Contribution

Table 1: ADAMTS13 Levels, Activities and Autoantibodies, VWF Levels and Tissue Factor Levels of Citrated Plasma from Patients with TTP, HIV-positive People on cART and HIV-positive People not on cART

<table>
<thead>
<tr>
<th>Test</th>
<th>Unit</th>
<th>TTP (Mean±SD)</th>
<th>HIV+ on cART (Mean±SD)</th>
<th>HIV+ not on cART (Mean±SD)</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADAMTS13 levels</td>
<td>ng/ml</td>
<td>144±109</td>
<td>934±245</td>
<td>627±109</td>
<td>520–1,060</td>
</tr>
<tr>
<td>ADAMTS13 activity</td>
<td>ng/ml</td>
<td>366±97</td>
<td>735±230</td>
<td>421±260</td>
<td>481–785</td>
</tr>
<tr>
<td>ADAMTS13 autoantibodies</td>
<td>% of positive patients</td>
<td>50</td>
<td>50</td>
<td>83</td>
<td>none</td>
</tr>
<tr>
<td>VWF antigen levels</td>
<td>%</td>
<td>494±130</td>
<td>317±187</td>
<td>767±378</td>
<td>50–150</td>
</tr>
<tr>
<td>Tissue factor levels</td>
<td>% of patients with increased levels</td>
<td>36</td>
<td>50</td>
<td>87</td>
<td>none</td>
</tr>
</tbody>
</table>

ADAMTS13 = a disintegrin and metalloprotease with thrombospondin type 1 repeats; cART = combination antiretroviral therapy; SD = standard deviation; TTP = thrombotic thrombocytopenic purpura; VWF = von Willebrand factor.

Figure 1: Proposed Mechanism for the Initial Onset of HIV-associated Thrombotic Thrombocytopenic Purpura

A recent study of 24 patients with HIV-associated TTP emphasises the importance of the prompt initiation/re-initiation of cART in parallel with plasma exchange and steroid treatment that lead to prompt remission.11 Adjunct immunomodulatory agents such as rituximab have been used with some success in refractory cases.12 It has been reported that patients with HIV-associated TTP seem to be far more responsive to plasma infusion treatment regimens than their HIV-negative counterparts, suggesting a different pathophysiology.13

Pathophysiology of HIV-associated Thrombotic Thrombocytopenic Purpura

The pathophysiology and initial onset of TTP in HIV-positive patients are not fully understood. In 1994, Cruccu et al. suggested that the aetiology of thrombocytopenia in HIV-infected patients may be due to direct infection of megakaryocytes by the virus, immune-mediated destruction, impaired haematopoiesis, toxic effects from medications and microangiopathic anaemia syndromes.14 It is now generally accepted that HIV-associated TTP is an acquired form of ADAMTS13 deficiency. Acquired ADAMTS13 deficiency is often due to the presence of autoantibodies directed against ADAMTS13.14,15 These autoantibodies to ADAMTS13 play a pivotal role in the pathogenesis of acquired TTP. By decreasing the function of ADAMTS13, the autoantibodies impair the cleavage of ULVWF into smaller sizes, leading to the formation of platelet-VWF thrombi in the microcirculation. Only limited data are available regarding the presence of autoantibodies in HIV-associated TTP patients. In one study, only 38 % of patients with reduced ADAMTS13 activity had inhibitory antibodies. This was measured by using the residual collagen binding assay and performing mixing studies with normal plasma.14 The same group also found only a mild reduction of ADAMTS13 levels in patients with HIV-associated TTP and an increase in VWF levels in all patients.

We measured ADAMTS13 levels, activity and autoantibodies in 40 patients diagnosed with HIV-associated TTP at the Haematology Clinic of the Universitas Hospital in Bloemfontein, South Africa. As a control group, we used the plasma of 104 HIV-positive persons visiting the local primary healthcare clinic. The control HIV group was divided into two groups, depending on whether they were on cART or not. The ADAMTS13 levels and autoantibodies were measured using the respective enzyme-linked immunosorbent assay (ELISA) kits from American Diagnostica (US). The ADAMTS13 activities were measured using the Actifluor™ ADAMTS13 fluorescence resonance energy transfer (FRET) assay (also from American Diagnostica). We also measured the VWF levels using antibodies from Dako (South Africa) and tissue factor levels in plasma with an ELISA kit from American Diagnostica. The results are summarised in Table 1.

We found that the ADAMTS13 levels extensively decreased in TTP patients. HIV infection on its own did not seem to have a significant influence on ADAMTS13 levels. However, HIV-positive persons who were not on cART seemed to have slightly lower ADAMTS13 levels than those who were on cART, although the levels were still in the normal range. It is possible that less metalloproteases such as ADAMTS13 are synthesised in these patients, since micronutrient deficiencies are widespread and compound the effects of HIV disease.15 Our findings regarding ADAMTS13 levels differed from those of another group in South Africa. They detect normal ADAMTS13 levels in one-third of adult cases with HIV-associated TTP.16 The activities of ADAMTS13 that we measured using the FRET assay did not correlate with the ADAMTS13 levels in all groups. However, the lowest activities were found in the TTP patients. The assay might not be as suitable as the ADAMTS13 levels to diagnose the presence of HIV-associated TTP. We also found that only 50 % of our TTP patients presented with autoantibodies to ADAMTS13. This correlates with what is reported in the literature.14 An interesting finding was that most of the HIV-positive persons who were not on cART also presented with high amounts of autoantibodies against ADAMTS13. We thus suggest that antibody formation against ADAMTS13 is a result of the weakened immune system in HIV. Similar to what is reported in the literature, we found increased VWF levels in patients with HIV-associated TTP.16 The VWF antigen levels were
greatly increased in HIV-positive patients who were not on cART. This could be due to an HIV-associated inflammatory state or concomitant infection. Similar to the WVF levels, we found that most (87 %) of the HIV-positive women who were not on cART had increased tissue factor levels, which is known to be a risk factor for thrombosis. We found that only 36 % of the TPT patients presented with increased tissue factor levels. This is probably because these patients were all on cART.

We propose a mechanism for the initial onset of HIV-associated TTP (see Figure 1). HIV type 1 (HIV-1) infection is associated with inflammation and elevated levels of inflammatory cytokines such as gamma interferon and tumour necrosis factor alpha (TNF-α) and beta (TNF-β). It is known that inflammatory cytokines, such as TNF-α and interleukin-1, 6 and 8, have profound stimulatory effects on the endothelial release of ULWVF18. In addition, the synthesis of ADAMTS13 that cleaves the ULWVF is inhibited.19 This might ultimately lead to the deficiency of ADAMTS-13 and the overexpression of ULWVF, resulting in the initiation of TPT. Furthermore, HIV-infection is associated with widespread micronutrient deficiencies that might cause decreased ADAMTS13 synthesis.20 Autoantibodies to ADAMTS13 are also present in HIV-positive patients as a result of the weakened immune system. An inhibitory antibody might also cause the initial onset of HIV-associated TTP.

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Laboratory Diagnosis of HIV-Associated Thrombotic Thrombocytopenic Purpura

The diagnosis of TTP rests on evidence of microangiopathic haemolytic anaemia (MAHA) and thrombocytopenia in the absence of disseminated intravascular coagulation and other known causes of thrombotic microangiopathy. Highly specific diagnostic tools such as plasma levels of ADAMTS13 are recommended, but are not always routinely available for immediate clinical diagnosis. The presence of schistocytes on a blood smear is the morphologic hallmark of the disease. A schistocyte count of 1 % or more in conjunction with thrombocytopenia suggests TTP if other causes of MAHA have been excluded.21 We suggest the use of ADAMTS13 levels as a confirmatory test of HIV-associated TTP. The measurement of ADAMTS13 activity levels using the FRET assay is expensive and does not necessarily confirm the disease. The exact role of inhibitory autoantibodies to ADAMTS13 remains uncertain and further investigation is required.

Conclusion

HIV-associated TTP is a heterogeneous disorder and its initial onset is still not clear. We propose that infection with HIV might trigger the disease through the inflammatory process. Inflammatory cytokines stimulate the release of extreme amounts of VWF.22 Extremely high levels of WVF were found in the plasma of HIV-positive persons who are not on HAART as well as in TPT plasma. The cytokines also downregulate the release of the WVF-cleaving protease ADAMTS13.23 We found very low levels of ADAMTS13 in TPT plasma. This might be sufficient to cause thrombotic microangiopathy and precipitate an acute episode of TTP even in the presence of normal cleaving protease activity. Furthermore, the release of WVF may be sufficient to overwhelm the capacity of the cleaving protease and result in a ‘consumptive deficiency’, as speculated by Gunther et al.14 An important finding in HIV-associated TTP that differs from acquired TTP is the fact that not all patients present with autoantibodies to ADAMTS13. More specific methods might be needed to identify autoantibodies, especially inhibitory autoantibodies, in these patients. Inhibitory antibodies to ADAMTS13 might also trigger the disease. We could finally show that neither HIV-infection per se, nor the use of HAART, has any effect on ADAMTS13 levels, making it a useful diagnostic tool to diagnose HIV-positive patients with TPT. The increased tissue factor levels in HIV-positive persons who are not on cART indicate the thrombotic potential in HIV that might also contribute to the initial onset of HIV-associated TTP.

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