Microangiopathic haemolytic anaemia resembling thrombotic thrombocytopenic purpura in systemic lupus erythematosus: the role of ADAMTS13

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Abstract
Thrombotic thrombocytopenic purpura (TTP) is a rare but frequently fatal complication of SLE. It occurs in the context of both active and inactive lupus and carries a worse overall prognosis than idiopathic acquired TTP. Recent advances in the knowledge and treatment of TTP do not seem to have brought similar improvements in the management and outcome of TTP in SLE. The illumination of the role of the von Willebrand factor multimer protease, ADAMTS13 in idiopathic TTP continues to enhance our comprehension of the pathogenesis of the disease and has contributed to improvements in diagnosis and management. We explore the overlap of TTP and SLE, and discuss the current understanding of the involvement of ADAMTS13 and its implications for patients with this uncommon form of microangiopathic haemolytic anaemia.

Key words: Microangiopathic haemolytic anaemia, Thrombotic thrombocytopenic purpura, Systemic lupus erythematosus.

Introduction
SLE is an autoimmune disorder characterized by the production of diverse autoantibodies and multisystem pathology. The generalized immune activation associated with SLE leads to protein manifestations of the disease [1]. The disease can, therefore, mimic other autoimmune syndromes whose mechanisms of pathogenesis are more narrowly defined. For example, patients with SLE can develop autoimmune nephritis, interstitial lung disease, autoimmune thrombocytopenia and aplastic anaemia. Some authors have examined the presentation of aplastic anaemia in SLE and found it to vary in many ways from that of idiopathic aplastic anaemia [2]. Similarly, a large body of literature has evolved on the presentation and management of SLE immune nephritis [3]. Thrombocytopenia in SLE can occur through a variety of mechanisms involving anti-platelet autoantibodies, the aPL syndrome and a thrombotic thrombocytopenic purpura (TTP)-like syndrome among others. Thus, SLE can, through a number of immune mechanisms, mimic other well-defined autoimmune syndromes. However, because the mechanisms of disease presentation can differ in the SLE-associated forms of these clinical syndromes compared with the idiopathic/classic forms, responses to therapy in SLE variants are often less predictable. In TTP, both autoimmune and non-autoimmune modes of pathogenesis have been described [4]. Due to the extremely complex mechanisms of tissue injury that occur in SLE, it can infrequently simulate the clinical presentation of TTP, presumably through immune-mediated processes. We briefly review the current understanding of TTP in SLE and highlight the differences between this condition and idiopathic TTP.

TTP: diagnosis and pathophysiology
TTP is a clinical syndrome classically characterized by a pentad of microangiopathic haemolytic anaemia (MAHA), thrombocytopenic purpura, fever, neurological abnormalities and renal disease that was first described by Moschcowitz [5]. Although the disease is rare, affecting ~3.7 cases per 1 million residents [6], some populations appear to be more susceptible to developing TTP, including patients with SLE, 1–4% of whom develop the disease [7]. Untreated TTP is almost uniformly fatal, but the recent
introduction of plasma exchange has improved the survival to between 80 and 90% [8]. A favourable outcome still depends on the early initiation of treatment. Relapses are very common in TTP patients who survive the initial episode, occurring in perhaps 30-60% of cases [4].

The differential diagnosis of TTP is extensive and includes diseases of autoimmune and non-autoimmune pathogenesis including haemolytic uraemic syndrome (HUS), immune thrombocytopenic purpura (ITP), autoimmune haemolytic anaemia (AIHA), APS, eclampsia, disseminated intravascular coagulation (DIC), MAHA associated with scleroderma and paroxysmal nocturnal haemoglobinuria (PNH). The absence of schistocytes in APS and the elevation of fibrin degradation products in DIC help to differentiate those conditions from TTP. While the diagnostic criteria for TTP include manifestations of MAHA, thrombocytopenic purpura, fever, neurological abnormalities and renal disease, an incomplete presentation does not rule out the diagnosis. Waiting for the evolution of the complete pentad can significantly delay early treatment and worsen patient outcomes. It is now accepted that thrombocytopenia as defined by a platelet count of <100 x 10^9/l and MAHA defined by the presence of schistocytes in peripheral blood smears are sufficient grounds to make a clinical diagnosis of TTP, as long as there are no other causes such as AIHA, DIC, cancer, eclampsia, drug toxicity, stem cell transplantation or malignant hypertension. A positive Coomb’s test, high serum lactate dehydrogenase levels and low-serum haptoglobin level would be unexpected findings in TTP and would suggest an alternative diagnosis. Fever, the presence of neurological symptoms such as seizures and altered mental status, and signs of altered renal function, are symptoms that often appear in more advanced presentations and occur later in the course of the disease. The fine points of diagnosing TTP have been comprehensively reviewed elsewhere [9].

Acute TTP episodes develop when there is high shear stress in the microcirculation, and von Willebrand’s factor (vWF) and platelets are prone to form aggregates. This propensity of vWF and platelets to form microvascular thrombi is mitigated by a disintegrin and metalloprotease with thrombospondin type 1 motif 13 (ADAMTS13), which cleaves vWF. Deficiency of ADAMTS13, due in part to autoimmune inhibitors in patients with acquired TTP and mutations of the ADAMTS13 gene in hereditary cases, leads to ultra-large vWF multimers that aggregate with platelets to cause microvascular thrombi [4, 10]. Histological staining of tissue affected by microvascular deposits and multiple occlusive thrombi. Thrombi can occur in the small arteries, arterioles and capillaries of several sites, especially the heart, brain, kidneys, mesenteric vessels, retina and adrenal glands. Immunohistochemical staining of affected tissues demonstrates thrombi that are strongly positive for vWF antigen but only weakly positive for fibrinogen or fibrin. The platelet-rich nature of the thrombi is revealed by electron microscopy, which shows tightly packed platelets with little fibrin [11].

Tests for ADAMTS13 antigen, anti-ADAMTS13 antibody and inhibitors of ADAMTS13 metalloprotease activity are available in some institutions but their role in diagnosis is not standardized. Severe ADAMTS13 deficiency is defined as <5% of ADAMTS13 activity in plasma. This can occur in the hereditary form of TTP, the Upshaw–Schulman syndrome, inherited as an autosomal recessive trait. In acquired idiopathic TTP, severe ADAMTS13 deficiency is the result of circulating antibodies or inhibitors of ADAMTS13 activity. Although the pathogenesis of acquired idiopathic TTP is well described, only about two-thirds to three-quarters of patients clinically diagnosed with acute idiopathic TTP have been found to be severely deficient in ADAMTS13 activity [12]. Differences in assay techniques may help to explain the discrepancies. However, the differences may also reflect our incomplete understanding of the biochemistry involved. The story for secondary forms of TTP is much less clear cut, with less of a correlation of disease activity with ADAMTS13 levels. The appellation idiopathic is therefore tentatively maintained.

The treatment of TTP involves the early use of plasma exchange with fresh frozen plasma [8]. Refractory cases may benefit from the addition of vincristine sulphate, CYC, AZA and IVIG [13, 14]. The use of cryoprecipitate-poor plasma has not been found to improve the response in adults with TTP [15]. Lastly, rituximab, an mAb to CD20, which depletes B lymphocytes, has been used in relapsing or refractory TTP [14].

**TTP in SLE**

Patients with SLE who develop TTP, do significantly worse than those with idiopathic TTP. One study recorded 34% mortality in reported cases of TTP in SLE alone [16] and another reported a much higher mortality of 62.5% [17]. It is very likely that due to the reporting bias in favour of positive outcomes, the mortality of TTP in SLE is even higher than these figures portray. The management of TTP in SLE has been modelled around the protocols used in idiopathic TTP. In addition, because of the evidence of active SLE in many of these patients, immunosuppressive medications traditionally used for SLE, such as CYC and AZA, have been employed. Some have suggested that the early use of CYC in patients with other evidence of SLE activity may result in more favourable outcomes in patients with concomitant TTP [7]. However, this approach has not met with universal acceptance and the care of patients with TTP in SLE remains a real challenge.

The dismal outcome of TTP in SLE is clearly fostered by our lack of understanding of the pathophysiology of the syndrome as it occurs in lupus. Possible explanations for the poor outcome have included the multisystem nature of lupus and the overall high disease burden occurring together with TTP. Lupus affects the CNS, kidneys, bone marrow, formed blood elements and cytokine release frequently enough in aggregate fashion that its presentation can mimic that of TTP when thrombotic MAHA occurs simultaneously. Others have, therefore, suggested that...
the syndrome is completely different in SLE and has to be considered a separate disease entity [18]. Yet others have suggested that the complex mechanisms of disease in lupus including, for example, the production of antibodies to ADAMTS13 protease may negatively impact on the outcome of TTP in lupus. Understanding the precise mechanisms is further complicated by the fact that the overall severity of SLE does not seem to correlate with the onset or prognosis of TTP [16].

**The role of ADAMTS13 in TTP in SLE**

In recent years, the more coherent comprehension of the aetiology of idiopathic TTP mentioned above has enhanced our understanding of TTP at the molecular level and is making an impact on the management of the disease. The role of vWF has emerged as a revolutionary insight into how the disease occurs. As noted before, large multimers of vWF characterize idiopathic TTP and are found in the microvasculature where they are processed by the cleaving protease ADAMTS13 under conditions of shear stress [4]. These multimers in turn facilitate the aggregation and activation of platelets, which drive the microvascular angioopathy. However, the role of ADAMTS13 in TTP in CTDs and SLE has not been as clearly defined.

Assays have been developed that can detect the presence of enzyme inhibitors, and quantitative and functional deficiencies of the ADAMTS13 enzyme associated with TTP. Some authors maintain that when performed correctly these assays seem to identify the majority of patients with idiopathic TTP [4]. Some have suggested that the absence of a reduction in enzyme activity at the time of diagnosis is associated with a significantly worse prognosis in TTP [12]. This seems to suggest that there are clinical subsets of disease, disease variants or even completely different diseases with similar manifestations, which perhaps require novel approaches to management. Expressed another way, patients with severe deficiency of ADAMTS13 activity could be seen as different in pathogenesis, response to treatment and prognosis from those without severe deficiency of protease activity. Therefore, the presence of a laboratory assay (measurement of ADAMTS13 activity) that might separate patients with idiopathic TTP in terms of prognosis has generated much excitement in the field. For example, Ahmed et al. [19] published a case of ulcerative colitis with TTP in which ADAMTS13 levels correlated perfectly with platelet recovery levels and patient clinical improvement following plasma exchange and vincristine administration. Similarly, Fakhouri et al. [20] have shown that the use of rituximab is associated with removal of ADAMTS13 inhibitor activity and improvement of clinical outcomes in TTP. Therefore, it would seem that in patients who have demonstrable inhibitor activity or functional deficiencies in the protease, conventional treatments afford a very favourable prognosis. However, in patients whose disease occurs in the presence of fully functional ADAMTS13, the outlook appears grimmer, including those with SLE and normal ADAMTS13 activity. This is similar to the dismal outlook of patients who develop transplant-associated microangiopathic anaemia after allogeneic stem cell transplantation. These patients appear to lack the severe compromise of ADAMTS13 activity that characterizes idiopathic TTP. The mortality of this condition is also high at 60–90% [21]. These patients do not respond favourably to plasma exchange.

Despite these reports of a clear association between ADAMTS13 activity and TTP presentation in idiopathic disease, several authors have suggested that the relationship may not be as straightforward as inferred. Therefore, the role of ADAMTS13 in idiopathic TTP remains controversial and there are no recommendations for the use of ADAMTS13 activity assays for therapeutic decisions or to gauge prognosis and patient outcomes. Thus, counter to the initial assumption that low ADAMTS13 activity would be more common in idiopathic TTP and that such patients would more likely respond to plasma exchange as referred to in the studies mentioned above, some studies have shown that the level of ADAMTS13 activity does not necessarily differ between secondary and idiopathic forms of TTP and that the activity of ADAMTS13 has no predictive value for response to therapy [22].

A study by Matsuyama et al. [23] looked at the specific role of ADAMTS13 in thrombotic microangiopathies in CTDs. The evidence suggests that some differences exist in the levels of vWF and ADAMTS13 between patients with or without underlying CTD, but that these differences do not sufficiently explain the increase in TTP-like disease in these syndromes. Overall, what few data there are seem to suggest that TTP in the context of CTD, in general, and SLE, in particular, is not associated with the trend to low ADAMTS13 activity reported in the idiopathic disease [24]. Furthermore, there does not appear to be an increase in neutralizing antibody to ADAMTS13 in the CTDs [24]. The possibility of neutralizing antibodies being important in the inhibition of ADAMTS13 and the development of a relative deficiency of the protease, has been the justification for the use of rituximab in refractory TTP in lupus. Although rituximab has been used successfully to treat TTP in SLE unresponsive to other interventions [25, 26], the numbers of patients are small and large series have not been published because of the rarity of the disease. It therefore remains to be seen whether B-cell targeting or depletion strategies will be uniformly effective in TTP in SLE.

**Beyond ADAMTS13: other mechanisms of microangiopathy in TTP in lupus**

A number of lupus-related mechanisms deserve attention as potential modulators of the process of microangiopathy in lupus-associated TTP. These pathways may help to explain why ADAMTS13 does not appear to take centre stage in TTP in lupus as it has been suggested to do in the majority of idiopathic TTP cases. Gunther and Dhlamini [27] observed that D-dimer was significantly elevated in TTP patients with HIV compared with non-HIV-related TTP, suggesting a role for D-dimer in...
the pathogenesis of HIV-related TTP. The authors hypothesize that the significant damage to endothelial cells seen in HIV/AIDS might encourage the formation of D-dimer. Since endothelial cell activation and damage are common in lupus, it remains to be seen whether D-dimer elevation would typify TTP in SLE. It has also been observed that patients with lupus irrespective of their aPL status are more likely to thrombose, if they have high levels of D-dimer. High levels of D-dimer in turn correlated with the presence of infections and flares of lupus disease activity [28]. Unfortunately, authors have not detailed D-dimer levels in their reports of TTP in SLE and no conclusive statements can be made on the differences between levels of D-dimer in lupus-related TTP and idiopathic TTP.

It has also been suggested that some of the clinical features of TTP might result from the removal of nitric oxide from the circulation by the nitric oxide scavenger free haemoglobin. Excess of haemoglobin not removed by haptoglobin during intravascular haemolysis acts as a powerful binding agent for nitric oxide, thus interfering with its vasodilator action and its ability to regulate microvascular tone. Furthermore, haemoglobin might be an inhibitor of ADAMTS13 activity, which could further encourage the build-up of vWF multimers and platelet aggregation [29]. The specific role of nitric oxide in microvascular haemolysis in TTP in SLE has not been clarified.

It has been proposed that the APS leads to both microvascular thrombosis and microangiopathic involvement in multiple organs. This latter finding can make it hard to distinguish the APS from other MAHAs including TTP. Obviously, in lupus patients, a continuum of pathological mechanisms is involved in their clinical presentation. This not only poses challenges for diagnosis but also complicates the management of MAHA in lupus [30]. The overlap in pathogenic mechanisms at the microvascular level is what makes catastrophic APS [31] such a close mimic of TTP. Austin et al. [32] studied 52 stable APS patients and noted a reduction in ADAMTS13 activity. However, the reductions in activity were mild and ADAMTS13 levels were normal suggesting a loss of function of the antigen. Few of the patients had ADAMTS13 antibodies with neutralizing activity although half of them had ADAMTS13 antibodies. There was reduced clearance of vWF consistent with the reduction in activity found. However, there did not seem to be any correlation between ADAMTS13 activity and clinical phenotype. The study suggests that perhaps in a subset of patients with APS, ADAMTS13 may play a role in the aetiopathogenesis of MAHA and account for the similarities with idiopathic TTP.

The pro-coagulant environment in APS may be aided in part by increased activity of plasminogen activator inhibitor (PAI) [33], which has been demonstrated in patients with the LA and more generally in some SLE patients. In addition to the previous observation, Musial et al. [34] have described an increase in thrombin generation after small skin-vessel injury in subjects with lupus and aPLs that are only partially compensated for by enhanced anti-thrombin III and other fibrinolytic activity.

Another interesting hypothesis related to the possible resistance of vWF to proteolysis in the inflammatory state is supported by in vitro evidence [35, 36]. The release of reactive oxygen species and free radicals by neutrophil activity exposes vWF cleavage site amino acids to hypochlorous acid and peroxynitrite. Oxidized vWF has been shown to be resistant to cleavage by ADAMTS13 at least in vitro. Therefore, it is quite possible that with the pro-inflammatory climate of lupus, vWF could be rendered resistant to the activity of ADAMTS13, predisposing to thrombosis. Notably, oxidation of vWF amino acid residues by peroxynitrite did not inhibit the ability of purified vWF multimers to agglutinate platelets activated by ristocetin [35].

Endothelial cell activation through anti-endothelial cell antibodies, complement activation and other immunological pathways, are processes that characterize lupus microangiopathy. Together with the non-ADAMTS13-mediated reactions detailed above, these interactions could offer insights into how the microangiopathic process might differ in SLE from idiopathic TTP and why the differences in pathogenesis, pathology and clinical presentation as well as response to therapy noted before might occur.

Conclusion

The literature suggests an excess of TTP in the SLE population [37], which we think is explained by the expected rare incidence of TTP in this population in addition to the phenomenon of thrombotic MAHA associated with severe lupus that mimics idiopathic TTP because of the multi-system nature of SLE. Infection might in large part be responsible for the excess mortality [38]. Our understanding of the biochemistry of ADAMTS13 protease and its role in TTP has made a significant impact on our understanding of the pathogenesis of idiopathic TTP. However, the relationship of ADAMTS13 to the TTP-like syndromes seen in CTDs in general, and lupus in particular, still needs clarification. The differences may be due at least in part to the non-standardized assay techniques used today. Although further elucidation and confirmation are required, the current TTP literature generally suggests that the idiopathic form of the disease is related to low levels of ADAMTS13 activity and is responsive to plasma exchange. In contrast, the TTP-like syndrome associated with CTDs including SLE appears to be more refractory to plasma exchange (Table 1) and carries a worse prognosis than idiopathic TTP. The precise mechanisms of ADAMTS13 involvement in these haemolytic syndromes clearly need to be better defined. Cases of TTP in SLE need to be documented and ADAMTS13 levels need to be measured in all cases of suspected TTP, preferably before treatment initiation and certainly before plasma exchange, using universally standardized assays. Hopefully, the knowledge gained would allow improved classification and management of the variety of presentations of TTP. In the interim, given the significant differences in presentation and outcome of idiopathic TTP and TTP in lupus, we
propose that the TTP-like presentation of some forms of severe lupus be properly termed lupus-related TTP-like MAHA to distinguish it from the more classically defined idiopathic and hereditary forms of TTP.

**Rheumatology key messages**

- Unlike idiopathic TTP, TTP in SLE is frequently fatal.
- Understanding the pathogenesis, including the role of ADAMTS13, would greatly improve the management of TTP in SLE.

**Disclosure statement:** The authors have declared no conflicts of interest.

**References**


**Table 1 Clinical differences between idiopathic TTP and TTP in SLE**

<table>
<thead>
<tr>
<th>Clinical presentation</th>
<th>Idiopathic TTP</th>
<th>TTP in SLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex predominance</td>
<td>M = F</td>
<td>Female predominance remains</td>
</tr>
<tr>
<td>Response to plasma exchange</td>
<td>Good</td>
<td>Poor</td>
</tr>
<tr>
<td>Need for immunosuppressives</td>
<td>Some cases</td>
<td>Most cases</td>
</tr>
<tr>
<td>Relapse rate</td>
<td>++</td>
<td>+++ [99]</td>
</tr>
<tr>
<td>Morbidity</td>
<td>Moderate</td>
<td>Lupus renal disease is frequent. Patients are sicker overall, many require intensive care</td>
</tr>
<tr>
<td>Mortality</td>
<td>10%</td>
<td>Higher: 30–60%</td>
</tr>
<tr>
<td>ADAMTS13 activity</td>
<td>Low</td>
<td>Variable</td>
</tr>
<tr>
<td>Endothelial cell activation</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

++: high; +++: very high.


