Advantages and limits of ADAMTS13 testing in the prognostic assessment of thrombotic thrombocytopenic purpura

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Summary

Thrombotic thrombocytopenic purpura (TTP) is a rare but severe disease characterized by mechanical hemolytic anemia and consumptive thrombocytopenia leading to disseminated microvascular thrombosis that causes signs and symptoms of organ ischemia and functional damage. After the elucidation of the pathophysiology of TTP, thanks to the demonstration of the congenital or acquired (autoimmune) plasma deficiency of the von Willebrand factor cleaving metalloprotease A Disintegrin And Metalloprotease with ThromboSpondin 1 repeats (ADAMTS13), a number of laboratory assays for measuring ADAMTS13 and related autoantibodies have been developed. Current knowledge on the diagnostic and prognostic value of ADAMTS13 and anti-ADAMTS13 assays is summarized in this review.

Thrombotic thrombocytopenic purpura (TTP) is a rare disease (five to 10 cases per year per million) characterized by the massive formation of platelet rich-thrombi in the microcirculation of multiple organs [1–3]. It affects both sexes, although the incidence is two to three times higher among females [4]. Several reports of TTP have followed the initial description in 1924 by Moschowitz [5], leading to an earlier definition based upon the following pentad of symptoms: fever, mechanical haemolytic anemia, thrombocytopenia, central nervous system and renal impairment [6]. However, neither fever nor neurologic abnormalities and renal impairment are constant symptoms, especially during the early stage of the disease, thus leading to the currently accepted diagnostic definition of TTP consisting of the dual presence of mechanical haemolytic anemia with fragmented erythrocytes and consumptive thrombocytopenia (< 100 × 10⁹/L) with exclusion of alternative causes [4,7].
The pathogenesis of TTP remained unknown until the beginning of the 1980s, when Joel Moake observed that plasma from a patient with a chronic recurrent form of TTP contained ultralarge (UL) molecular weight multimers of the platelet-adhesive glycoprotein von Willebrand factor (ULVWF) [8]. These ULVWF forms are normally present in the endothelium and platelets but not in plasma. Once released from abnormally stimulated endothelial cells, ULVWF form string-like structures that in turn cause platelet adhesion, aggregation and microvascular thrombosis. Hemolytic anemia is caused by mechanical erythrocyte damage, particularly in blood flow conditions characterized by high shear stress such as those occurring in the microcirculation [3]. Moake hypothesized that the deficiency of a depolymerase was responsible for the presence of ULVWF in chronic recurrent TTP [8], but the agent responsible for regulating the multimeric structure of VWF was not identified until 1996, when Furlan et al. [9] and Tsai [10] independently isolated from human plasma a metal-dependent protease able to cleave the peptide bond between the tyrosine at position 1605 and the methionine at position 1606 in the central A2 domain of VWF. The next year, the VWF-cleaving protease was found to be deficient in the plasma of patients with congenital TTP [11]. Soon after, patients with acquired idiopathic TTP were reported to have severe VWF-cleaving protease deficiency caused by IgG autoantibodies that inhibit enzyme activity [12,13].

During the next few years, the VWF-cleaving protease was purified [14,15], cloned [16,17], and named ADAMTS13 because it belongs to the recently discovered A Disintegrin And Metalloprotease with Thrombospondin 1 repeats (ADAMTS) family (it was the 13th member) of metalloproteases [18]. During more recent years, there has been much progress in understanding how ADAMTS13 regulates the biologic function of VWF, but there is still some uncertainty on how to use and apply clinically this knowledge. In particular, a major question pertains to the diagnostic and prognostic value of ADAMTS13 and anti-ADAMTS13 testing in TTP, issues that will be addressed in this review article.

**Diagnosis of thrombotic thrombocytopenic purpura**

There are two different forms of TTP, congenital and acquired [3]. Congenital TTP, caused by mutations in the ADAMTS13 gene (which is located on chromosome 9q34 and codes for the metalloprotease), is an extremely rare (incidence 1:1,000,000) autosomal recessive disease which manifests often, but not exclusively, at birth or during childhood [19–22]. The acquired forms can be distinguished into two main types: immune-mediated forms, due to autoantibodies against ADAMTS13 [23–25], and those perhaps secondary to massive endothelial stimulation that results in the release of ULVWF multimers in amounts exceeding the system’s cleaving ability, despite the presence of normal or only mildly reduced plasma levels of ADAMTS13 [26]. The most common physiological or pathological conditions associated with the immune-mediated forms, which are often accompanied by severe ADAMTS13 deficiency (plasma levels less than 5 to 10% of normal), are pregnancy, infections, autoimmune diseases and the use of such drugs as ticlopidine and clopidogrel. The most frequent conditions associated with TTP presenting with normal or mildly reduced levels of ADAMTS13 (greater than 10%) are metastatic tumors, sepsis, organ transplantation (particularly allogeneic bone marrow transplantation and solid organ transplants) and the use of such drugs as cyclosporine, mitomycin and α-interferon [27]. In some instances, TTP occurs as a single, sporadic acute episode, but there are also chronic recurrent forms (20–30% of the cases), which have a genetic basis or are associated with the sustained persistence of anti-ADAMTS13 autoantibodies [3].

The strategy of using plasma ADAMTS13 levels to manage TTP stems from the current availability of assays that include measurement of ADAMTS13 activity, ADAMTS13 antigen and neutralizing or non-neutralizing anti-ADAMTS13 autoantibodies. The functional assays traditionally used to measure ADAMTS13 activity in plasma involve the incubation of plasma samples with a source of full-length VWF followed by and then the measurement of the amount of residual, uncleaved, higher molecular weight VWF [28–30]. These assays are sensitive (lower detection limit: 3–6% of ADAMTS13 activity) and quite reproducible but cumbersome, time consuming and performed in non-physiological conditions, because denaturating agents are required to promote the susceptibility of full-length VWF cleavage by ADAMTS13. ADAMTS13 activity can also be measured using the fluorescence resonance energy transfer (FRET) technique, a rapid test which employs a truncated synthetic 73-amino-acid VWF peptide as substrate for the determination of ADAMTS13 activity (FRETS-WF73 assay) [31,32]. The assays based on VWF peptides are more sensitive (lower detection limit: 1% to 3% of ADAMTS13 activity), reproducible, facile, rapid (4 hours) and performed in absence of denaturating agents, but they employ non-physiological substrates. ADAMTS13 antigen methods are also available using enzyme-linked immunosorbent assay (Elisa) techniques. They are sensitive to very low levels of ADAMTS13 antigen, but their clinical applications and diagnostic usefulness are still poorly investigated. Autoantibodies that inactivate the enzymatic activity of ADAMTS13 are detected by means of functional assays that measure the capacity of test plasma to neutralize the protease activity in normal plasma, using a principle similar to that of the so called Bethesda assay of anti-factor VIII alloantibodies in hemophilia [33]. Finally, non-neutralizing autoantibodies to ADAMTS13 can be detected by Western blotting or by measuring the amount of immunoglobulin that binds to microtitre plates coated with recombinant ADAMTS13 [34]. These assays are rapid, and when used in combination with functional assays, can establish whether a patient has neutralizing or non-neutralizing antibodies to ADAMTS13.
A number of studies have assessed the diagnostic value of plasma ADAMTS13 testing in acute TTP [35,36]. After the original publications of Furlan et al. [12] and Tsai and Lian [13], who claimed that all patients with TTP have a severe plasma deficiency of the protease, the results obtained by Veyradier et al. [37] only partially confirmed the original findings, because severe ADAMTS13 deficiency was found in 71% of patients clinically diagnosed with TTP. The high diagnostic value of finding severe ADAMTS13 deficiency was subsequently challenged by several studies, which reported that the protease was severely deficient in a variable proportion of patients clinically diagnosed with TTP, ranging from as few as 18% to 72% [38–42]. The controversial issue of the diagnostic specificity of low ADAMTS13 levels in acute TTP is still debated and the current prevailing opinion is that, while undetectable or very low plasma levels of enzymatic activity (less than 10%) establish unequivocally a diagnosis of inherited or acquired TTP, not all patients appropriately diagnosed with TTP on the basis of clinical and laboratory signs and symptoms present with severe protease deficiency. As mentioned above, the cases of TTP more frequently associated with normal or moderately reduced ADAMTS13 (between 10% and 40% of normal) are those secondary to other diseases or conditions, such as allogeneic bone marrow transplantation, HIV infection, chemotherapy, metastatic cancer and sepsis [40].

**Prognosis of thrombotic thrombocytopenic purpura**

A number of studies have evaluated ADAMTS13 activity and anti-ADAMTS13 as predictors of outcome during the acute phase or after remission in patients with acquired TTP [39–50].

### Acute phase

A critical problem for the management of patients with TTP is whether or not ADAMTS13 or anti-ADAMTS13 testing during the acute phase of the disease helps to predict important short-term outcomes such as clinical and laboratory remission and mortality. In a prospective study conducted on 142 patients with TTP, Vesely et al. [40] found that patients with severe ADAMTS13 deficiency achieved remission more frequently than those without severe deficiency (84% vs. 55%) and had a lower mortality rate (16% vs. 45%). Similar results in terms of relationship between ADAMTS13 values during the acute episode, remission and mortality were obtained in a smaller prospective study by Zheng et al. [42] and in several retrospective studies (table I) [39,41,44]. Overall, those data suggest that, during acute TTP, a severe deficiency of plasma ADAMTS13 is associated with a greater likelihood of favourable short-term outcomes (remission and survival). Conversely, TTP cases with detectable ADAMTS13 activity are associated with a high mortality. It cannot be excluded, however, that the latter finding is simply due to the fact that patients with detectable ADAMTS13 develop TTP in association with more severe and life-threatening underlying diseases or conditions such as metastatic cancer and allogeneic bone marrow transplantation [40].

Most of the aforementioned studies also evaluated the short-term prognostic value of anti-ADAMTS13 testing. In the study by Zheng et al. [42], all patients (8/8) without detectable anti-ADAMTS13 achieved a complete remission, while one death was detected among the four patients with anti-ADAMTS13. Mori et al. [39] reported that complete remission was reached in all the eight patients without detectable anti-ADAMTS13.

### Table I

<table>
<thead>
<tr>
<th>Study design</th>
<th>Severe vs. non-severe ADAMTS13 deficiency (%)</th>
<th>Presence vs. absent anti-ADAMTS13 (%)</th>
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<tbody>
<tr>
<td>Remission</td>
<td>Death</td>
<td>Relapse</td>
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<tr>
<td>Mori, 2002 [39]</td>
<td>Retrospective</td>
<td>85 vs. 20</td>
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<tr>
<td>Vesely, 2003 [40]</td>
<td>Prospective</td>
<td>84 vs. 55</td>
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<tr>
<td>Zheng, 2004 [42]</td>
<td>Prospective</td>
<td>82 vs. 49</td>
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<tr>
<td>Raife, 2004 [44]</td>
<td>Retrospective</td>
<td>–</td>
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<tr>
<td>Copped, 2004–2005 [41,45]</td>
<td>Retrospective</td>
<td>–</td>
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<tr>
<td>Böhm, 2005 [46]</td>
<td>Retrospective</td>
<td>–</td>
</tr>
<tr>
<td>Kremer Hovinga, 2010 [50]</td>
<td>Prospective</td>
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while of the six patients with detectable anti-ADAMTS13, four (67%) obtained a response and two (33%) died. In the study conducted by Coppo et al. [45], deaths (4/21, 19%) were observed only in patients with detectable inhibitor, while all cases with undetectable anti-ADAMTS13 (12/12) evolved favourably obtaining a complete remission. Finally, in a study including both patients with a first episode of TTP and those with relapses, Böhml et al. [46] found that all TTP cases with undetectable anti-ADAMTS13 achieved a complete response to treatment, while 16% of 18 patients with anti-ADAMTS13 died. Overall, these data would indicate that inhibitors are associated with a worse prognosis, although the small sample size of all these studies demands great caution in interpreting the results.

## Relapse

Due to the high rate of relapse (at least 20–30% of cases) occurring in patients who survive the acute initial episode of TTP, many studies were focused on the search of predictors for TTP recurrence [43]. With this goal, the value of ADAMTS13 and anti-ADAMTS13 testing was evaluable in predicting the likelihood of recurrence in TTP patients, during both the acute phase and first remission of TTP. So far no single prospective study has been of adequate size, but data pooled from those of Vesely et al. [40], and Zheng et al. [42] yielded a relapse rate of 37% in patients with severe ADAMTS13 deficiency, compared with 6% in those without. Similarly, the results from three small prospective studies [37,39,41] also indicate that ADAMTS13 deficiency caused by autoantibodies is associated with a relapsing course of TTP (43%), while patients without detectable antibodies during acute TTP rarely relapse (5%). In a prospective cohort study, Ferrari et al. [47] investigated 32 patients who had low plasma levels of ADAMTS13 activity at the time of the first acute episode of TTP and subsequently achieved remission. In them, the presence of high levels of inhibitory anti-ADAMTS13 IgG at presentation was associated with the persistence of undetectable ADAMTS13 activity in remission, which was in turn predictive of relapses within 18 months. These results were in accordance with those published by Peyvandi et al. [48] in a retrospective cohort study on 109 TTP patients. Survivors of an acute TTP episode with severely reduced ADAMTS13 levels and/or anti-ADAMTS13 antibodies during remission had an approximately threefold greater likelihood of developing another episode of TTP than patients with higher protease activity and no antibody. That low ADAMTS13 activity during clinical remission is a key predictor for TTP relapse was also reported by Jin et al. [49]. Finally, the recent update of the Oklahoma TTP Registry conducted by Kremer Hovinga et al. [50] confirms once more that the relapse rate is greater among patients with severe ADAMTS13 deficiency (< 10%) than among those with ADAMTS13 activity ≥ 10% (41% vs. 4%). In addition, among patients with severe ADAMTS13 deficiency, an inhibitor titre of 2 Bethesda Units or more is associated with a shorter survival ($P = 0.05$) [50]. Overall, it appears that severe ADAMTS13 deficiency and the presence of anti-ADAMTS13 antibodies at relatively high titres during acute TTP or during remission are associated with a higher risk of recurrence.

## Conclusions

The prognostic value of ADAMTS13 testing is still a controversial issue. Although the available literature data suggest that the detection of an anti-ADAMTS13 antibody is associated with increased treatment refractoriness and death rate, the number of patients evaluated is too limited to draw firm conclusions. Additional studies are also required to elucidate the relationship between the inhibitor titre and short-term outcomes. The finding that severe ADAMTS13 deficiency and the presence of anti-ADAMTS13 antibodies during acute TTP or first remission are associated with an increased risk of relapses is rather consistent in the frame of several retrospective studies, but more prospective studies on larger populations of TTP patients are warranted. Future trials assessing the role of prophylactic immunosuppressive therapy during remission in order to prevent relapses are also much awaited.

**Disclosure of interest:** the authors declare that they have no conflicts of interest concerning this article.

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