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CHAPTER 4

Residual ADAMTS13 activity in ADAMTS13-deficient thrombotic thrombocytopenic purpura: an emerging concept

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Abstract

Thrombotic thrombocytopenic purpura (TTP) is a rare life-threatening thrombotic microangiopathy characterized by acute episodes of widespread microvascular thrombosis. The discovery that the plasmatric activity of the von Willebrand factor (VWF) cleaving protease, ADAMTS13, is severely deficient in a great proportion of individuals with TTP partially clarified the pathophysiology of the disease. However, the finding of severe deficiency of ADAMTS13 alone is unable to fully explain the clinical heterogeneity of patients with TTP. The recent development of methods that measure ADAMTS13 activity with great analytical sensitivity and precision offers the opportunity to study the spectrum of ADAMTS13 activity below 10% (herein defined as “residual ADAMTS13 activity”). Recent exploratory studies on residual ADAMTS13 activity suggest that the amount of residual activity of ADAMTS13 might be a major determinant of the clinical heterogeneity of TTP in patients with severe ADAMTS13 deficiency. In this article, we review the recent findings on residual ADAMTS13 activity and their implications for research and clinical practice in the field.

Introduction and scope of this review

Thrombotic thrombocytopenic purpura (TTP) is a rare life-threatening thrombotic microangiopathy characterized by acute episodes of widespread microvascular thrombosis.¹ The discovery that the plasmatic activity of the von Willebrand factor (VWF) cleaving protease, ADAMTS13, is severely deficient in a great proportion of individuals with TTP partially clarified the pathophysiology of the disease.² Severe ADAMTS13 deficiency is defined as an activity of the protease below 10% of normal, with normal values usually ranging from 50% to 150%. Severe ADAMTS13 deficiency is either due to circulating anti-ADAMTS13 autoantibodies (i.e. acquired deficiency)³ or, less frequently, to recessively inherited mutations of *ADAMTS13* (i.e. congenital deficiency).⁴ Although not all of the patients with a clinically defined TTP present with severely reduced ADAMTS13, the finding of severe ADAMTS13 deficiency has been consistently shown to define a distinct subgroup of the disease, with peculiar behavior and clinical features.⁵⁻⁷ In these patients, the pathologic presence in the circulation of ultralarge VWF multimers that remain uncleaved is regarded as the mechanism responsible for VWF-mediated platelet aggregation and thrombosis.⁸ The peculiar clinical characteristics of TTP with severe ADAMTS13 deficiency include a relatively mild acute-disease prognosis, with better response to plasma exchange (PEX) and lower episode-fatality rate, and high tendency to recur.^{6,9} In spite of the recognized importance of measuring ADAMTS13 activity at acute disease presentation, the finding of severe deficiency of ADAMTS13 alone is unable to fully explain the clinical heterogeneity of patients with TTP. For example, patients with auto-antibody mediated severe deficiency of ADAMTS13 may

remain in remission for years, in spite of unmeasurable or very low ADAMTS13 activity and persistence of anti-ADAMTS13 autoantibodies.⁹ Also, some patients with congenital severe deficiency of ADAMTS13 develop their first disease episode in their adult age, whereas others have early-onset disease and frequent recurrences.^{10, 11} The recent development of methods that measure ADAMTS13 activity with great analytical sensitivity and precision offers the opportunity to study the spectrum of ADAMTS13 activity below 10%.^{11, 12} Residual ADAMTS13 activity is herein used to define the amount of activity below 10% that is detectable in patients with severe ADAMTS13 deficiency. Recent exploratory studies found that patients with severe deficiency of ADAMTS13 show a range of residual plasmatic activity and that residual activity is associated with clinically relevant endpoints.¹³⁻¹⁶ This suggests that the amount of residual activity of ADAMTS13 might be a major determinant of the clinical heterogeneity of TTP in patients with severe ADAMTS13 deficiency. In this article, we review the recent findings on residual ADAMTS13 activity and their implications for research and clinical practice in the field. The review will focus only on TTP with severely deficient ADAMTS13 activity.

TTP with severe deficiency of plasmatic ADAMTS13 activity as a distinct subtype of the disease

TTP was originally described by Eli Moschcowitz in 1924 as a syndrome with a pentad of clinical manifestations: thrombocytopenia, microangiopathic hemolytic anemia, neurologic symptoms, renal involvement and fever.¹⁷ It was later recognized that fever, neurologic symptoms or renal involvement are not always

present during TTP, so that TTP is currently defined by the presence of thrombocytopenia and microangiopathic hemolytic anemia and by the exclusion of an alternative diagnosis.¹⁸ The pathophysiology of TTP remained unexplained for many decades until studies in the early 1980s demonstrated that patients with chronic relapsing TTP had a defect in their ability to cleave unusually large VWF multimers.¹⁹ Subsequently, in the late 1990s it was found that the plasma of patients with the disease was severely deficient of von Willebrand factor (VWF) cleaving protease activity.⁸ Further studies identified anti-ADAMTS13 antibodies³ and mutations in ADAMTS13⁴ as causes of severely reduced ADAMTS13 activity. The discovery of the role of ADAMTS13 sparked investigations on laboratory measurements related to the protease. A number of observational studies demonstrated that ADAMTS13 deficiency is not invariably present in patients with a diagnosis of TTP, but also that patients with severe deficiency of ADAMTS13 have distinct clinical characteristics and prognosis.^{5-7, 9, 20-22} Severe ADAMTS13 deficiency in TTP patients is associated with idiopathic rather than secondary disease (i.e., disease associated with clinical conditions such as cancer, bone marrow transplantation or use of certain drugs), lower platelet count at presentation and lower prevalence of renal involvement.^{5, 6, 9, 22} Patients with severely reduced ADAMTS13 activity at acute disease presentation have milder acute disease prognosis with better response to PEX and lower episode fatality rate than those with ADAMTS13 above 10%.^{5, 6, 9} This association is caused by the poor prognosis of secondary TTP forms in which severe ADAMTS13 deficiency has low prevalence (e.g., bone-marrow transplantation associated TTP or cancer-associated TTP). Patients with severe

deficiency are also more prone to recurrence.^{9, 14, 20, 23} A recent study found a 41% percent recurrence risk at 7.5 years in TTP survivors who presented with severe ADAMTS13 deficiency, whereas survivors with activity above 10% had a recurrence rate of only 4%.⁹ Studies on ADAMTS13-related measurements as markers for recurrence are summarized in Table 1. Also ADAMTS13-related laboratory measurements other than ADAMTS13 activity were shown to be associated with disease outcomes. These markers include ADAMTS13 antigen levels and IgG titre, inhibitor titre, Ig subclass and specificity of anti-ADAMTS13 antibodies.^{6, 9, 20, 23-26} However, the value of these markers as predictors after accounting for ADAMTS13 activity is uncertain. This prompted researchers to look for additional prognostic markers that could help predict the risk of acute disease morbidity and mortality and the imminence of disease recurrence.²⁷

Residual ADAMTS13 activity in congenital and acquired ADAMTS13 deficiency

Until recently the quantification of ADAMTS13 plasmatic activity relied on assays that were developed in order to distinguish patients with severely reduced from patients with normal or slightly reduced protease activity. Assays standardization, and limitations in sensitivity, linear behavior and reproducibility were well recognized issues affecting early-developed methods.²⁸ The performance of ADAMTS13 assays improved through the years, with the development of new methods and improvement of existing ones.²⁹ Recently, assays able to measure residual ADAMTS13 activity with great analytical

sensitivity (limit of detection [LOD] of up to 0.5%) have been developed.^{11, 12} In particular, a method based on SELDI-TOF mass spectrometry for the detection of cleavage product showed linear behavior for the measurement of activities below 10% and great reproducibility.¹³ The assay was employed in a number of exploratory studies that sought for associations between residual ADAMTS13 plasmatic activity and disease outcomes.¹³⁻¹⁶

Residual ADAMTS13 activity in congenital TTP

Congenital TTP (also known as Upshaw-Schulman syndrome, OMIM #274150) is caused by mutations in *ADAMTS13* and accounts for less than 10% of all cases of TTP. As noted, the phenotype of congenital TTP is variable in its clinical severity.^{10, 11} A study was recently conducted in which the residual activity of ADAMTS13 was measured by SELDI-TOF mass spectrometry in a cohort of 29 patients with congenital TTP.¹⁵ The study was designed to test the hypothesis that genetically-determined patterns of residual ADAMTS13 activity were responsible for the variable clinical phenotype. The study found that a residual plasmatic activity of ADAMTS13 was measurable in 90% of the study participants. It also showed that the amount of residual plasmatic activity of ADAMTS13 measured by SELDI-TOF mass spectrometry is negatively associated with the clinical severity of the phenotype (as assessed by the age of disease onset, the annual rate of TTP episodes and the prescription of FFP prophylaxis). Residual plasmatic activity levels below 2-3% pinpointed patients with adverse clinical outcomes (i.e. age of onset below 18 years, annual rate of TTP episodes greater than 1 and need for FFP prophylaxis). Although these discriminative levels should be

validated in an independent cohort before being introduced in clinical practice, the results of the study suggest that the use of a sensitive ADAMTS13 activity measurement could be used to tailor individual management, in particular concerning the decision of whether or not to implement long-term FFP prophylaxis. The results also provide pathophysiological insights. A model of congenital TTP is suggested whereby congenital TTP patients with a higher residual plasmatic activity of ADAMTS13 are protected from disease onset until they come across a strong challenge to their fragile ADAMTS13-VWF balance (e.g. pregnancy, infection, surgery). In contrast, patients with a low or with no residual activity are more vulnerable to environmental challenges, developing frequent disease episodes. Consistent with this model is the relationship between residual ADAMTS13 activity and the age of first disease episode of congenital TTP patients. Patients with higher residual activity had an older age of onset (Figure 1A), although a few patients had early age of onset in spite of relatively high residual activity. This may be due to the presence of genetic modifiers or to a varying impact of environmental triggering conditions (Figure 1B); most importantly, none of the patients with low residual activity had adult age of onset (Figure 1C). Additional support for this model came from the measurement of residual activity during acute TTP and remission in a patient with congenital TTP.¹⁶ While activity was detectable in multiple remission samples, a drop to undetectable levels was observed during an acute disease episode. Overall, these studies highlight an important contribution of residual ADAMTS13 as a determinant of disease severity in congenital TTP.

Residual ADAMTS13 activity in acquired TTP

In patients with acquired TTP, the amount of residual ADAMTS13 activity in patients with severely deficient ADAMTS13 (activity below 10%) was associated with both subsequent TTP exacerbation (i.e. reappearance of disease within 30 days of the remission of a previous event)¹⁴ and recurrence (i.e. reappearance of disease after 30 days).¹³ In the former study, mean ADAMTS13 activity at presentation was significantly lower in patients who suffered exacerbations than in those who did not (mean residual ADAMTS13 of 1.4% vs 2.9%). Receiver operating characteristic curve analysis of all study subjects indicated that the discriminative level of residual ADAMTS13 activity for the risk of exacerbation was 1.15%. In the latter study, the association between residual ADAMTS13 activity and disease recurrence was obtained within a group of patients who already had a diagnosis of severely reduced ADAMTS13. This indicates that the measurement of ADAMTS13 in TTP patients is informative of the risk of recurrence also for activities below 10%. The relationship between ADAMTS13 activity and risk for recurrence is characterized by an exponential curve (Figure 2). This pattern confirms the importance of being able to measure low ADAMTS13 activity, as the risk of recurrence witnesses a steep increase for decreasing activity below levels of 20% and especially below 10%. Anecdotic reports on the risk of pregnancy-associated TTP support these results, showing that women who develop TTP associated with pregnancy have very low levels of ADAMTS13 activity (below 2.5%) at the beginning of their pregnancy.

Future directions

The concept that the residual activity of ADAMTS13 in patients with ADAMTS13-deficient TTP is an important determinant of disease severity and of clinically relevant outcomes has implications for future research in the field. The development of a new generation of assays able to measure in a fast, cost effective and reproducible way ADAMTS13 activity at low concentrations is warranted in order to apply residual activity measurement to large-scale research and in clinical practice. Similarly sensitive and precise assays of ADAMTS13 antigen levels might help calculating the specific activity of mutated ADAMTS13 in congenital patients in whom residual plasmatic activity has been quantified. In this group of patients the benefit of measuring residual ADAMTS13 repeatedly and that of using the recently identified discriminative levels for clinical decisions will be the focus of future research. In the field of acquired TTP, the exponential increase of TTP recurrence risk for decreasing values of ADAMTS13 activity implies that much of predictive power of measuring ADAMTS13 activity may stand in a tail of the ADAMTS13 activity spectrum (which is poorly ascertained by most of the currently available assays). The hypothesis merits further and thorough testing, as researchers in the field of TTP might happen to already have in hand the clinical marker they were looking for.

Figures

Figure 1. Relationships between residual ADAMTS13 activity and age of disease onset in congenital TTP. Increasing residual activity is associated with older age of onset (A). A few patients with high residual activity have early age of onset (B). None of the patients with low residual activity have late disease onset (C).

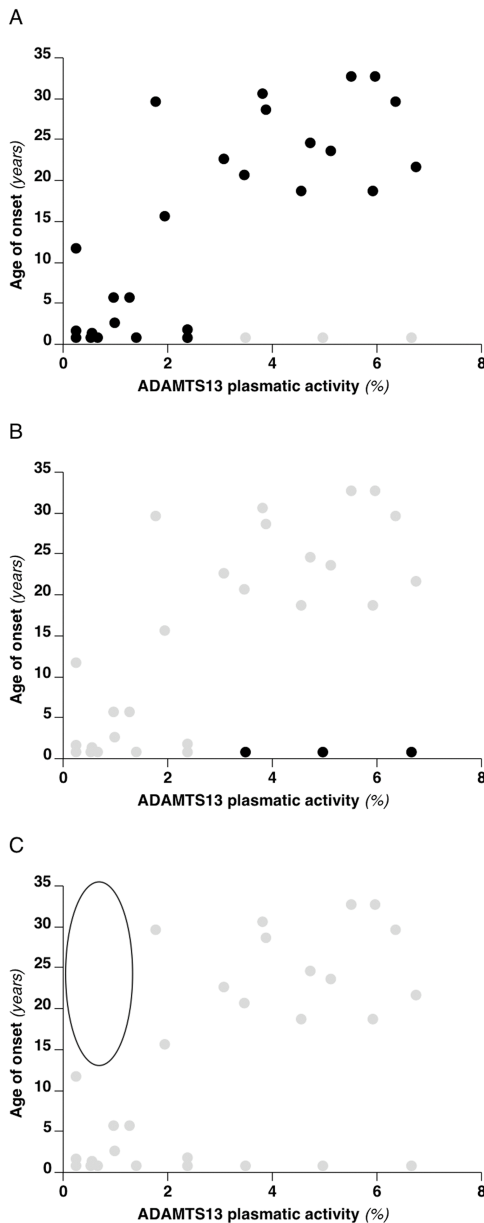
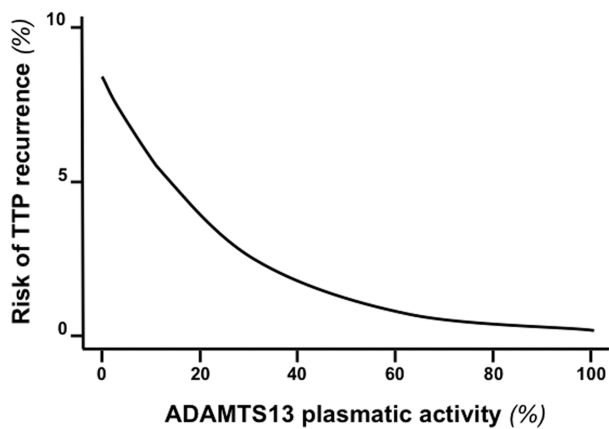


Figure 2. Exponential decay of the risk of thrombotic thrombocytopenic purpura recurrence for increasing levels of ADAMTS13 activity.



Tables

Table 1. Studies that investigated ADAMTS13-related measurements in thrombotic thrombocytopenic purpura.

Reference	Desing	Timing of measurement	Marker	Endpoint	Result
Ferrari et al. Blood 2007	Cohort	Acute TTP	Anti-ADAMTS13 inhibitor levels	Acute disease mortality	Inhibitor at presentation not associated with death
		Acute TTP	Anti-ADAMTS13 Ig class	Acute disease mortality	High-IgA titres associated with death
		Remission	ADAMTS13 activity	Recurrence	Severe ADAMTS13 deficiency increases risk
Peyvandi et al. Haematologica 2008	Case-control	Remission	ADAMTS13 activity	Recurrence	Severe ADAMTS13 deficiency increases risk
		Remission	ADAMTS13 antigen	Recurrence	Severe antigen deficiency not associated with increased risk
		Remission	Anti-ADAMTS13 autoantibodies	Recurrence	Presence of antibodies increases risk
Jin et al. BJH 2008	Cohort	Remission	ADAMTS13 activity	Recurrence	Decreasing ADAMTS13 levels associated with increased risk
Ferrari et al. JTH 2009	Case-control	Acute TTP	Anti-ADAMTS13 IgG subclasses	Recurrence	High IgG4 associated with recurrence
Kremer-Hovinga et al. 2010	Inception cohort	Acute TTP	ADAMTS13 activity	Acute disease mortality	Severely deficient patients had higher survival but no statistical significance
		Acute TTP	ADAMTS13 activity	Recurrence	Severe ADAMTS13 deficiency increases risk (RR=10)
		Acute TTP	Anti-ADAMTS13 inhibitor levels	Acute disease mortality	High inhibitor associated with increased mortality in patients with severe ADAMTS13 deficiency
		Acute TTP	Anti-ADAMTS13 Ig class	Acute disease mortality	High-IgA titres associated with death

TTP indicates thrombotic thrombocytopenic purpura; RR, relative risk.

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