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Acquired thrombotic thrombocytopenic purpura: ADAMTS13 activity, anti-ADAMTS13 autoantibodies and risk of recurrent disease

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Thrombotic thrombocytopenic purpura (TTP), first described by Moschcowitz in 1924,[1] has long been known as a generally fatal acute disease occurring in previously healthy subjects[2]. Despite its rarity, over the past eight decades it has attracted the interest of many researchers and clinicians whose many hypotheses as to its pathophysiological mechanisms have been widely discussed (for reviews see refs. #3-7). The outcome of affected patients has been dramatically improved by the largely empirical introduction of plasma therapy in the 1970s,[8] and a prospective study by the Canadian Apheresis Study Group demonstrated the superiority of plasma exchange with fresh frozen plasma (FFP) replacement over simple FFP infusion.[9] However, in spite of the use of plasma exchange and FFP replacement (and corticosteroids in many patients), the mortality from acute TTP is still high even today, with some 10-20% of patients dying from the acute disease episode.[5]

In 1982, Moake *et al.*[10] suggested that unusually large von Willebrand factor multimers (ULVWF), observed in plasma during the remission phase in 4 patients with a chronically relapsing form of TTP, were responsible for the recurring platelet clumping in the microvasculature of their patients. They hypothesized that a deficiency of a VWF depolymerase was causing the persistence in plasma of these highly adhesive ULVWF multimers.[10] In 1996, such a "depolymerase", initially named von Willebrand factor-cleaving protease, was partially purified from plasma[11,12] and later shown to be a member of the ADAMTS (A disintegrin and metalloprotease with thrombospondin type 1 repeats) family of proteases, denoted ADAMTS13.[13-16] In 1997, a complete deficiency of VWF-cleaving protease (ADAMTS13) activity was reported by Furlan *et al.*[17] in 4 patients, including 2 brothers, with chronic relapsing TTP, and in 1998, two independent retrospective studies showed that 20/24[18] and 37/37[19] patients with a clinical diagnosis of acute

sporadic TTP had a severe deficiency of VWF-cleaving protease activity, in most cases associated with a circulating IgG inhibitor against the VWF-cleaving protease.[18,19]

Astonishingly, 23 patients diagnosed with hemolytic uremic syndrome (HUS) had normal or subnormal VWF-cleaving protease activity.[18] This was rather unexpected because clinically it is often difficult or impossible to distinguish between these two thrombotic microangiopathies.[5] Interestingly, in the report of Tsai and Lian,[19] all 7 patients investigated in remission after the acute TTP episode showed normalized or near-normalized VWF-cleaving protease activity, and the IgG autoantibodies inhibiting the protease during the acute disease phase had disappeared in all investigated patients. In our report, however, only 5 out of 15 patients studied in remission from acute sporadic TTP had fully normalized VWF-cleaving protease activity, 5 remaining severely deficient and in 6 out of the 15 an inhibitor was still present during remission.[18]

Several critical reports in the early 2000s suggested that VWF-cleaving protease deficiency was not a specific finding for TTP, but occurred also in disseminated intravascular coagulation (DIC), thrombocytopenic disorders other than TTP, various inflammatory conditions, and even in healthy controls.[20,21] However, in these studies, VWF-cleaving protease activity was either not properly quantitated or was only moderately decreased, in clear contrast to the severely deficient activity found in many patients with acute TTP. In a study from our laboratory on 68 patients with thrombocytopenia due to various causes other than TTP or HUS, none had VWF-cleaving protease activity <10% of normal plasma even though some had values of <30%.[22] Recently, a study from Japan[23] found severely decreased ADAMTS13 activity (<5% of normal plasma) in 17 out of 109 patients with sepsis-induced DIC, whereas in our investigation of 40 patients with severe sepsis or septic shock, none had ADAMTS13 activity <24% as assessed by two different methods.[24] In line with our data is a study on 30 septic patients by Martin *et al.*[25]

Therefore, it seems that a severely deficient ADAMTS13 activity (lower than 5-10% of the activity in normal plasma) is a rather specific finding for a thrombotic microangiopathy, most often clinically diagnosed as TTP.[26,27]

Whereas patients with a sporadic TTP often have an acquired, autoantibody-mediated severe ADAMTS13 deficiency, many subjects have been identified in the past few years with a hereditary ADAMTS13 deficiency, and in almost all cases a double heterozygous or homozygous ADAMTS13 gene mutation has been identified.[28-32]

Over the last decade, several cohorts of patients with an acquired TTP have been investigated for ADAMTS13 related parameters, most often in the form of retrospective multicenter studies (Table 1). It becomes clear from these studies that only about 60% of patients diagnosed with acute idiopathic TTP show a severely deficient ADAMTS13 activity, as measured by various static activity assays.[18,19,33-39] It cannot be excluded, of course, that plasma therapy, given to some patients of these case series prior to the initial blood sampling for ADAMTS13 measurement, may have slightly reduced the proportion of patients with severely deficient ADAMTS13 activity. Still, the question is raised whether cases of TTP with versus TTP without autoantibody-mediated severe ADAMTS13 deficiency represent in fact different diseases, both in terms of pathophysiology and possibly also concerning treatment needs.

Table 1. Proportion of patients with severely deficient ADAMTS13 acctivity (defined as <5% of normal or <10% in ref. #39) in reported TTP case series (from ref. #27).

Authors, year[ref]	*Design of study*	*Severely deficient/ total (n/n)*	*Sensitivity (%)*
Furlan *et al.* 1998[18]	Retrospective, multicenter	26/30*	86
Tsai, Lian 1998[19]	Retrospective	37/37*	100
Veyradier *et al.* 2001[33]	Prospective multicenter	47/66*	71
Mori *et al.* 2002[34]	Retrospective	12/18*	66
Vesely *et al.* 2003[35]	Inception cohort single center	16/48**	33
Matsumoto *et al.* 2004[36]	Multicenter	56/108**	52
Kremer Hovinga *et al.* 2004[37]	Multicenter	56/93***	60
Zheng *et al.* 2004[38]	Single center prospective	16/20**	80
Peyvandi *et al.* 2004[39]	Multicenter	48/100*	48

**Patients classified as having acute TTP; **patients classified as having acute idiopathic TTP; ***patients with first attack or relapse of acute idiopathic TTP.*

Theoretically, this may well be the case because the clinical definition of TTP[2,5] (consumptive thrombocytopenia, microangiopathic hemolysis with schistocytes on the blood smear, elevated LDH, with or without organ ischemia causing, e.g. neurological dysfunction or renal impairment, and with or without fever) is not very specific and it is conceivable that various pathophysiological mechanisms may lead to these clinical signs and symptoms and laboratory features. Alternatively, the way of measuring ADAMTS13 activity under static conditions using plasma-derived or recombinant full length multimeric VWF or a recombinant 73 amino acid VWF peptide containing the physiological cleavage site[40] as substrates may not fully reflect the *in vivo* function of ADAMTS13. *In vivo,* ADAMTS13 probably cleaves newly synthesized ULVWF multimers on the endothelial cell surface of the microcirculation under high shear stress conditions.[41,42] Indeed, some preliminary data suggest that at least in

some TTP patients with autoantibodies to ADAMTS13 as measured by ELISA, ADAMTS13 function may be normal in a static assay but severely impaired in a flow based assay.[43]

Some of the studies listed in Table 1 report sufficient details on patients to allow a comparison of clinical and laboratory manifestations in those with versus those without severe acquired ADAMTS13 deficiency. Mori *et al.*[34] found that only 2 out of 12 patients with severe ADAMTS13 deficiency died, whereas 4 out of 6 without severely deficient protease died despite plasma exchange and corticosteroid treatment. On the other hand, data from the Oklahoma TTP-HUS registry[35] showed that among 48 patients with idiopathic TTP the response to plasma exchange and FFP replacement was similar in the 16 patients with a severe acquired ADAMTS13 deficiency and in the 32 without. Also, there was no difference in mortality from acute TTP between the two groups. Later relapses, however, were more frequently observed in those with autoantibody-mediated severe ADAMTS13 deficiency (6 out of 14 patients) compared with only 2 relapses in 25 patients without severe protease deficiency at the initial disease episode. Similarly, Zheng *et al.*[38] reported that among 20 patients with idiopathic TTP, 3 out of 16 with severe acquired ADAMTS13 deficiency died but none of the 4 without severely decreased ADAMTS13 activity. A much higher mortality (10/17 patients), however, was found in patients with secondary thrombotic microangiopathy, associated with hematopoietic stem cell transplantation, neoplasia or anticancer agents. Notably, none of these 17 patients had severe ADAMTS13 deficiency.[38]

It is evident, therefore, that plasma exchange with FFP replacement remains mandatory for all patients with a clinical diagnosis of idiopathic TTP. Furthermore, the absence of severe ADAMTS13 deficiency, as measured by current static assays, should not be used to argue against the use of this treatment which had been shown to be highly effective several years before the discovery of ADAMTS13.[9]

A large proportion of patients surviving an acquired TTP attack will suffer from one or more relapses over the following months or years.[35,44-46] The Oklahoma TTP-HUS registry, which includes and follows all consecutive patients from a defined geographical area with a clinical diagnosis of TTP-HUS (except pediatric diarrhea-positive typical HUS) and referred to the Oklahoma Blood Institute for plasma exchange treatment, shows that relapses occur mainly in patients with severe autoantibody-mediated ADAMTS13 deficiency at the initial disease episode,[35] and that relapses most often occur during the first year after the initial TTP attack.[46] Nevertheless, relapses may also occur many years after disease onset.[44]

Previously, splenectomy had been empirically found to be useful in patients with relapsing disease and a Dutch study convincingly demonstrated that the frequency of recurrences in 24 patients significantly dropped after splenectomy.[45] On the basis of present knowledge of the pathophysiology, it seems likely that splenectomy may eliminate anti-ADAMTS13 producing B-cell clones, as demonstrated in a few patients studied before and after splenectomy for relapsing or plasma-refractory acquired TTP.[47-49]

In contrast to the initial report by Tsai and Lia,[19] which showed the absence of inhibitory autoantibodies and normalized ADAMTS13 activity in all investigated patients achieving remission from acute acquired TTP, other authors observed that several patients remained severely deficient in ADAMTS13 activity and retained ADAMTS13 inhibitors in their plasma despite obvious full recovery from clinical and laboratory features of TTP.[18,37,38,50] This suggests that severe acquired

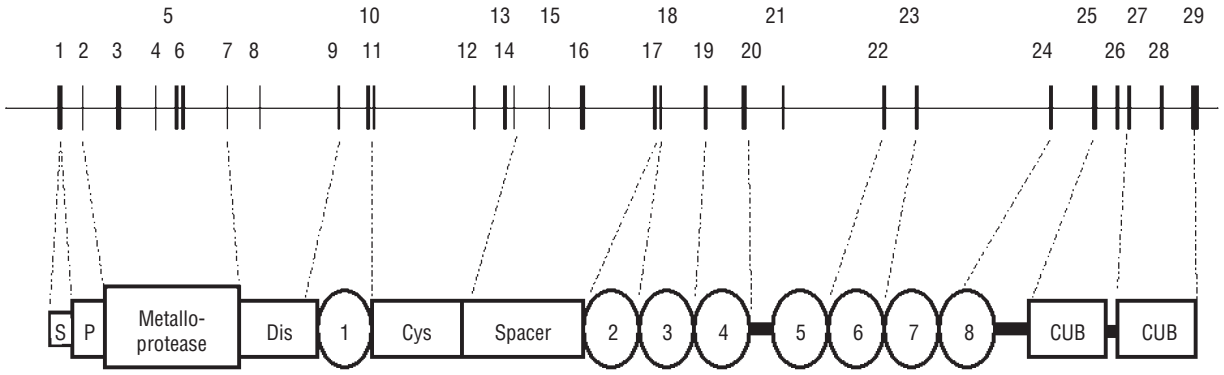

Figure 1. Gene structure and protein domains of ADAMTS13. The ADAMTS13 gene consists of 29 exons (upper panel), encoding the ADAMTS13 protein (lower panel), consisting of a signal peptide (S), a propeptide (P), a metalloprotease domain, a disintegrin domain (Dis), 8 thrombospondin type 1 domains (1-8), a cysteine-rich (Cys) and spacer domain, and two CUB domains. Adapted from Zheng *et al.*[16] Reactivity of autoantibodies towards different ADAMTS13 domains is described in the text.

ADAMTS13 deficiency per se may not be sufficient to cause TTP, but that some trigger, such as an inflammatory or infectious condition, may also be necessary to bring about acute disease manifestations.

A paper by Peyvandi and co-workers in this issue of the journal[51] investigates the most important clinical question as to whether persisting or recurring severe ADAMTS13 deficiency and/or autoantibodies towards ADAMTS13 during remission increase the risk of recurring disease for survivors of acute TTP. Based on their findings, they suggest that the persistence of severe ADAMTS13 functional deficiency and/or circulating anti-ADAMTS13 autoantibodies during clinical remission increase the risk of recurrence in the 109 patients studied over a period from 1994 to 2006 by approximately three-fold. This is very valuable information which suggests the important role of these autoantibodies and deficient ADAMTS13 activity for the pathogenesis of TTP. However, the study, as clearly stated by the authors, has limitations. Due to its retrospective nature, plasma samples during remission were available from only two thirds of the 178 patients originally included with acquired TTP. Furthermore, plasma was collected at variable time points after achieving remission (median 10 months; range 1-96 months) and the observation period for patients remaining free of relapse was only 22 months (median; range 13-134 months), whereas in cases of relapse, this occurred 21 months (median; range 2-144 months) after the initial disease episode. As the authors mention, only a prospective multicenter cohort study with repeated blood sampling at pre-defined time intervals will clearly demonstrate the predictive value of measuring ADAMTS13 activity, inhibitors, and autoantibodies by ELISA during follow-up of survivors. Interestingly, another recent study[50] on a cohort of 35 adult French patients undergoing a first episode of acute idiopathic TTP and having had severe ADAMTS13 deficiency (<5% of normal plasma) similarly addressed the prognostic significance of various ADAMTS13 related parameters measured at disease onset. The 3 patients who died from the initial TTP attack despite plasma exchange and corticosteroid therapy all showed several anti-ADAMTS13 Ig isotypes as measured by ELISA, including very high titers of IgA anti-ADAMTS13 autoantibodies, whereas the functional inhibitor titer was not predictive of mortality. During an 18-month-follow-up, 6 of the 32 surviving patients relapsed with acute TTP. High levels of inhibitory anti-ADAMTS13 IgG autoantibodies at initial presentation were found to be associated with persisting severe ADAMTS13 deficiency during remission and this, in turn, was predictive of relapse.

Therefore, a prospective multicenter study with blood sampling at pre-defined time intervals addressing the relapse rate and its predictability based on ADAMTS13 related parameters and potential triggering events is much needed for a disease with a recurrence rate of up to 50% and a fatality rate of up to 20%. If a high titer inhibitor, a combination of several Ig isotypes, and/or the persistence or reappearance of severe ADAMTS13 functional deficiency during remission do indeed sensitively predict disease relapse, interventional strategies, e.g. immunosuppression by corticosteroids, rituximab, or splenectomy should be evaluated for their prophylactic efficacy.

There remain many more questions to be addressed. Acquired TTP is an autoimmune disorder, at least in those patients with an autoantibody-mediated severe ADAMTS13 deficiency. Why do some seemingly healthy individuals generate an autoimmune response towards ADAMTS13 at all? We have observed identical twin sisters suffering from an acute autoantibody-mediated severe ADAMTS13 deficiency and clinical TTP one year apart.[52] This raises the possibility that genetic factors may predispose some subjects to this autoimmune disorder.

Epitope mapping studies revealed that IgG autoantibodies directed against the cysteine-rich/spacer domains (Figure 1) were present in all investigated patients with TTP and acquired severe ADAMTS13 deficiency,[53-55] and Luken *et al.*[55] localized an epitope on the 130 amino acids of the spacer domain recognized by all 6 investigated patients. However, Klaus *et al.*[54] also found antibodies towards the CUB domain, the metalloprotease/disintegrin/first thrombospondin type 1 repeat, the thrombospondin type 1/2-8 domain and even to the propeptide in variable proportions of the 25 investigated patients, and Luken *et al.*[55] demonstrated antibodies reacting with the thrombospondin type 1/2-8/domain in 1 out of 7 patients. Whereas antibodies directed to the spacer domain seem to inhibit ADAMTS13 functional activity in the classical static *in vitro* assays,[53-55] since the spacer domain is necessary for VWF cleavage by ADAMTS13,[53,56] the significance of autoantibodies towards other epitopes must still be further investigated. It is certainly possible that they may disturb the *in vivo* function of ADAMTS13 or accelerate the clearance of this vital protease without being inhibitory *in vitro*.[57] Also, as outlined above, the apparent damaging effects of high titer IgA autoantibodies to ADAMTS13[50] need to be confirmed and some pathophysiological explanation found.

The progress made in research on TTP over the past ten years has been amazing but, as in many other areas of biomedical research, the recent discoveries raise new questions which probably far outweigh the substantial knowledge gained.

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