

Cover Page



Universiteit Leiden



The handle <http://hdl.handle.net/1887/20119> holds various files of this Leiden University dissertation.

Author: Lotta, Luca Andrea

Title: Pathophysiology of thrombotic thrombocytopenic purpura : pathophysiology of thrombotic thrombocytopenic purpura : the "two-hit" paradigm

Date: 2012-11-13

SECTION II

CHAPTER 5

Pathogenesis and treatment of acquired idiopathic thrombotic thrombocytopenic purpura

Flora Peyvandi, Roberta Palla, **Luca A Lotta**.

Adapted from *Haematologica* 2010;95:1444-7.

Thrombotic thrombocytopenic purpura (TTP) is a rare thrombotic disease characterized by episodes of thrombocytopenia and microangiopathic haemolytic anemia due to disseminated microvascular thrombosis. TTP was first described in 1924 by Moschowitz as a disease presenting with a pentad of signs and symptoms (anaemia, thrombocytopenia, fever, hemiparesis and haematuria).¹ Post-mortem examination showed widespread thrombi, mainly composed of platelets, in the terminal circulation of several organs. The description of von Willebrand factor (VWF) multimers of unusually large size in the plasma of patients with TTP represented a turning point for the understanding of the disease pathophysiology.^{2, 3} The presence in plasma of the highly platelet-adhesive ultralarge multimers of VWF provided a plausible explanation for the platelet- and VWF-rich thrombi observed in the small vessels of TTP patients. Then, studies in the late 1990s independently demonstrated the severe deficiency of a specific VWF cleaving-protease in the plasma of patients with recurrent TTP.⁴ This protease was identified as the thirteenth member of the ADAMTS (a disintegrin and metalloprotease with thrombospondins 1 repeats) family of metalloproteases, ADAMTS13.⁵⁻⁷ Severe ADAMTS13 deficiency can be due to mutations in the ADAMTS13 gene (congenital TTP, see review⁸) or to anti-ADAMTS13 autoantibodies (autoimmune TTP).⁹⁻¹¹ The antibody-mediated severe deficiency of ADAMTS13 can be detected in most patients with idiopathic TTP (i.e. TTP occurring without associated clinical conditions/events), whereas its prevalence is much lower in the secondary forms of TTP (i.e. TTP associated with pregnancy, infections, autoimmune diseases and the use of drugs such as ticlopidine and clopidogrel).¹² It should also be mentioned that there are

idiopathic TTP cases with only slightly deficient or even normal ADAMTS13 levels at presentation, but these cases are not object of the present article and we will use idiopathic and autoantibody-mediated TTP as synonyms.

Epidemiology and clinical course of idiopathic TTP

The incidence of idiopathic TTP is estimated to be 4.5/1 million person/years, higher in blacks than in whites and Asians and with a male to female ratio of 1:2, similarly to other autoimmune diseases.¹³ Idiopathic TTP tends to have a less severe acute-disease prognosis, but much higher risk of recurrent disease in comparison to secondary forms.^{14, 15} The overall mortality of TTP was higher than 90%, but has decreased to 8-30% after the introduction of plasma exchange (PEX), which is the treatment of choice of acute TTP episodes.^{16, 17}

The lower mortality of idiopathic TTP (21% vs 39% in the frame of the Oklahoma TTP registry)¹⁵ is probably due to the higher response to PEX of patients with auto-antibodies and to the mortality related to associated conditions in secondary cases.¹⁸ Up to 40% of patients with TTP develop recurrent episodes of the disease, with higher risk for recurrences in patients with severe ADAMTS13 deficiency and anti-ADAMTS13 autoantibodies during acute episodes. The cumulative risk for recurrence at 7.5 years from the first episode in patients with ADAMTS13 activity below 10% at presentation was estimated to be 41%, 10 times that of patients with activity above 10% (4% risk at 7.5 years).¹⁶ Persistence of ADAMTS13 deficiency and autoantibodies during disease remission is also associated with increased risk for recurrence.^{19, 20}

Anti-ADAMTS13 antibody characterization

Anti-ADAMTS13 autoantibodies have been the focus of several research efforts trying to characterize their immunoglobulin (Ig) subclass, specificity and mechanisms of action. Early studies distinguished two classes of anti-ADAMTS13 autoantibodies: inhibitory and non-inhibitory antibodies. Inhibitory antibodies are present in 50-90% and their mechanism of action is the inhibition of ADAMTS13-mediated proteolysis of VWF.^{21, 22} When non-inhibitory antibodies are also measured, the anti-ADAMTS13 autoantibodies are detectable in the majority of patients.¹⁰ The core binding site for VWF, located in the spacer domain of ADAMTS13 and consisting of aminoacid residues Tyr572-Asn579 and Arg657-Gly666, represents the target site of the autoantibodies found in the plasma of several TTP patients.^{23, 24} The mechanism of action of non-inhibitory antibodies has been proposed to be the opsonisation and enhanced clearance of ADAMTS13, but this mechanism has never been proven.²¹ Studies on the class of anti-ADAMTS13 autoantibodies showed they are usually of IgG type, particularly IgG1 and IgG4 subtypes,^{10, 25} but in a few cases autoantibodies of IgA and/or IgM isotype were also found.¹⁹ Most anti-ADAMTS13 IgG found in TTP patients were demonstrated to be directed against the spacer domain, but additional antibodies against other ADAMTS13 domains were also detected.^{26, 27} However, these studies were conducted in small patient cohorts. Zheng et al. in this issue of *Haematologica* report the first study of antibody specificity on a relatively large group of patients with TTP.²⁸ They found that, although almost all patients with IgG had antibodies directed against the N-terminal ADAMTS13 domains (Cys-rich through spacer), up to 46% of TTP patients also had

antibodies towards the C-terminal ADAMTS13 domains (TSP1-5 through CUB). Moreover, two patients had antibodies against the C-terminal domains of ADAMTS13, but not against the N-terminal domains. These findings suggest a functional role of C-terminal domains of ADAMTS13 *in vivo*, also in light of the importance of these domains in the VWF-ADAMTS13 interaction under fluid shear stress. Importantly, Zheng et al.²⁹ combined antibody specificity with clinical data, showing that patients with antibodies against ADAMTS13 C-terminal domains had lower platelet counts at presentation. This is not the first time anti-ADAMTS13 antibody features are found to correlate with clinical outcomes in TTP. Patients with IgG subclasses 4 were found to be more likely to have disease recurrence than patients with IgG subclass 1.²⁸ Patients with high inhibitor titers were found to have worse acute-disease prognosis.²⁹ Consistently, patients with high levels of IgG (both inhibitory and non-inhibitory) were found to have higher likelihood of developing cardiac involvement and, hence, poorer prognosis in comparison to patients with low IgG levels.³⁰ All these findings indicate that different antibody features might be associated with clinical outcomes in TTP, but more comprehensive studies should be carried out before antibody characterization can be introduced in routine clinical practice of TTP. Moreover there remain other questions to be addressed. Acquired TTP is an autoimmune disorder, at least in those patients with an autoantibody-mediated severe ADAMTS13 deficiency, but the mechanisms involved in the loss of tolerance of the immune system against ADAMTS13 remain unknown. The higher incidence of autoimmune idiopathic TTP in specific ethnical groups such as Afro-Caribbeans, as well as the report of idiopathic TTP in two monozygotic

twins both developing anti-ADAMTS13 antibodies,³¹ strongly argues in favor of a genetic predisposition even in the acquired form of the disease. In the last year, two groups independently demonstrated an association between human leukocyte antigen (HLA) alleles and idiopathic TTP: HLA-DR and HLA-DQ typing suggests an underlying genetic risk for the development of TTP in Europeans.^{32, 33} As for the antibody characterization, confirmation of these results in larger groups of patients and in other ethnic groups are required prior of the introduction of HLA typing in the control of the disease.

Treatment and clinical trials in idiopathic TTP

PEX remains the treatment of choice of acute episodes of TTP.³⁴ As mentioned, its introduction greatly reduced the disease mortality and it has been proven superior to plasma infusion.¹⁷ Several different immunosuppressive drugs (corticosteroids, cyclosporine, azathioprine and, recently, rituximab) are added by many centers to PEX, with the rationale that they help stopping antibody production in autoimmune cases, but their efficacy has never been confirmed by large clinical trials. In addition to these treatments, novel drugs have been developed or are undergoing pre-clinical development that could potentially be used in idiopathic TTP along with PEX. These could tackle different aspects of TTP pathophysiology (Table 1). First, it is possible to reduce or abolish the production of anti-ADAMTS13 autoantibodies with anti-CD20 monoclonal antibodies that target B-lymphocytes (e.g. rituximab, but other more potent compound are being developed).³⁵ Second, it could be in principle possible to restore VWF cleavage in patients with severe ADAMTS13 deficiency with the

use of recombinant ADAMTS13. Third, novel compounds that inhibit VWF binding to platelet glycoprotein Ib-alpha have been developed that could block VWF-mediated platelet activation. There is hope in the TTP community that these novel therapeutic strategies be able to reduce the persistently high disease mortality. However, the availability of these new options generates new challenges for clinicians who have to deal with TTP patients. These include uncertainties on the safety of the drugs in this delicate clinical setting and on the subgroup(s) of patients (idiopathic, secondary, TTP with prominent renal impairment, etc.) that could benefit from the treatment. The efficacy and safety of these novel therapeutic strategies will need to be assessed in the frame of large clinical trials, a challenge for clinical scientists who work on this rare disease. Idiopathic TTP incidence is such that anyone willing to carry out a 3-year clinical trial involving roughly 100 patients would need to be able to cover a population of approximately 7 million people (assuming an incidence of 4.5/1 million person/years). The choice of the clinical end-point will similarly be a challenge: mortality is ~10-20% which makes it a hardly targetable end-point with small sample sizes. Such surrogate endpoints as the incidence of stroke, renal failure, myocardial infarction, time to platelet recovery or clinical remission may be adequate for the definition of therapeutic efficacy but there are few data available from cohort studies that could inform the design of clinical trials employing these endpoints. The picture is made even more complicated by the heterogeneity in the pathophysiologic background of TTP. The inclusion in a TTP trial of secondary cases, patients with atypical hemolytic uremic syndrome, patients at first TTP episodes or recurrence may in principle conceal the effect of a treatment which is

highly effective in a subgroup of TTP patients (e.g. only those with anti-ADAMTS13 autoantibodies). Recently, a phase 2 double-blind, placebo-controlled, clinical trial of intravenous ARC1779, an inhibitor of VWF binding to platelet glycoprotein Ib-alpha, was stopped due to slow recruitment (clinicaltrials.gov identification number NCT00726544). New trials are nonetheless being designed and carried out. These will be critical to the efforts of translating preclinical achievements in improvements in the care of this rare, but still life-threatening thrombotic disease.

Table. Novel drugs developed or undergoing pre-clinical development that could potentially be used in idiopathic TTP along with plasma exchange.

Pathogenic Mechanism	Drug class	Drug(s)	Mechanism of action	Stage of development
Production of anti-ADAMTS13 auto antibodies	Anti-CD20 antibodies	Rituximab. Other drugs under development	B-lymphocyte depletion	Available
Impaired VWF cleavage	Recombinant replacement products	recombinant ADAMTS13	Replacement of deficient ADAMTS13	Pre-clinical development
VWF binding to platelets and VWF-mediated thrombosis	VWF-platelet interaction inhibitors	ARC1770 and ALX-0681	Blockage of VWF-mediated platelet aggregation	Phase 2 of clinical development

TTP, thrombotic thrombocytopenic purpura; VWF, von Willebrand factor.

References

1. Moschcowitz E. An acute febrile pleiochromic anemia with hyaline thrombosis of the terminal arterioles and capillaries: an undescribed disease. 1925. *Mt Sinai J Med.* 2003; **70**(5): 352-5.
2. Moake JL, Rudy CK, Troll JH, Weinstein MJ, Colannino NM, Azocar J, et al. Unusually large plasma factor VIII: von Willebrand factor multimers in chronic relapsing thrombotic thrombocytopenic purpura. *The New England journal of medicine.* 1982; **307**(23): 1432-5.
3. Moake JL. Thrombotic microangiopathies. *The New England journal of medicine.* 2002; **347**(8): 589-600.
4. Furlan M, Robles R, Solenthaler M, Wassmer M, Sandoz P, Lammle B. Deficient activity of von Willebrand factor-cleaving protease in chronic relapsing thrombotic thrombocytopenic purpura. *Blood.* 1997; **89**(9): 3097-103.
5. Gerritsen HE, Robles R, Lammle B, Furlan M. Partial amino acid sequence of purified von Willebrand factor-cleaving protease. *Blood.* 2001; **98**(6): 1654-61.
6. Fujikawa K, Suzuki H, McMullen B, Chung D. Purification of human von Willebrand factor-cleaving protease and its identification as a new member of the metalloproteinase family. *Blood.* 2001; **98**(6): 1662-6.
7. Zheng X, Chung D, Takayama TK, Majerus EM, Sadler JE, Fujikawa K. Structure of von Willebrand factor-cleaving protease (ADAMTS13), a metalloprotease involved in thrombotic thrombocytopenic purpura. *The Journal of biological chemistry.* 2001; **276**(44): 41059-63.
8. Lotta LA, Garagiola I, Palla R, Cairo A, Peyvandi F. ADAMTS13 mutations and polymorphisms in congenital thrombotic thrombocytopenic purpura. *Human mutation.* 2010; **31**(1): 11-9.
9. Furlan M, Robles R, Galbusera M, Remuzzi G, Kyrle PA, Brenner B, et al. von Willebrand factor-cleaving protease in thrombotic thrombocytopenic purpura and the hemolytic-uremic syndrome. *The New England journal of medicine.* 1998; **339**(22): 1578-84.
10. Tsai HM, Lian EC. Antibodies to von Willebrand factor-cleaving protease in acute thrombotic thrombocytopenic purpura. *The New England journal of medicine.* 1998; **339**(22): 1585-94.
11. Tsai HM, Li A, Rock G. Inhibitors of von Willebrand factor-cleaving protease in thrombotic thrombocytopenic purpura. *Clinical laboratory.* 2001; **47**(7-8): 387-92.

12. Veyradier A, Obert B, Houllier A, Meyer D, Girma JP. Specific von Willebrand factor-cleaving protease in thrombotic microangiopathies: a study of 111 cases. *Blood*. 2001; **98**(6): 1765-72.
13. Terrell DR, Williams LA, Vesely SK, Lammle B, Hovinga JA, George JN. The incidence of thrombotic thrombocytopenic purpura-hemolytic uremic syndrome: all patients, idiopathic patients, and patients with severe ADAMTS-13 deficiency. *Journal of thrombosis and haemostasis : JTH*. 2005; **3**(7): 1432-6.
14. Zheng XL, Kaufman RM, Goodnough LT, Sadler JE. Effect of plasma exchange on plasma ADAMTS13 metalloprotease activity, inhibitor level, and clinical outcome in patients with idiopathic and nonidiopathic thrombotic thrombocytopenic purpura. *Blood*. 2004; **103**(11): 4043-9.
15. Hovinga JA, Vesely SK, Terrell DR, Lammle B, George JN. Survival and relapse in patients with thrombotic thrombocytopenic purpura. *Blood*. 2010; **115**(8): 1500-11; quiz 662.
16. Shepard KV, Bukowski RM. The treatment of thrombotic thrombocytopenic purpura with exchange transfusions, plasma infusions, and plasma exchange. *Seminars in hematology*. 1987; **24**(3): 178-93.
17. Rock GA, Shumak KH, Buskard NA, Blanchette VS, Kelton JG, Nair RC, et al. Comparison of plasma exchange with plasma infusion in the treatment of thrombotic thrombocytopenic purpura. Canadian Apheresis Study Group. *The New England journal of medicine*. 1991; **325**(6): 393-7.
18. George JN. Clinical practice. Thrombotic thrombocytopenic purpura. *The New England journal of medicine*. 2006; **354**(18): 1927-35.
19. Ferrari S, Scheiflinger F, Rieger M, Mudde G, Wolf M, Coppo P, et al. Prognostic value of anti-ADAMTS 13 antibody features (Ig isotype, titer, and inhibitory effect) in a cohort of 35 adult French patients undergoing a first episode of thrombotic microangiopathy with undetectable ADAMTS 13 activity. *Blood*. 2007; **109**(7): 2815-22.
20. Peyvandi F, Lavoretano S, Palla R, Feys HB, Vanhoorelbeke K, Battaglioli T, et al. ADAMTS13 and anti-ADAMTS13 antibodies as markers for recurrence of acquired thrombotic thrombocytopenic purpura during remission. *Haematologica*. 2008; **93**(2): 232-9.
21. Tsai HM, Raoufi M, Zhou W, Guinto E, Grafos N, Ranzurmal S, et al. ADAMTS13-binding IgG are present in patients with thrombotic thrombocytopenic purpura. *Thrombosis and haemostasis*. 2006; **95**(5): 886-92.
22. Rieger M, Mannucci PM, Kremer Hovinga JA, Herzog A, Gerstenbauer G, Konetschny C, et al. ADAMTS13 autoantibodies in patients with thrombotic

microangiopathies and other immunomediated diseases. *Blood*. 2005; **106**(4): 1262-7.

23. Luken BM, Turenhout EA, Kaijen PH, Greuter MJ, Pos W, van Mourik JA, et al. Amino acid regions 572-579 and 657-666 of the spacer domain of ADAMTS13 provide a common antigenic core required for binding of antibodies in patients with acquired TTP. *Thrombosis and haemostasis*. 2006; **96**(3): 295-301.

24. Jin SY, Skipwith CG, Zheng XL. Amino acid residues Arg(659), Arg(660), and Tyr(661) in the spacer domain of ADAMTS13 are critical for cleavage of von Willebrand factor. *Blood*. 2010; **115**(11): 2300-10.

25. Scheiflinger F, Knobl P, Trattner B, Plaimauer B, Mohr G, Dockal M, et al. Nonneutralizing IgM and IgG antibodies to von Willebrand factor-cleaving protease (ADAMTS-13) in a patient with thrombotic thrombocytopenic purpura. *Blood*. 2003; **102**(9): 3241-3.

26. Klaus C, Plaimauer B, Studt JD, Dorner F, Lammler B, Mannucci PM, et al. Epitope mapping of ADAMTS13 autoantibodies in acquired thrombotic thrombocytopenic purpura. *Blood*. 2004; **103**(12): 4514-9.

27. Luken BM, Turenhout EA, Hulstein JJ, Van Mourik JA, Fijnheer R, Voorberg J. The spacer domain of ADAMTS13 contains a major binding site for antibodies in patients with thrombotic thrombocytopenic purpura. *Thrombosis and haemostasis*. 2005; **93**(2): 267-74.

28. Zheng XL, Wu HM, Shang D, Falls E, Skipwith CG, Cataland SR, et al. Multiple domains of ADAMTS13 are targeted by autoantibodies against ADAMTS13 in patients with acquired idiopathic thrombotic thrombocytopenic purpura. *Haematologica*. 2010; **95**(9): 1555-62.

29. Ferrari S, Mudde GC, Rieger M, Veyradier A, Kremer Hovinga JA, Scheiflinger F. IgG subclass distribution of anti-ADAMTS13 antibodies in patients with acquired thrombotic thrombocytopenic purpura. *Journal of thrombosis and haemostasis : JTH*. 2009; **7**(10): 1703-10.

30. Hughes C, McEwan JR, Longair I, Hughes S, Cohen H, Machin S, et al. Cardiac involvement in acute thrombotic thrombocytopenic purpura: association with troponin T and IgG antibodies to ADAMTS 13. *Journal of thrombosis and haemostasis : JTH*. 2009; **7**(4): 529-36.

31. Studt JD, Kremer Hovinga JA, Radonic R, Gasparovic V, Ivanovic D, Merkler M, et al. Familial acquired thrombotic thrombocytopenic purpura: ADAMTS13 inhibitory autoantibodies in identical twins. *Blood*. 2004; **103**(11): 4195-7.

32. Coppo P, Busson M, Veyradier A, Wynckel A, Poullin P, Azoulay E, et al. HLA-DRB1*11: a strong risk factor for acquired severe ADAMTS13 deficiency-related idiopathic thrombotic thrombocytopenic purpura in Caucasians. *Journal of thrombosis and haemostasis : JTH.* 2010; **8**(4): 856-9.
33. Scully M, Brown J, Patel R, McDonald V, Brown CJ, Machin S. Human leukocyte antigen association in idiopathic thrombotic thrombocytopenic purpura: evidence for an immunogenetic link. *Journal of thrombosis and haemostasis : JTH.* 2010; **8**(2): 257-62.
34. George JN. How I treat patients with thrombotic thrombocytopenic purpura: 2010. *Blood.* 2010; **116**(20): 4060-9.
35. Scully M, Cohen H, Cavenagh J, Benjamin S, Starke R, Killick S, et al. Remission in acute refractory and relapsing thrombotic thrombocytopenic purpura following rituximab is associated with a reduction in IgG antibodies to ADAMTS-13. *British journal of haematology.* 2007; **136**(3): 451-61.