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

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CHAPTER 3

Clinical case: drop of residual plasmatic activity of ADAMTS13 to undetectable levels during acute disease in a patient with adult-onset congenital thrombotic thrombocytopenic purpura

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Congenital thrombotic thrombocytopenic purpura (TTP) (also known as Upshaw-Schulman syndrome, OMIM #274150) is a rare, recessively inherited thrombotic microangiopathy. The disease is characterized by a congenital severe deficiency of ADAMTS13 plasmatic activity caused by mutations in the *ADAMTS13* gene.[1-4] Congenital TTP displays a heterogeneous clinical severity with respect to age of disease onset, frequency of acute disease episodes and need for fresh frozen plasma (FFP) prophylaxis.[1-4] We recently found that residual ADAMTS13 activity, measured by highly-sensitive surface-enhanced laser desorption/ionization time-of-flight (SELDI-TOF) mass spectrometry,[5] is inversely associated with the severity of the disease. Low residual activity measured during disease remission was associated with a history of early disease onset, a high annual rate of TTP episodes and prescription of FFP prophylaxis.[5] These results suggest that congenital TTP patients who have some residual plasmatic activity of ADAMTS13 (we found 2-3% to be the critical level) are protected from early disease and frequent recurrences, developing the disease only when the fragile balance between residual ADAMTS13 and its cleavage substrate, the pro-thrombotic ultralarge forms of von Willebrand factor (VWF), is shifted towards ultralarge VWF release by environmental perturbations (e.g. infections, traumas, pregnancy, etc.). According to this "two-hit" model, one would expect to see ADAMTS13 activity drop during acute disease even in patients who during remission have some degree of residual plasmatic activity. However, in the aforementioned study residual ADAMTS13 activity was measured only during disease remission.[5] In this paper, we report the case of an Italian patient with congenital TTP who developed one single acute episode of

the disease in her adult life. The patient had been included in the study of residual ADAMTS13 during remission, but the clinical history of the patient is hereby presented in full and we also report the results of SELDI-TOF-based ADAMTS13 activity measurement during and after the acute disease episode. The patient provided informed consent and the study was approved by the institutional review board of the Fondazione IRCCS Ca' Granda – Ospedale Maggiore Policlinico.

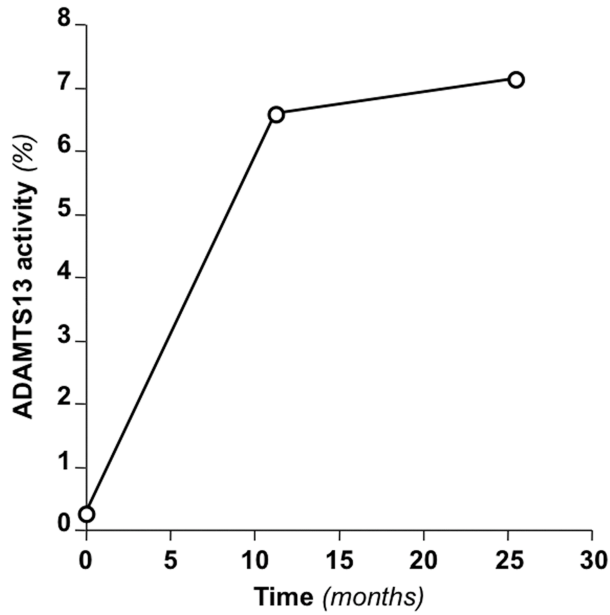
At the age of 32, during the 19th week of her first pregnancy, the patient was admitted to the gynecology department of her local hospital with epigastric pain, fatigue and headache. Complete blood count showed anemia (hemoglobin: 8.7 g/L) and thrombocytopenia (platelet count: 42 x 10^9/L) with increased reticulocyte count and presence of schistocytes at the blood smear. Lactate dehidrogenase and indirect bilirubin levels were also above the upper normal value, direct Coombs tests was negative. There was no sign of renal involvement (creatinine: 79 μmol/L). Both alanine and aspartate transaminases were elevated. While elevation of liver enzymes suggested a diagnosis of HELLP, the presence of schistocytes supported a diagnosis of TTP. Plasma exchange (PEX) was implemented, with rapid increase of platelet counts. A total of 5 daily PEX procedures were performed. After the 5th procedure the patient developed an allergic reaction with bronchospasm, which led to the administration of corticosteroids and to the temporary suspension of PEX. At this time, fetal distress developed and the patient agreed to the therapeutic termination of pregnancy. Platelet counts dropped to 59 x 10^9/L and PEX was resumed. A total of 7 procedures were carried out, with resolution of the disease symptoms and

correction of laboratory abnormalities. After remission was achieved, the patient was referred to the outpatient clinic for thrombotic microangiopathies of the Angelo Bianchi Bonomi Hemophilia and Thrombosis Center. ADAMTS13 assays hereby mentioned have been described elsewhere.[6, 7] Measurement of ADAMTS13 plasmatic activity by collagen binding assay on plasma samples collected during the first and second visits to the clinic, as well as on a sample that had been collected at the time of acute disease showed severely deficient plasmatic activity of ADAMTS13 (i.e. activity below 10% of normal). ADAMTS13 antigen levels were severely reduced. No anti-ADAMTS13 autoantibody was detectable by western blotting or ELISA in any of the samples, indicating congenital ADAMTS13 deficiency. *ADAMTS13* gene analysis by PCR and Sanger sequencing identified two novel missense mutations, not present in the dbSNP database. The two mutations were a c.578G>A substitution in exon 6 of *ADAMTS13* leading to a p.R193Q protein change in the metalloprotease domain and a c.3283C>T substitution in exon 25 leading to a p.R1095W protein change in the eighth thrombospondin-1-like domain of ADAMTS13. Both changes were predicted to be potentially damaging for protein function by Polyphen 2 (URL: http://genetics.bwh.harvard.edu/pph2/) and SIFT (http://sift.jcvi.org/) software and were not present in 198 alleles of thrombosis free individuals sequenced in the frame of the DVT-Milan study. A diagnosis of congenital TTP was made. The patient remained asymptomatic during follow-up, with no need for FFP prophylactic infusion. In order to assess whether or not the patient had measurable ADAMTS13 activity during acute disease and remission, the residual plasmatic activity of ADAMTS13 was quantified by SELDI-TOF

mass spectrometry.[5] A detectable ADAMTS13 cleavage activity was found in the two samples collected during disease remission months after the last PEX procedure, whereas it was undetectable during acute disease before the implementation of PEX (Figure). Herein, we have reported the measurement of residual plasmatic activity of ADAMTS13 by SELDI-TOF mass spectrometry in a patient with congenital TTP during both remission and acute disease. During remission, ADAMTS13 activity was detectable, with values above 5%. Consistent with the patient's clinical history, similar residual activity levels were shown to be associated with adult-onset disease and low-tendency towards recurrence. By contrast, activity during acute disease was completely abolished, consistent with the hypothesis that onset of acute TTP is associated with a drop in ADAMTS13 levels. Pregnancy, a recognized TTP-triggering condition that is physiologically associated with reduction in ADAMTS13 and raise in VWF plasmatic levels, is likely to have precipitated the onset of acute disease in the patient. In conclusion, this case report of a patient with congenital TTP highlighted the reduction of ADAMTS13 activity to undetectable levels in a patient with otherwise detectable residual ADAMTS13. This case is consistent with the paradigm of a 'two-hit' model in TTP, whereby a triggering agent can cause the onset of acute disease by abrogating residual activity in patients with already severely-reduced plasmatic ADAMTS13.

Figures

Figure 1. Residual ADAMTS13 activity measured by SELDI-TOF mass spectrometry at different moments of the clinical course.



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