The third form of thrombotic thrombocytopenic purpura shows up again: what is it, does it even exist, how to find out? Comment on "Acquired thrombotic thrombocytopenic purpura without detectable anti-ADAMTS13 antibodies: a possible underlying autoimmune mechanism" and on "The different faces of thrombotic thrombocytopenic purpura"

In this issue of *Haematologica* a Letter by Delphine Simon *et al*., on behalf of the French Centre National de Référence des Microangiopathies Thrombotiques (CNR-MAT), again brings up the existence and characteristics of a third form of thrombotic thrombocytopenic purpura (TTP), labeled as TTP of unidentified pathophysiology (uTTP).[1] This reiterated important insight is accompanied by a comment by a TTP specialist, Paul Knöbl.[2] Many questions concerning this potentially important but so far incompletely understood variant of TTP remain and I will try to put this uTTP variant into perspective and propose further steps to confirm its existence and unravel its secrets.

TTP was first described in 1924 by Moschcowitz[3] and the history of extensive research, elucidation of the pathophysiology, and the enormous therapeutic advances for this rare - and during half a century universally fatal - disease has been reviewed by many groups.[4-12] In all these reviews two separate forms of TTP are generally distinguished: (i) a rare congenital or hereditary form (cTTP or hTTP) caused by biallelic variants of the *ADAMTS13* gene giving rise to a severe constitutional deficiency of ADAMTS13 (A Disintegrin And Metalloprotease with ThromboSpondin type 1 motifs, number 13), the specific von Willebrand factor (VWF)-cleaving metalloprotease and (ii) a still rare but more common acquired form of TTP caused by autoantibodies inhibiting and/or clearing ADAMTS13 from plasma, generally termed autoimmune TTP (iTTP). In both forms of TTP, cTTP and iTTP, the defective proteolytic processing of VWF multimers results in accumulation of highly adhesive, unusually large VWF multimers leading to microvascular clumping of platelets and ischemia of multiple organs.[4,5] Treatment of acute cTTP episodes consists of plasma infusions and many patients need regular plasma prophylaxis to avoid recurring acute bouts.[13,14] More recently, recombinant human ADAMTS13 has become available for improved and facilitated treatment and prophylaxis of cTTP.[15] Acute iTTP episodes are managed by daily plasma exchanges with plasma replacement, immunosuppression with corticosteroids and rituximab, and the anti-VWF nanobody caplacizumab.[6-9] The International Working Group on TTP has proposed consensus definitions of the disease TTP, outlined the differential diagnosis between TTP and other thrombotic microangiopathies (TMA), and defined response to treatment and outcomes.[16,17] Moreover, under the auspices of the International Society on Thrombosis and Haemostasis, recommendations and guidelines for the diagnosis and treatment of TTP have been established.[18,19] In sum, TTP, both cTTP and iTTP, have been transformed from a generally deadly condition without any effective therapy until the 1960s to 1970s to two pathophysiologically well understood separate diseases, which can be effectively managed provided they are recognized promptly and diagnosed in a timely manner.[12]

Is there a third form of thrombotic thrombocytopenic purpura?

In 2016, Mariotte and colleagues published a large cross-sectional study on behalf of the French CNR-MAT on more than 900 adult patients hospitalized in the years 1999 to 2013 for a first bout of acute TMA associated with severely decreased functional activity of ADAMTS13.[20] From 772 patients clinical and laboratory data at admission, before starting plasma therapy, were available: 378 patients had idiopathic TTP without an accompanying clinical condition and 394 had non-idiopathic TTP, i.e. TTP with a concomitant disorder, such as an infection, autoimmune disease, pregnancy, cancer, organ transplantation, and certain drugs. Pathophysiologically, 585/772 (75%) were diagnosed as iTTP, 21/772 (3%) had cTTP, and 166/772 (22%) received a diagnosis of acquired TTP of unknown cause (uTTP). The pathophysiological diagnosis of iTTP in 75% of the patients was based on the detection of free anti-ADAMTS13 IgG antibodies in the plasma, cTTP in 3% was established based on lacking anti-ADAMTS13 antibodies and biallelic *ADAMTS13* genetic variants, whereas uTTP in 22% of the cohort was diagnosed in those with acquired severe ADAMTS13 deficiency without detectable anti-ADAMTS13 antibodies. Of note, most cases with an idiopathic TTP presentation had iTTP (345/378, 91%) whereas among the cases of non-idiopathic acquired TTP only 240/373 (64%) had iTTP and 133/373 had uTTP. Thus,

the diagnosis of uTTP was made predominantly in cases of non-idiopathic TTP and was associated with sepsis, cancer, infection with human immunodeficiency virus, transplantation and other conditions.[20]

In 2023, Joly *et al*. presented a new cohort of 125 adult patients with a first episode of acute non-idiopathic TTP included in the French TMA registry from 2012 to 2016.[21] Forty-nine of these 125 patients had circulating anti-ADAMTS13 IgG antibodies and were classified as having iTTP, whereas 76/125 lacked such antibodies and were diagnosed with uTTP. The conformation of ADAMTS13 in plasma was studied and compared in the uTTP and iTTP patients of this cohort. The ADAMTS13 conformation is determined with the so-called 1C4 enzyme-linked immunosorbent assay (ELISA) using immobilized monoclonal antibody 1C4 directed to a spacer epitope that is hidden in closed ADAMTS13 but binds the protease in its open conformation. An ADAMTS13 antigen concentration of ≥0.03 μg/mL (about 3% of normal) is needed for assaying its conformation.[22,23] Because of the generally low ADAMTS13 concentration in acute TTP, the ADAMTS13 conformation index could be established in only 59/76 uTTP and 30/49 iTTP cases. An open ADAMTS13 conformation (conformation index >0.5) is a hallmark of iTTP[22] and was found in 23/30 (76%) of the iTTP cases but in only 8/59 (14%) of the uTTP cases.[21]

Now, Simon *et al*. report yet another cohort of uTTP patients from the French registry, retrospectively collected over the past two decades.[1] Two-hundred and seventy-three of 1,325 (21%) acute TTP patients met the criteria for acquired uTTP, having an acute TMA, severely deficient ADAMTS13 activity, and undetectable anti-ADAMTS13 antibodies (<25 U/mL with the Technozym® ADAMTS13-INH ELISA kit, Technoclone, Vienna, Austria). Again, about 70% of these 273 uTTP patients had non-idiopathic TTP with associated infection, often with septic shock, cancer, organ or hematopoietic stem cell transplantation, systemic autoimmune disease or other conditions. The mortality was exceptionally high (82/273, 30%). During follow-up of the 191 survivors of the 273 patients of the initial uTTP cohort, 40 developed anti-ADAMTS13 antibodies during follow-up, either in remission or at acute relapse, and they were recategorized as having iTTP, leaving 151 survivors with a diagnosis of uTTP. Comparison of the 151 surviving uTTP and 40 surviving iTTP patients showed that the former were older and more often had non-idiopathic TTP with associated infection, cancer and other conditions; they also had worse renal function, and less acute recurrent TTP bouts during follow-up as compared with the iTTP group (see Table 1 in Simon *et al*.[1]).

Taken together, these three reports from the French National Reference Center for TMA suggest that a sizable proportion of some 20-25% of patients with acute acquired, severely ADAMTS13-deficient TTP may not fit the diagnosis of classical autoimmune TTP, commonly labeled as iTTP.

Questions raised concerning thrombotic thrombocytopenic purpura of unidentified pathophysiology

Most published series of TTP patients distinguish two forms of TTP, cTTP and the more common iTTP.[13,14,24-30] Similarly, reviews[5,6,10-12] and guidelines on diagnosis and management[18,19,31-33] mention these two disease variants. One may wonder, therefore, why this uTTP disease variant has only been identified in the large French TMA reference center registry. An initial diagnosis of uTTP in the abovementioned reports from the CNR-MAT[1,20,21] affected roughly 25% of TTP patients. Even though some 20% of initial uTTP diagnoses may be revised to iTTP when autoantibodies become detected during long-term follow-up, either in remission or at an acute relapse,[21] it nevertheless remains difficult to explain why uTTP has so far not been identified in other major cohorts. How can this entity of uTTP, rather common in non-idiopathic TTP, be overlooked in other experienced TMA centers?

Could different sensitivities of assays for measuring anti-ADAMTS13 antibodies or using variable cut-offs for positivity of the same ELISA give an explanation? The commercial Technozym® ADAMTS13-INH-ELISA used by the French centers is commonly also used by other groups. It should be realized that this ELISA only detects free autoantibodies of the IgG class and may miss IgM and/or IgA antibodies. Moreover, if all antibodies are bound to ADAMTS13 and circulate in plasma as immune complexes with the protease,[34] no free autoantibodies can be detected. Therefore, a negative assay for free anti-ADAMTS13 antibodies in plasma or serum does not strictly rule out an iTTP. Measuring functional inhibitors using Bethesda-like assays[24] is not done by many groups and is not expected to give positive results in the absence of anti-ADAMTS13 antibodies by ELISA.

Another concern relates to the ADAMTS13 activity assay. Could at least some patients from the French TMA registry have been falsely diagnosed as having a severely deficient ADAMTS13 functional activity? In the report by Mariotte *et al*.[20] the FRETS-VWF73 assay[35] and, for patients in the early 2000s, a home-made ELISA, or rather an immune radiometric assay quantifying cleavage of whole-length VWF using two monoclonal antibodies, were used.[36] These assays in expert laboratories are not expected to give falsely low ADAMTS13 activity.[37] Severe hyperbilirubinemia results in falsely low ADAMTS13 activity with the FRETS-VWF73 method.[38] Alarming numbers of false positive diagnoses of severe ADAMTS13 deficiency were reported in a management study by Singh *et al*. for the HemosIL AcuStar automated chemiluminescent ADAMTS13 immunoassay.[39] Several patients admitted to the emergency unit with acute inflammatory conditions such as sepsis had severely deficient ADAMTS13 activity whereas simultaneously performed comparative ADAMTS13 activity assays on the same samples revealed only mildly reduced or even normal ADAMTS13 activity, thereby ruling out a diagnosis of TTP. However, it is unlikely

that this rapid HemosIL AcuStar assay or other automated rapid assays were used at some less experienced centers contributing patients to the French TMA registry.
Therefore, if false-positive TTP diagnoses can be confidently refuted and all these patients with uTTP[1,20,21] have true ADAMTS13 deficiency, the pathophysiological mechanism(s) underlying ADAMTS13 deficiency need(s) to be unraveled. This will be important not only for a formally correct diagnosis but may be most relevant for the therapeutic approaches in patients with uTTP.

Proposal for a prospective study to establish the existence and search for pathomechanisms of thrombotic thrombocytopenic purpura of unidentified pathophysiology

If the underlying pathophysiology in uTTP cases were indeed to involve a non-immune mechanism, one may imagine several possibilities resulting in severely deficient ADAMTS13 activity. As suggested by Simon *et al.*[1] non-immune destruction or consumption of ADAMTS13, deficient synthesis in liver failure, proteolytic degradation or catalytic inhibition of ADAMTS13, e.g. by interleukin-6,[40] may be considered.
I fully agree with Knöbl that carefully planned prospective studies will be needed and Table 1 in his Comment[2] lists many important items to be applied: prospective patient enrollment at hospital admission is mandatory. A strict protocol must be in place for clinical monitoring, blood sampling, laboratory analyses and biomaterial sample storage for future investigations, e.g. citrated plasma, EDTA plasma, serum and samples for DNA- and/or RNA-based analyses. A professional biobank set-up, as reported for a prospective cohort study on elderly patients with venous thrombosis,[41] should be established. Necessarily, this needs to be a multicenter study with dedicated TTP specialist centers and all included patients should be followed-up, optimally for several years. This will allow to determine whether, similarly to the cohort reported by Simon *et al.*,[1] some 20% of patients will later receive a revised diagnosis of iTTP when autoantibodies against ADAMTS13 become positive and/or clinical relapses occur.
Such a proposed study needs broad academic input from many centers and needs to be funded. The International Working Group on TTP[16,17] may be optimally positioned to set up a Steering Committee preparing a study protocol and I would hope that, besides academically acquired funding, industry will substantially support such an investigation.

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