Acquired thrombotic thrombocytopenic purpura without detectable anti-ADAMTS13 antibodies: a possible underlying autoimmune mechanism

Thrombotic thrombocytopenic purpura (TTP) is characterized by severe thrombocytopenia, erythrocyte fragmentation and organ failure resulting from disseminated microvascular thrombosis.[1] The pathophysiology of TTP is based on a severe deficiency of ADAMTS13 (A Disintegrin And Metalloprotease with ThromboSpondin type-1 repeats, member 13), the specific von Willebrand factor-cleaving protease.[2-4] In up to 50% of cases, acute TTP occurs in the context of pre-existing or concomitant clinical conditions such as infections, systemic autoimmune diseases, cancers and drug intake.[5] ADAMTS13 deficiency is either inherited with recessive mutations of the encoding *ADAMTS13* gene (congenital TTP) or acquired,[2-5] and in this case mostly due to autoantibodies directed against ADAMTS13 (immune-mediated TTP, iTTP). Anti-ADAMTS13 antibodies are predominantly of the IgG class[6] and have different non-exclusive effects on the enzyme, including inhibition of the proteolytic activity of ADAMTS13, and excessive ADAMTS13 clearance through the formation of immune complexes.[2] Anti-ADAMTS13 antibodies can also alter the conformation of ADAMTS13 *in vitro* to an open structure.[7] These antibodies are usually transient and disappear during clinical remission, concomitantly with the recovery of ADAMTS13 activity. In up to 25% of patients with acquired TTP, however, anti-ADAMTS13 antibodies are not identified, and ADAMTS13 is frequently in a closed conformation;[8] therefore, the mechanism resulting in ADAMTS13 deficiency remains unidentified (uTTP). In these patients, whether standard treatment, including full immunosuppression with corticosteroids and rituximab, but also anti-VWF strategies (caplacizumab),[9] should be given is still controversial.

Here, we provide further insights into the clinical presentation and outcome of uTTP to identify features suggestive of an underlying autoimmune process consistent with the final diagnosis of iTTP, which should prompt initiation of a standard treatment.[9]

We studied 273 patients with acute TTP without anti-ADAMTS13 antibodies (uTTP) at diagnosis. During follow-up, anti-ADAMTS13 antibodies were found positive in 40 patients (21%). These patients were typically younger, with more neurological features, more severe thrombocytopenia and rarely had associated contexts (mostly pregnancy or systemic autoimmune diseases), as compared to patients with persistently undetectable anti-ADAMTS13 antibodies. They also more frequently experienced clinical or ADAMTS13 relapse.

We retrospectively analyzed patients with a diagnosis of uTTP at first presentation recruited in the French Reference Center for Thrombotic Microangiopathies (*www.cnr-mat.fr*) within the last two decades. Diagnostic criteria for acquired uTTP included at baseline: (i) presence of mechanical hemolytic anemia; (ii) acute peripheral thrombocytopenia (platelets <150x10^9/L) in the absence of another identifiable cause of thrombocytopenia; (iii) severe ADAMTS13 deficiency (activity <10%), and (iv) undetectable serum anti-ADAMTS13 IgG (<25 IU/mL) (Technozym® ADAMTS13-INH ELISA kit, Technoclone, Vienna, Austria). Anti-ADAMTS13 IgG were available after 5 to 7 days. Written informed consent was obtained from all patients according to the Declaration of Helsinki. The local ethics committee of Hospital Saint Antoine approved the study.

Among 1,325 patients with severe ADAMTS13 deficiency at presentation, we focused on 273 patients with uTTP, representing 20% of the national cohort (Figure 1). In this population, the female-to-male ratio was 1.3 and the median age was 51 years (interquartile range [IQR], 34-66). Neurological, cardiac and digestive manifestations were present in 52%, 19% and 36% of cases, respectively. Two hundred and eleven (77%) patients received daily therapeutic plasma exchange. The other patients were treated only with fresh-frozen plasma and treatment of associated factors. These patients correspond to the oldest patients in the cohort, treated in the 2000s, before the recent recommendations. Moreover, this therapeutic choice was also related to the presence of associated factors leading to the suspicion of a non-immune mechanism of ADAMTS13 deficiency. Only 40 (15%) patients received rituximab as first-line therapy. Three patients received caplacizumab. Seventy percent of uTTP patients had an associated condition (infections [41%, including 60% with septic shock], cancer [19%], organ or hematopoietic stem cell transplantation [12%], systemic autoimmune diseases [9%], liver failure [7%], pregnancy [7%], human immunodeficiency infection [3%], or drug intake - clopidogrel [1%]). As a result, overall survival was poor in this group, with 82 (30%) patients dying during the acute episode (Figure 1).

Among 191 surviving patients with uTTP, anti-ADAMTS13 antibodies were found positive following the acute phase in 40 (21%) cases (Figure 1), either during the systematic follow-up (18 cases) or during a relapse (22 cases), therefore suggesting the final diagnosis of iTTP. Due to missing data, we were not able to measure the impact of previous treatment on time to positive ADAMTS13 antibodies. These 40 patients rarely had an associated condition (22% of cases; mostly pregnancy or a systemic autoimmune dis-

ease). They were also younger (*P*<0.0001), had more severe cytopenia (*P*=0.002), had more neurological features (45% of cases, *P*=0.0001), included fewer cases with renal failure (*P*=0.04), had more severe deficiency in ADAMTS13 activity (ADAMTS13 activity ≤5% in 80% of patients, *P*=0.008) and had more clinical (*P*<0.0001) or biological (*P*<0.0001) relapses as compared to patients with persistently undetectable anti-ADAMTS13 antibodies. Accordingly, they received significantly more rituximab infusions at initial diagnosis than patients with persistently undetectable anti-ADAMTS13 antibodies (*P*=0.006) (Table 1).

Normalization of ADAMTS13 activity during follow-up was documented for 38 out of the 40 patients in the iTTP group and 69 patients in the uTTP. The missing data, particularly for the uTTP group, can be explained by a unique TTP episode, leading to loss of follow-up. The median time to achieve ADAMTS13 activity >10% was significantly longer in the iTTP group (46 days; IQR, 25-293) than in the uTTP group (34.5 days; IQR, 11-69.2; *P*=0.05).

Our observations support the view that patients with acquired TTP of unidentified mechanism (uTTP) (20% of our total cohort) should be reconsidered as having iTTP in 21% of cases, as anti-ADAMTS13 antibodies were found to be present during their follow-up. The rate of uTTP in our study was higher than in another TTP registry.[10]

We acknowledge that the absence of antibodies at first presentation of TTP may be related to a failure to detect these antibodies rather than a true absence. This could be due to a lack of sensitivity of techniques for detecting anti-ADAMTS13 antibodies *in vitro*. Additionally, the presence of anti-ADAMTS13 IgG complexed to the ADAMTS13 antigen in immune complexes might be present *in vivo* but undetectable *in vitro*.[11] Furthermore, IgM or IgA isotypes, which are described in 20% of patients, may not be detected.[12] Moreover, the positivity of anti-ADAMTS13 IgG antibodies may vary from one flare to another in some patients. Additionally, some antibodies may recognize cryptic epitopes whose identification, dependent on circumstances, is more complex. Mutations or polymorphisms in the *ADAMTS13* gene could contribute to this lack of detection. Finally, due to

Table 1. Initial demographic, clinical and biological characteristics of patients with a final diagnosis of immune-mediated thrombotic thrombocytopenic purpura *versus* acquired thrombotic thrombocytopenic purpura of unidentified mechanism.

	uTTP N=151	iTTP N=40	*P*
Age in years, median (IQR)	51 (39-66)	33 (25-45)	<0.0001
Sex ratio (F/M)	1.7 (90/61)	2 (27/13)	0.46
Idiopathic TTP, N (%)	32 (21)	31 (78)	<0.0001
Non-idiopathic TTP, N (%)	119	9	
Pregnancy	15 (13)	5 (56)	0.57
Auto-immune disease	16 (13)	2 (22)	0.37
Infection	68 (57)	1 (11)	<0.0001
Cancer	20 (17)	0	0.008
Others	33 (27.7)	1 (11)	0.002
Clinical presentation at initial diagnosis, N (%)			
Neurological involvement	23 (15)	18 (45)	0.0001
Fever	63 (42)	5 (13)	0.0004
Cardiac disorders	25 (16)	8 (20)	0.64
Renal failure	103 (68)	20 (50)	0.04
Hemoglobin, g/dL, median (IQR)	8 (6.7-9)	9.2 (7.5-11)	0.002
Platelet count, x10^9/L, median (IQR)	24 (15-45)	13 (10-25)	0.002
Serum creatinine, μmol/L, median (IQR)	194 (80-377)	116 (74-134)	<0.0001
ADAMTS13 activity, N (%)			0.008
6-9%	60 (40)	7 (18)	
≤ 5%	86 (57)	32 (80)	
Clinical/biological relapse, N (%)	29 (19)	32 (80)	<0.0001
Clinical relapse	18 (62)	22 (69)	< 0.0001
ADAMTS13 relapse	11 (38)	10 (31)	0.006
Rituximab treatment, N (%)			
At initial presentation	16 (11)	9 (23)	0.006
At relapse	18 (12)	22 (55)	< 0.0001
Pre-emptive	11 (7)	10 (25)	0.003

uTTP: acquired thrombotic thrombocytopenic purpura of unidentified mechanism; iTTP: immune-mediated thrombotic thrombocytopenic purpura; IQR: interquartile range; F: female; M: male; TTP: thrombotic thrombocytopenic purpura; ADAMTS13: a disintegrin and metalloprotease with thrombospondin type-1 repeats, member 13.

the retrospective nature of our study, some patients only had one assay of anti-ADAMTS13 antibodies.

This hypothesis of an initial failure to detect autoantibodies is limited to the 21% of patients with positive antibodies during follow-up. However, our results cannot be explained solely by a technical defect in antibody detection. We identified two populations with different clinical and biological profiles at diagnosis, and different evolutionary profiles. These are indeed two distinct populations, associated with different pathophysiological mechanisms of ADAMTS13 deficiency, whose identification at initial diagnosis should enable personalized management.

Due to the retrospective nature of our study, some biological parameters could not be studied, particularly functional tests for the identification of ADAMTS13 inhibitor and ADAMTS13 conformation, as these tests are not routinely performed. This is an inherent bias in these studies. We cannot affirm that the absence of anti-ADAMTS13 antibody assessed using an enzyme-linked immunosorbent assay excludes the presence of ADAMTS13 inhibitor measured by the Bethesda assay or plasma mixing assay. In a previous study by our team,[5] based on the same French registry, no inhibitor was found, with results probably the same in our work if available. A recent study has shown a positive correlation between the measurement of ADAMTS13 activity and the titer of anti-ADAMTS13 antibodies, as well as between the measurement of ADAMTS13 activity and the titer of ADAMTS13 inhibitor.[13] Furthermore, it has already been shown that anti-ADAMTS13 IgG antibodies are more frequently detected than ADAMTS13 inhibitor, and that patients without detectable ADAMTS13 inhibitor have positive anti-ADAMTS13 IgG antibodies.[10] It is, therefore, unlikely that these patients without anti-ADAMTS13 IgG antibodies had a detectable ADAMTS13 inhibitor.

Regarding the conformation of ADAMTS13, recent research has demonstrated that ADAMTS13 appears to have a closed conformation during uTTP and an open conformation during iTTP,[8] suggesting that this biological parameter should be included as a marker of iTTP in future prospective studies. The pathophysiology of uTTP without anti-ADAMTS13 antibodies has not been studied. One of the hypotheses is a non-immune mechanism of destruction and consumption of ADAMTS13. In fact, patients with uTTP without anti-ADAMTS13 antibodies mostly have only one episode of TTP, related to a specific clinical circumstance responsible for the deficiency of ADAMTS13. In the literature, a deficiency of ADAMTS13 synthesis has been reported in liver failure,[14] degradation of ADAMTS13 by proteases (plasmin or thrombin) has been observed in sepsis[15] and, finally, catalytic inhibition of ADAMTS13 by free hemoglobin or interleukins has been described. It is evident that the pathophysiological mechanisms in uTTP are not yet wholly elucidated, as isolated sepsis does not appear to be a sufficient circumstance to trigger TTP. Those cases of uTTP must be a unique episode of TTP.

In conclusion, the absence of detectable anti-ADAMTS13 antibodies at baseline in TTP adult patients should not systematically rule out the diagnosis of iTTP. In these patients, usual features of iTTP (young age, cerebral involvement, severe thrombocytopenia, severe ADAMTS13 activity deficiency) with no other associated context than a history of systemic autoimmune disease or pregnancy, should prompt consideration of the diagnosis of iTTP. (*Online Supplementary Figure S1*).

Considering our findings, we assessed some recommenda-

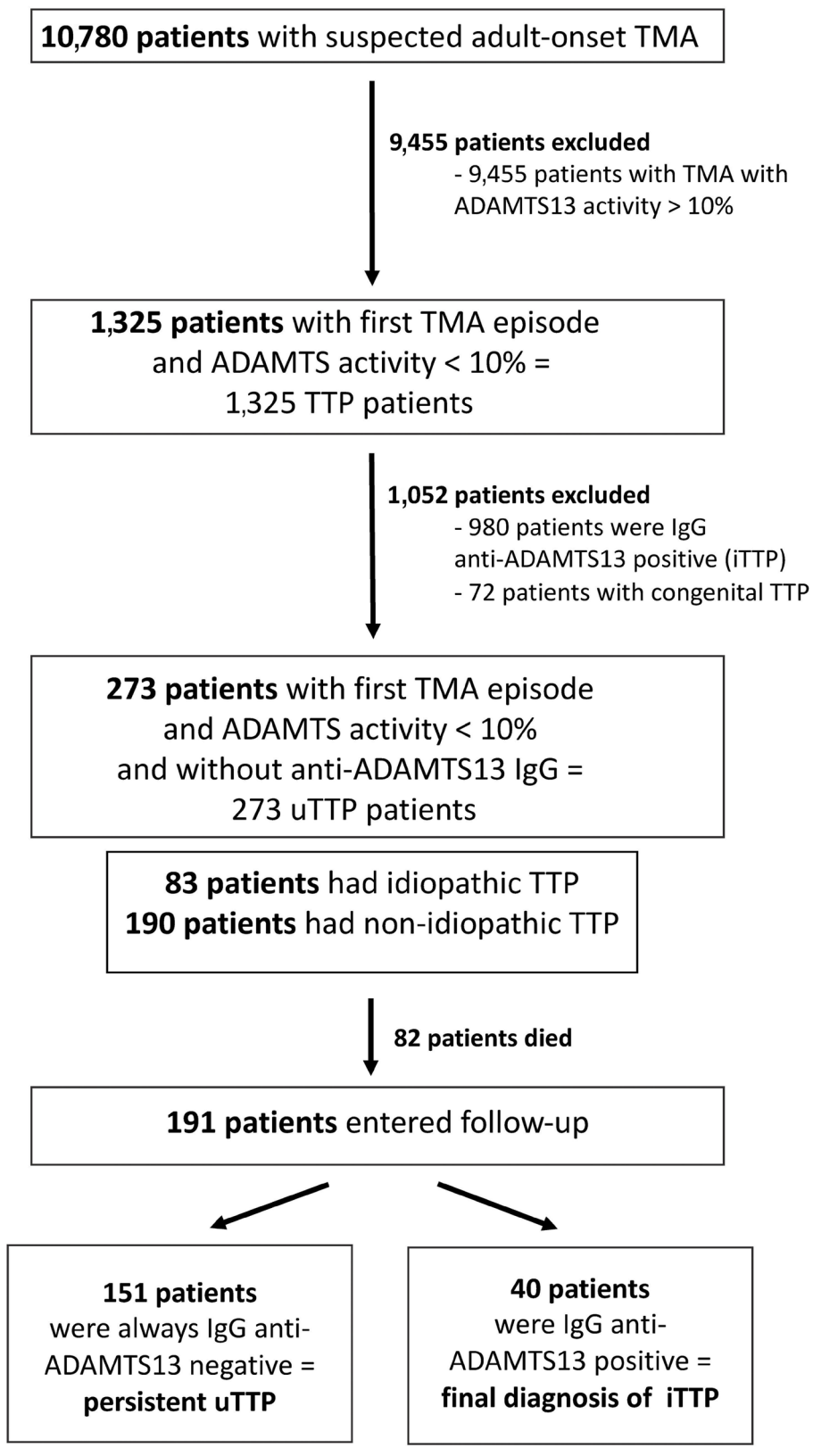

Figure 1. Flow-chart of the study population. TMA: thrombotic microangiopathy; ADAMTS13: a disintegrin and metalloprotease with thrombospondin type-1 repeats, member 13; TTP: thrombotic thrombocytopenic purpura; iTTP: immune-mediated thrombotic thrombocytopenic purpura; uTTP: thrombotic thrombocytopenic purpura of unidentified mechanism.

tions, which will require future prospective projects to be confirmed, given the severity and mortality of TTP. We suggest that patients exhibiting clinical characteristics similar to those of patients with iTTP (such as young age, neurological features, severe thrombocytopenia, severe ADAMTS13 activity deficiency and idiopathic TTP) should be treated as if they had iTTP, i.e,, with therapeutic plasma exchange, corticosteroids, rituximab, and caplacizumab.

A potential future phase III trial could be designed to compare mortality and relapse rates between two therapeutic strategies based on patients' profiles: one arm receiving standard treatment (therapeutic plasma exchange, corticosteroids, rituximab, and caplacizumab) for patients with uTTP and clinical characteristics of iTTP, and the other arm receiving only therapeutic plasma exchange and caplacizumab for patients with uTTP, lacking anti-ADAMTS13 antibodies and clinical characteristics of iTTP. The integration of recombinant ADAMTS13 into the therapeutic strategy for iTTP and uTTP remains to be defined.[16] The biological monitoring for this study should be more comprehensive, including measurements of ADAMTS13 activity, anti-ADAMTS13 antibodies, identification of ADAMTS13 inhibitor, and ADAMTS13 conformation.

Authors

Delphine Simon,[1*] Mathilde Leclercq,[1,2*] Bérangère Joly,[3,4] Agnès Veyradier,[3,4] Paul Coppo[5,6] and Ygal Benhamou[1,7]

[1]Département de Médecine Interne, CHU Charles Nicolle, Rouen; [2]Université de Rouen, INSERM U1234 PANTHER, Rouen; [3]Département d'Hématologie Biologique, Hôpital Lariboisière, AP-HP, Paris; [4]Equipe d'Accueil 3518, Institut de Recherche Saint-Louis, Université de Paris, Paris; [5]Centre de Référence des Microangiopathies Thrombotiques, Service d'Hématologie, AP-HP et Sorbonne Université, Paris; [6]INSERM Unité Mixte de Recherche (UMRS) 1138, Centre de Recherche des Cordeliers, Paris and [7]Université de Rouen, INSERM U1026 EnVI, Rouen, France

DS and ML contributed equally as first authors.

Correspondence:
Y. BENHAMOU - ygal.benhamou@chu-rouen.fr

https://doi.org/10.3324/haematol.2024.285391

Received: March 8, 2024.
Accepted: September 18, 2024.
Early view: September 26, 2024.

Disclosures

BJ has received consulting fees from Takeda, payment or honoraria for lectures, presentations, speakers' bureaus, manuscript writing or educational events for LFB-Biomédicaments, Sanofi and Takeda and support for attending meetings and/or travel from Sanofi, Takeda and Alexion. PC has received payment or honoraria for lectures, presentations, speakers' bureaus, manuscript writing or educational events from Sanofi, Takeda and Alexion. ML has received support for attending meetings and/or travel from Horus and Pfizer. AV is a member of a French advisory board on caplacizumab for Sanofi and on recombinant ADAMTS13 for Takeda. YB is a member of an advisory board for Sanofi.

Contributions

DS performed research, analyzed data and wrote the manuscript. ML analyzed data and wrote the manuscript. BJ performed research. AV, PC and YB supervised the study.

Acknowledgments

The authors thank Raïda Bouzid, Centre de Référence des Microangiopathies Thrombotiques, for her help with collecting data for the manuscript.

Data-sharing statement

Data are available on request.

References

1. Kremer Hovinga JA, Coppo P, LämmleB, Moake JL, Miyata T, Vanhoorelbeke K. Thrombotic thrombocytopenic purpura. Nat Rev Dis Primers. 2017;3:17020.
2. Levy GG, Nichols WC, Lian EC, et al. Mutations in a member of the ADAMTS gene family cause thrombotic thrombocytopenic purpura. Nature. 2001;413(6855):488-494.
3. Georges JN, Nester CM. Syndromes of thrombotic microangiopathy. N Engl J Med. 2014;371(7):654-666.
4. Joly BS, Coppo P, Veyradier A. Thrombotic thrombocytopenic purpura. Blood. 2017;129(21):2836-2846.
5. Mariotte E, Azoulay E, Galicier L, et al. Epidemiology and pathophysiology of adulthood-onset thrombotic microangiopathy with severe ADAMTS13 deficiency (thrombotic thrombocytopenic purpura): a cross-sectional analysis of the French national registry for thrombotic microangiopathy. Lancet Haematol. 2016;3(5):237-245.
6. 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. J Thromb Haemost. 2009;7(10):1703-1710.
7. Roose E, Schelpe AS, Tellier E, et al. Open ADAMTS13, induced by antibodies, is a biomarker for subclinical immune-mediated thrombotic thrombocytopenic purpura. Blood. 2020;136(3):353-361.
8. Joly BS, Roose E, Coppo P, Vanhoorelbeke K, Veyradier A. ADAMTS13 conformation is closed in non-immune acquired thrombotic thrombocytopenic purpura of unidentified

pathophysiology. Haematologica. 2023;108(2):638-644.
9. Coppo P, Bubenheim M, Azoulay E, et al. A regimen with caplacizumab, immunosuppression, and plasma exchange prevents unfavorable outcomes in immune-mediated TTP. Blood. 2021;137(6):733-742.
10. Scully M, Yarranton H, Liesner R, et al. Regional UK TTP registry: correlation with laboratory ADAMTS 13 analysis and clinical features. Br J Haematol. 2008;142(5):819-826.
11. Lotta LA, Valsecchi C, Pontiggia S, et al. Measurement and prevalence of circulating ADAMTS13-specific immune complexes in autoimmune thrombotic thrombocytopenic purpura. J Thromb Haemost. 2014;12(3):329-336.
12. Scheiflinger F, Knöbl P, Trattner B, 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):3241-3243.
13. Saito K, Sakai K, Kubo M, et al. Persistent ADAMTS13 inhibitor delays recovery of ADAMTS13 activity in caplacizumab-treated Japanese patients with iTTP. Blood Adv. 2024;8(9):2151-2159.
14. Uemura M, Fujimura Y, Matsumoto M, et al. Comprehensive analysis of ADAMTS13 in patients with liver cirrhosis. Thromb Haemost. 2008;99(6):1019-1029.
15. Crawley JT, Lam JK, Rance JB, Mollica LR, O'Donnell JS, Lane DA. Proteolytic inactivation of ADAMTS13 by thrombin and plasmin. Blood. 2005;105(3):1085-1093.
16. Bendapudi PK, Foy BH, Mueller SB, et al. Recombinant ADAMTS13 for immune thrombotic thrombocytopenic purpura. N Engl J Med. 2024;390(18):1690-1698.