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

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Running Title: Acquired TTP without anti-ADAMTS13 antibodies.

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Summary:

In up to 25% of patients with acquired TTP, anti-ADAMTS13 antibodies are not identified, the mechanism resulting from ADAMTS13 deficiency remains unidentified (uTTP). In this study, we provide further insights on clinical presentation and outcome of uTTP.

In patients with baseline undetectable anti-ADAMTS13 antibodies, usual features of iTTP (young age, cerebral involvement, severe thrombocytopenia) with no other associated context than a history of systemic autoimmune disease or pregnancy, should prompt to consider the diagnosis of iTTP.

Keywords:

Thrombotic thrombocytopenic purpura, immune-mediated thrombotic thrombocytopenic purpura, anti-ADAMTS13 antibody.

Thrombotic thrombocytopenic purpura (TTP) is characterized by severe thrombocytopenia, erythrocyte fragmentation and organ failure resulting from disseminated microvascular thrombosis [1]. TTP pathophysiology is based on a severe deficiency in 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 can occur within 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 IgG class [6] and have different non-exclusive effects on the enzyme, including the inhibition of the proteolytic activity of ADAMTS13, and excessive ADAMTS13 clearance through immune complexes formation [2]. Anti-ADAMTS13 antibodies can also alter ADAMTS13 conformation to an opened structure *in vitro* [7]. These antibodies are usually transient and disappear during clinical remission, concomitantly with ADAMTS13 activity recovery. 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 from 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 performed is still controversial.

Here, we provide further insights on clinical presentation and outcome of uTTP to identify features suggestive of an underlying autoimmune process consistent with the final diagnosis of iTTP that should prompt to initiate 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 have associated contexts that mostly consist in pregnancy or systemic autoimmune diseases, as compared to patients with persistently undetectable anti-ADAMTS13 antibodies. They also experience more clinical or ADAMTS13 relapse.

We retrospectively analyzed patients with a diagnostic of uTTP at first presentation recruited in the French Reference Centre for Thrombotic Microangiopathies (www.cnr-mat.fr) within the last two decades. Diagnostic criteria for acquired uTTP included at baseline: (1)

presence of mechanical hemolytic anemia, (2) acute peripheral thrombocytopenia (<150 G/L) in the absence of another identifiable cause of thrombocytopenia, (3) severe ADAMTS13 deficiency (activity <10%), and (4) undetectable serum anti-ADAMTS13 IgG (< 25 UI/mL) (TECHNOZYM ® ADAMTS13-INH ELISA kit, Technoclone, Vienne, Autriche). 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 1325 patients with severe ADAMTS13 deficiency at presentation, we focused on 273 patients with uTTP, representing 20% of the national cohort. In this population, the women-to-men ratio was 1.3 F/1 M and the median (IQR) age was 51 years (34-66). Neurologic, 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 (TPE). 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 mechanisms 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), cancers (19%), organ or hematopoietic stem cell transplantation (12%), systemic autoimmune diseases (9%), liver failure (7%), pregnancy (7%), HIV infection (3%), or drug intake (clopidogrel) (1%)). As a result, overall survival was poor in this group, with eighty-two (30%) patients who died during the acute episode.

Among 191 surviving patients with uTTP, anti-ADAMTS13 antibodies were found positive following the acute phase in 40 (21%) cases, either during the systematic follow-up (n=18 cases) or during a relapse (n=22 cases), suggesting therefore the final diagnosis of iTTP. Due to missing data, we were not able to measure the impact between previous treatment and time to positive ADAMTS13 antibodies. These 40 patients rarely had an associated condition (22% of cases; mostly pregnancy or a systemic autoimmune disease). They also had a younger age ($p<0.0001$), more severe cytopenia ($p=0.002$), more neurologic features (45% of cases, $p=0.0001$), fewer renal failure ($p=0.04$), more severe deficiency in ADAMTS13 activity (ADAMTS13 activity ≤5% in 80% of patients, $p=0.008$) and more clinical ($p<0.0001$) or biological ($p<0.0001$) relapse, 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 collected 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 (IQR) to achieve ADAMTS13 activity >10% was significantly longer in the iTTP group (46 days [25-293] versus 34.5 days [11-69.25] in the uTTP group, p=0.05).

Our observations support the view that patients with acquired TTP of unidentified mechanism (uTTP) (20% of our total cohort) should be revisited as iTTP in 21% of cases, as anti-ADAMTS13 antibodies can be found positive during follow-up. The rate of uTTP in our study was higher than other 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 the retrospective nature of our study, some patients only have one dosage 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 patient management.

Due to the retrospective nature of our study, some biological parameters could not be studied, particularly functional tests for 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 with ELISA kit 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]. Therefore, it is unlikely that these patients without anti-ADAMTS13 IgG antibodies had a detectable ADAMTS13 inhibitor.

Regarding ADAMTS13 conformation, 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.

Pathophysiology of uTTP without anti-ADAMTS13 antibodies has not been studied. One of the hypotheses is a non-immune mechanism for destruction and consumption of ADAMTS13. In fact, patients with uTTP without anti-ADAMTS13 antibodies mostly have only one episode of TTP, related to specific clinical circumstance responsible for the deficiency of ADAMTS13. In the literature, a deficiency to synthesize ADAMTS13 has been reported in liver failure [14], degradation of ADAMTS13 by proteases (plasmin or thrombin) has been observed in sepsis [15]. Finally, catalytic inhibition of ADAMTS13 by free haemoglobin or interleukins has been described. It is evident that the entirety of the pathophysiological mechanisms in uTTP are not yet elucidated today, as isolated sepsis does not appear to be a sufficient circumstance to trigger TTP. Those 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 **(Supplementary Figure 1)**.

Considering our findings, we assessed some recommendations, that will require future prospective projects to confirm them, given the severity and mortality of TTP. We suggest that patients exhibiting clinical characteristics similar to iTTP (such as young age, neurological features, severe thrombocytopenia, severe ADAMTS13 activity deficiency and idiopathic TTP) should be treated as iTTP, i.e, TPE, corticosteroids, rituximab, and caplacizumab.

A potential future phase 3 trial could be designed to compare mortality and relapse rates between two therapeutic strategies based on patient profiles: one arm receiving standard treatment (TPE, corticosteroids, rituximab, and caplacizumab) for patients with uTTP and clinical characteristics of iTTP, and the other arm receiving only TPE 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 tools for this study should be more comprehensive, including measurements of ADAMTS13 activity, anti-ADAMTS13 antibodies, identification of ADAMTS13 inhibitor, and ADAMTS13 conformation.

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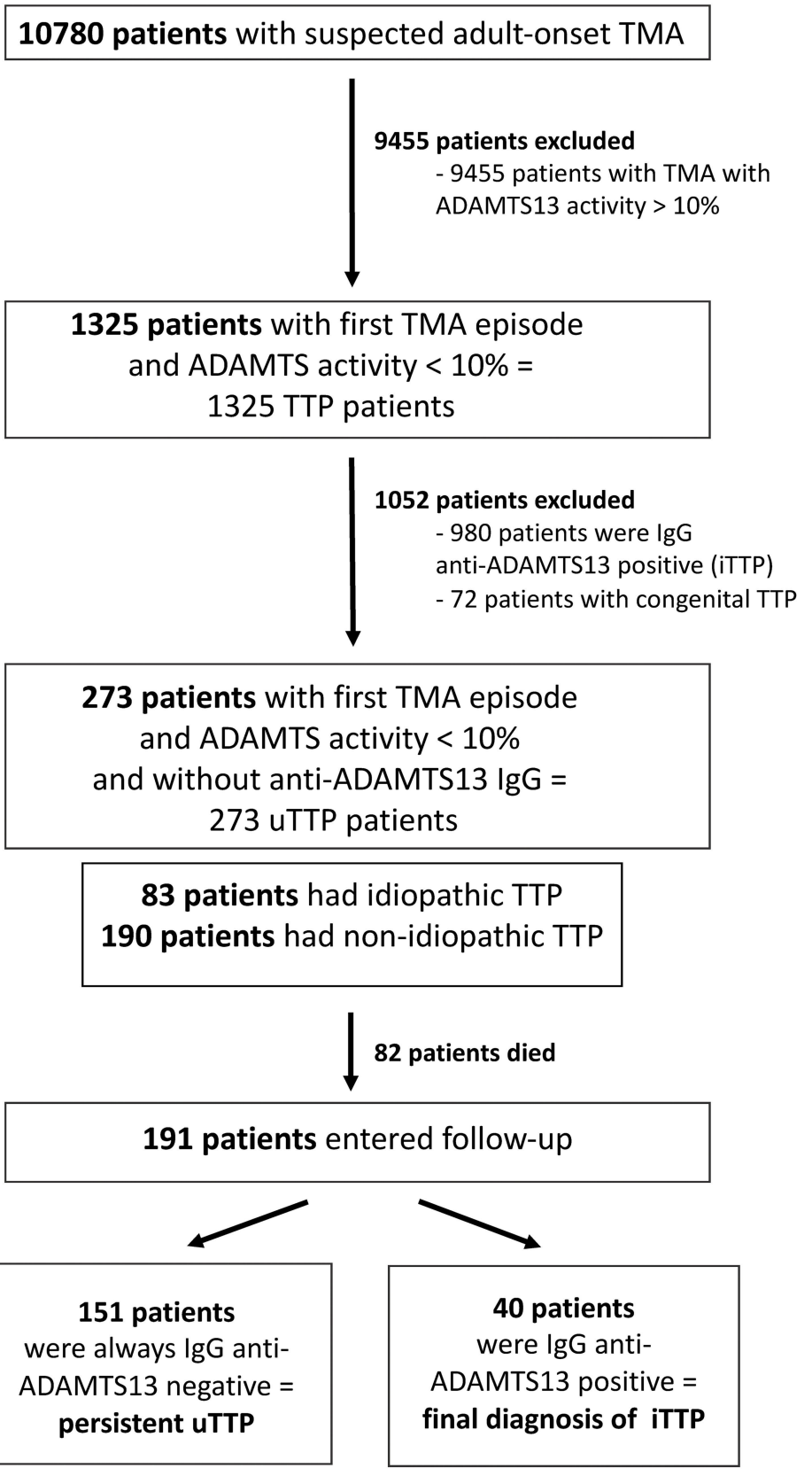
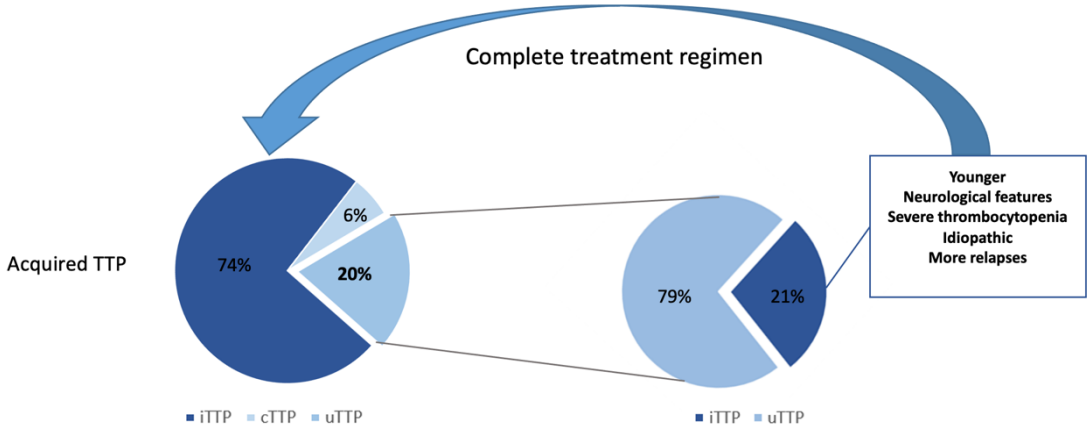
Table 1: Initial demographic, clinical and biological characteristics of patients with a final diagnosis of immune-mediated thrombotic thrombocytopenic purpura versus acquired thrombotic thrombocytopenic purpura of unidentified mechanism.

	uTTP (n=151)	iTTP (n=40)	p-value
Age (years)	51 (39-66)	33 (25-45)	< 0.0001
Sex ratio (W/M)	1.7 (90/61)	2 (27/13)	0.46
Idiopathic TTP	32 (21)	31 (78)	< 0.0001
Non Idiopathic TTP	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			
Neurologic 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
Haemoglobin (g/dL)	8 (6.7;9)	9.2 (7.5;11)	0.002
Thrombocytopenia (G/L)	24 (15-45)	13 (10;25)	0.002
Serum creatinine (µmol/L)	194 (80-377)	116 (74;134)	< 0.0001
ADAMTS13 activity			0.008
6-9%	60 (40)	7 (18)	
≤ 5%	86 (57)	32 (80)	
Clinical/Biological relapse	29 (19)	32 (80)	<0.0001
Clinical relapse	18 (62)	22 (69)	< 0.0001
ADAMTS13 relapse	11 (38)	10 (31)	0.006
Rituximab treatment			
Initial presentation	16 (11)	9 (23)	0.006
At relapse	18 (12)	22 (55)	< 0.0001
Preemptive	11 (7)	10 (25)	0.003

Data are presented as median (interquartile range) or number (percentage). TTP= thrombotic thrombocytopenic purpura. uTTP= acquired thrombotic thrombocytopenic purpura of unidentified mechanism. iTTP= immune-mediated thrombotic thrombocytopenic purpura.

Figure 1: Flow-chart of population.
TMA: thrombotic microangiopathy, TTP: thrombotic thrombocytopenic purpura, ADAMTS13: A Disintegrin And Metalloprotease with ThromboSpondin type-1 repeats, member 13), iTTP: immune mediated thrombotic thrombocytopenic purpura, uTTP: thrombotic thrombocytopenic purpura of unidentified mechanism.


10780 patients with suspected adult-onset TMA
9455 patients excluded
- 9455 patients with TMA with ADAMTS13 activity > 10%
1325 patients with first TMA episode and ADAMTS activity < 10% = 1325 TTP patients
1052 patients excluded
- 980 patients were IgG anti-ADAMTS13 positive (iTTP)
- 72 patients with congenital TTP
273 patients with first TMA episode and ADAMTS activity < 10% and without anti-ADAMTS13 IgG = 273 uTTP patients
83 patients had idiopathic TTP
190 patients had non-idiopathic TTP
82 patients died
191 patients entered follow-up
151 patients were always IgG anti-ADAMTS13 negative = **persistent uTTP**
40 patients were IgG anti-ADAMTS13 positive = **final diagnosis of iTTP**



Supplementary Figure 1: Acquired forms of thrombotic thrombocytopenic purpura in the French Reference Centre for Thrombotic Microangiopathies.