ADAMTS13 conformation is closed in non-immune acquired thrombotic thrombocytopenic purpura of unidentified pathophysiology

Thrombotic thrombocytopenic purpura (TTP) is a rare (prevalence of ~10-20 cases/million worldwide in 2022, incidence of ~1-2 new cases/million/year), relapsing and life-threatening thrombotic microangiopathy due to systemic platelet-rich thrombi of blood microvessels.[1,2] The cause of TTP was identified in 1998 as a severe functional deficiency (activity <10 IU/dL) of the enzyme ADAMTS13 (A Disintegrin And Metalloprotease with ThromboSpondin type 1 repeats, member 13).[3,4] The structure of ADAMTS13 includes 14 domains: metalloprotease (M), disintegrin-like (D), eight thrombospondin type 1 repeats (T1-T8), cysteine-rich (C), spacer (S) and two CUB (CUB1, CUB2).[5] Physiologically, ADAMTS13 prevents the systemic occlusion of the blood microvasculature with platelet-rich thrombi, by specifically cleaving the highly adhesive ultra-large multimers of von Willebrand factor (VWF), a glycoprotein crucial for platelet adhesion and aggregation at sites of vascular injury.[1-4]

In very rare cases, severe deficiency of *ADAMTS13* is due to bi-allelic mutations of the ADAMTS13 gene which cause congenital hereditary TTP.[1,2] In contrast, in a very large majority of TTP cases, severe deficiency of ADAMTS13 is an acquired condition, either via specific anti-ADAMTS13 polyclonal autoantibodies (ADAMTS13 antibodies) inducing a catalytic inhibition/accelerated clearance of ADAMTS13 or via other speculative mechanisms (defects of synthesis/secretion, excessive degradation, catalytic inhibition of ADAMTS13).[1,2] Thus, the pathophysiology of acquired TTP is either immune-mediated (iTTP, ~75% of cases) or unidentified (uTTP).[1,2,6] In terms of clinical presentation of acute events of TTP, about half occur without any other clinical context, defining *idiopathic* TTP. In contrast, the other half occurs with one or several associated and potentially triggering clinical conditions (mostly infections, autoimmune diseases, cancers, transplantation, drug treatment or pregnancy/post-partum) defining *non-idiopathic* TTP.[1,2,6] When crossing clinical presentation and pathophysiology, we previously showed that ~90% of cases of idiopathic TTP consist of iTTP whereas non-idiopathic TTP includes more balanced proportions of iTTP (~60%) and uTTP (~40%).[6] Consequently, uTTP are mostly found among non-idiopathic TTP.

In the 2010s, ADAMTS13 was shown to have a conformational plasticity.[7] In healthy individuals, ADAMTS13 circulates in a latent "closed" conformation governed by a local latency due to an interaction between its CUB1-CUB2 domains and its S domain which contains a cryptic highly immunodominant epitope[8-10] and by a local latency due to the presence of a gatekeeper triad that blocks the active site.[10] Upon physiological binding to VWF, ADAMTS13 adopts a short-lived, transient "open" conformation relieving global latency and allowing multiple interactions with VWF (molecular zipper model) and ultimately, its cleaving activity towards VWF (allosteric activation: relieving the gatekeeper triad).[8-10] ADAMTS13 conformation was recently investigated in three studies[11-13] gathering 102 patients with idiopathic iTTP in the acute phase and during follow-up in clinical remission. In the great majority of these iTTP patients: (i) ADAMTS13 conformation was open in acute phase;[11,12] (ii) ADAMTS13 was closed in remission provided that ADAMTS13 activity was normal (>50 IU/dL);[11,12] (iii) if open in remission, ADAMTS13 conformation could be returned to closed by preemptive rituximab treatment;[13] and (iv) ADAMTS[13] antibodies purified from iTTP patients could induce opening of ADAMTS13 *in vitro*.[13] Thus, a sustained open ADAMTS13 conformation appears to be a novel biomarker of acute and subclinical iTTP and appears to be closely linked to the presence of ADAMTS13 antibodies.[11-13]

The aim of the current study was to investigate whether ADAMTS13 was closed or open in uTTP. Closed ADAMTS13 in uTTP would further validate open ADAMTS13 as a biomarker for iTTP. Open ADAMTS13 in uTTP might imply that pathological triggers (distinct from ADAMTS13 antibodies) commonly found in TTP could induce a switch from the closed to the open conformation of ADAMTS13. To do so, we studied 125 patients with acute non-idiopathic TTP presenting with miscellaneous pathological contexts (autoimmunity, cancer, liver insufficiency, infection, drug treatment) including both cases of uTTP (n=76, group of interest) and iTTP (n=49, control group). The uTTP patients had an older median age, lower female/male ratio, more frequent renal disorders, and higher platelet count, ADAMTS13 antigen level and mortality. Interestingly, ADAMTS13 conformation was closed in 86.4% of uTTP cases but open in 76.7% of iTTP cases.

Adult patients with non-idiopathic TTP from the French Thrombotic Microangiopathy Registry (inclusion period 01.01.2012 – 12.31.2016) were enrolled if they met the following inclusion criteria: age >18 years old, inaugural acute thrombotic microangiopathy event associated with both an ADAMTS13 activity <10 IU/dL and another clinical situation at presentation (excluding pregnancy), available medical data and citrated plasma samples. Cases of hereditary TTP were excluded. Informed consent was obtained from each patient according to the Declaration of Helsinki. The study

Table 1. Demographic, clinical and biological features of 125 patients with non-idiopathic thrombotic thrombocytopenic purpura.

Demographic features	Non-idiopathic TTP		*P*
	uTTP (N=76)	iTTP (N=49)	
Age in years (range)§	61 (49 ; 68)	49 (38 ; 56)	**0.0005**
Sex ratio*	0.7 F / 1 M	1.6 F / 1 M	**0.03**
Associated conditions			
Autoimmmune diseases	13 (17.1 %)	19 (38.8 %)	**0.005**
Lupus erythematosus	5	10	
Rhumatoid polyarthritis	1	2	
Systemic sclerodermia	1	2	
Vascularitis	2	1	
Antiphospholipid syndrome	1	1	
Wegener disease	2	0	
Gougerot-Sjögren syndrome	0	1	
Hashimoto thyroitidis	0	1	
Primary biliary cirrhosis	1	0	
Sharp syndrome	0	1	
Cancers	24 (31.6 %)	8 (16.3 %)	**0.005**
Brain	0	1	
Lung	2	0	
Digestive tract	4	3	
Liver, biliary tractus, pancreas	5	0	
Kidney	0	1	
Uterus	1	1	
Prostate	2	1	
Hematoproliferative syndromes	10	1	
Severe liver insufficiency	5 (6.6 %)	3 (6.1 %)	NS
Infections	33 (43.4 %)	15 (30.6 %)	NS
Bacterial infections			
Lung	13	3	
Heart (endocarditis)	2	0	
Digestive tract	5	4	
Urinary tract	6	3	
Joints	1	0	
Viral infections			
Hepatitis C virus	1	0	
Human immunodeficiency virus	4	4	
Parasitic infections			
Malaria	1	0	
Chikungunya	0	1	
Drugs	1 (1.3 %)	4 (8.2 %)	NS
Tacrolimus	1	0	
Gemcitabine	0	1	
Clopidogrel	0	3	
Clinical presentation*			
Fever	33 (43.4 %)	16 (32.7 %)	NS
Neurological features	34 (44.7 %)	24 (49.0 %)	NS
Abdominal features	20 (26.3 %)	11 (22.4 %)	NS
Cardiac features	12 (15.8 %)	7 (14.3 %)	NS
Renal features	59 (77.6 %)	20 (40.8 %)	**<0.0001**

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Biological data			
Hemoglobin, g/dl (range)§	7.7 (7.0 ; 9.0)	7.7 (6.5 ; 8.7)	NS
Platelets x10⁹/L (range)§	28 (16 ; 60)	15 (9 ; 22)	**0.005**
Creatinine μmol/L (range)§	183 (110 ; 406)	107 (68 ; 148)	**0.005**
ADAMTS13 antigen μg/mL (range)§	0.20 (0.14 ; 0.27)	0.04 (0.02 ; 0.11)	**0.0007**
Open ADAMTS13 conformation *	8/59 (13.6 %)	23/30 (76.7 %)	**<0.0001**
Outcome*			
Death	6 (7.9 %)	1 (2.0 %)	NS

Data are presented as number (percentage) or median [interquartile range]. Comparisons from univariate analysis: §*t*-test, *χ^2 test. *P* values <0.05 are considered statistically significant. TTP: thrombotic thrombocytopenic purpura; uTTP: acquired non-immune TTP; iTTP: immune-mediated TTP; F: female; M: male. NS: not statistically significant.

was approved by the Ethics Committee of Pitié-Salpêtrière and Saint-Antoine Assistance Publique – Hôpitaux de Paris. ADAMTS13 activity was measured using the FRETS-VWF73 method (Peptide Institute Inc, Osaka, Japan) and ADAMTS13 IgG were titrated using the TECHNOZYM® ADAMTS13-INH enzyme-linked immunosorbent assay (ELISA) (Technoclone, Vienna, Austria), as previously described.[6] ADAMTS13 antigen levels and ADAMTS13 conformation were determined by our home-made 3H9- and 1C4-ELISA, respectively.[11] Quantitative parameters were reported as median (interquartile range) and qualitative parameters as number and percentage. Comparisons between uTTP and iTTP were performed using the Pearson χ^2 test and Fisher exact test as appropriate (Stat View version 8.0, Stanford, CA, USA). All tests were two-sided and *P* values less than 0.05 were considered statistically significant.

Among the 125 patients with non-idiopathic TTP enrolled, ADAMTS13 IgG were negative in 76 patients (uTTP, group of interest) and positive in 49 patients (iTTP, control group). The demographic, clinical and biological features of our TTP patients are presented in Table 1. Patients with uTTP were significantly older (median age, (61 *vs*. 49 years, respectively, *P*=0.0005) and had a lower female/male ratio (0.7 *vs*. 1.6, respectively; *P*=0.03). Overall, associated clinical contexts consisted in autoimmune diseases (n=32, 25.6%; mostly lupus erythematosus), cancers (n=32, 25.6%), severe liver insufficiency (n=8; 6.4%), infections (n=48, 38.4%) and drugs (n=5, 4.0%). Of note, autoimmune diseases were more frequent in iTTP (*P*=0.005) while cancers were more frequent in uTTP (*P*=0.005). The frequency of fever, neurological, digestive or cardiac ischemic symptoms was not significantly different between uTTP and iTTP. In contrast, uTTP patients had a significantly higher frequency of renal disorders (creatinine levels: 183 *vs*. 107 μmol/L, *P*=0.005), a significantly higher platelet count (28x10⁹/L *vs*. 15x10⁹/L; *P*=0.005) and a non-significantly higher mortality rate (n=6, 7.9% *vs*. n=1, 2.0%, respectively). ADAMTS13 antigen levels (Table 1, Figure 1A) were higher in uTTP patients (0.20 *vs*. 0.04 μg/mL, *P*=0.0007) although there was no influence of the associated clinical conditions (Figure 1B, C). Because a minimal antigen level of 0.03 μg/mL is mandatory to determine ADAMTS13 conformation,[11] 19 iTTP samples and 17 uTTP samples had to be excluded from the analysis of ADAMTS13 conformation. ADAMTS13 conformation was open in the majority of iTTP patients (23/30, 76.7%) and closed in the great majority of uTTP patients (51/59, 86.4%) (Table 1, Figure 1D). Additionally, the clinical condition associated with TTP had no influence on ADAMTS13 conformation, either in uTTP or in iTTP (Figure 1E, F).

The first aim of the current study was to investigate whether ADAMTS13 was closed or open in uTTP. Building on previous studies involving 102 patients with idiopathic iTTP found to be associated with an open ADAMTS13 conformation,[11-13] we here present original work focusing on 125 cases of non-idiopathic TTP, including both uTTP and iTTP. We confirm that uTTP and iTTP are distinct epidemiological, clinical and biological entities[6] and, for the first time, we show that uTTP is mostly associated with a closed ADAMTS13 conformation (Figure 2). In contrast, ADAMTS13 was open in the majority (76.7%) of our 30 patients with non-idiopathic iTTP. However, this 76,7% rate was surprisingly lower than the 92%-100% rate previously reported in 102 patients with idiopathic iTTP.[11-13] This difference may be explained by some heterogeneity of ADAMTS13 antibody epitopes as a function of the idiopathic or non-idiopathic presentation of TTP, the antibodies targeting the CS domains being likely the most efficient in maintaining an open ADAMTS13 conformation.[7,14,15]

With this study, we secondly investigated if pathological contexts commonly associated with non-idiopathic TTP could by themselves (independently of ADAMTS13 antibodies), convert ADAMTS13 from a closed to an open conformation. Our group of interest including 59 uTTP eligible for ADAMTS13 conformation testing exhibited a closed conformation in 86.4% of cases and thus showed that pathological contexts *per se* are not major triggers of ADAMTS13 opening. However, we surprisingly observed that some rare uTTP patients (8/59, 13.6%) did have an open ADAMTS13; most of them had infections or cancer (Figure 1E). Several mechanisms may be hypothesized to explain these exceptional cases:[7] firstly and most likely, ADAMTS13 antibodies (either IgG trapped in ADAMTS13/IgG immune complexes or IgM/IgA class antibodies) may be present *in vivo* but undetectable *in vitro* and these cases may correspond to under-

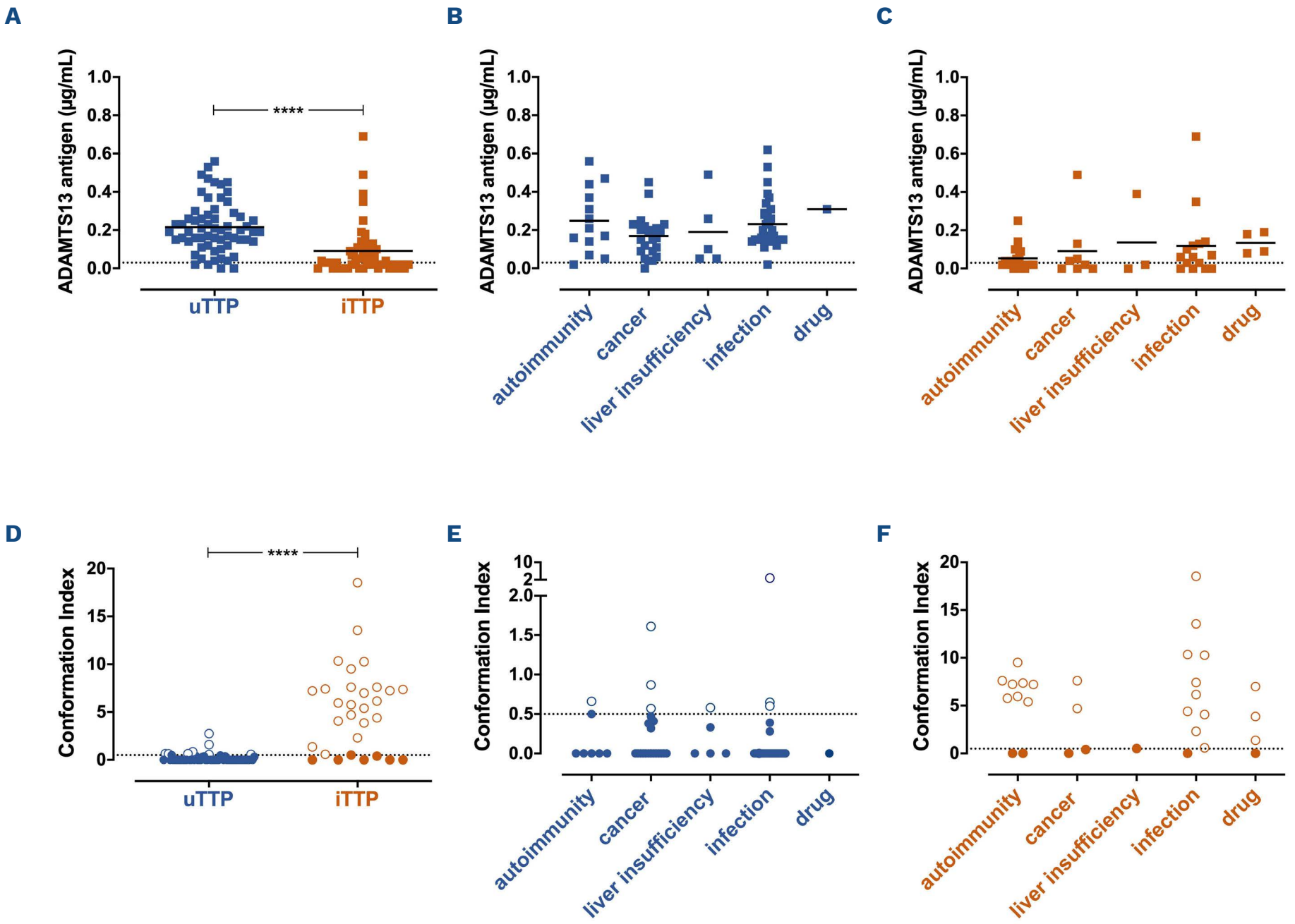

Figure 1. ADAMTS13 antigen levels and conformation in patients with non-idiopathic thrombotic thrombocytopenic purpura. (A-C) ADAMTS13 antigen levels in 125 patients with non-idiopathic thrombotic thrombocytopenic purpura (TTP). Each patient is represented by a dot. The continuous lines indicate mean values. The dotted lines represent the minimal ADAMTS13 antigen concentration (0.03 µg/mL) necessary to determine ADAMTS13 conformation. (A) ADAMTS13 antigen levels in cases of TTP of unidentified pathophysiology (uTTP; n=76) and immune-related TTP (iTTP; n=49). *P*<0.001 by *t*-test. (B) ADAMTS13 antigen levels in the uTTP samples associated with autoimmunity (n=13), cancer (n=24), liver insufficiency (n=5), infection (n=33) or drug treatment (n=1). (C) ADAMTS13 antigen levels in the iTTP samples associated with autoimmunity (n=19), cancer (n=8), liver insufficiency (n=3), infection (n=15) or drug treatment (n=4). (D-F) ADAMTS13 conformation in 89 patients with non-idiopathic TTP. A conformation index ≤0.5 corresponds to a closed ADAMTS13 (shown as ● in the figures), while a conformation index >0.5 corresponds to an open ADAMTS13 (shown as o in figures). The dotted line (conformation index 0.5) indicates the cut-off value between closed and open ADAMTS13. (D) ADAMTS13 conformation in uTTP (n=59) and iTTP (n=30) patients. *P*<0.0001 by Fisher test. (E) ADAMTS13 conformation in the uTTP samples associated with autoimmunity (n=7), cancer (n=21), liver insufficiency (n=5), infection (n=25), and drug treatment (n=1). (F) ADAMTS13 conformation in the iTTP samples associated with autoimmunity (n=10), cancer (n=4), liver insufficiency (n=1), infection (n=11) or drug treatment (n=4).

diagnosed iTTP; secondly, and less likely, hyper-elevated VWF levels, commonly observed during infections and cancers, may prolong the physiological opening of ADAMTS13; thirdly, post-translational modifications of ADAMTS13 structure induced by neutrophil-released substances during infections and inflammatory diseases (e.g., deglycosylation or citrullination/oxidation of CUB or M/S domain residues, respectively) may imbalance the stability of intra- and inter-domain interactions normally involved in the maintenance of a closed conformation.[7] These last two mechanisms remain very speculative (especially the role of hyper-elevated VWF levels) because, in the absence of any TTP context, ADAMTS13 conformation was found closed in patients with sepsis[11] or COVID-19 infection.[16]

To conclude, our study shows that TTP-associated triggers other than ADAMTS13 antibodies are poorly involved in the conversion of a closed to an open conformation of ADAMTS13. It also emphasizes the strong link between ADAMTS13 antibodies and an open ADAMTS13 conformation, even if the precise cause/consequence chronological process of this link remains unelucidated. This study also underlines that, besides the well-established congenital

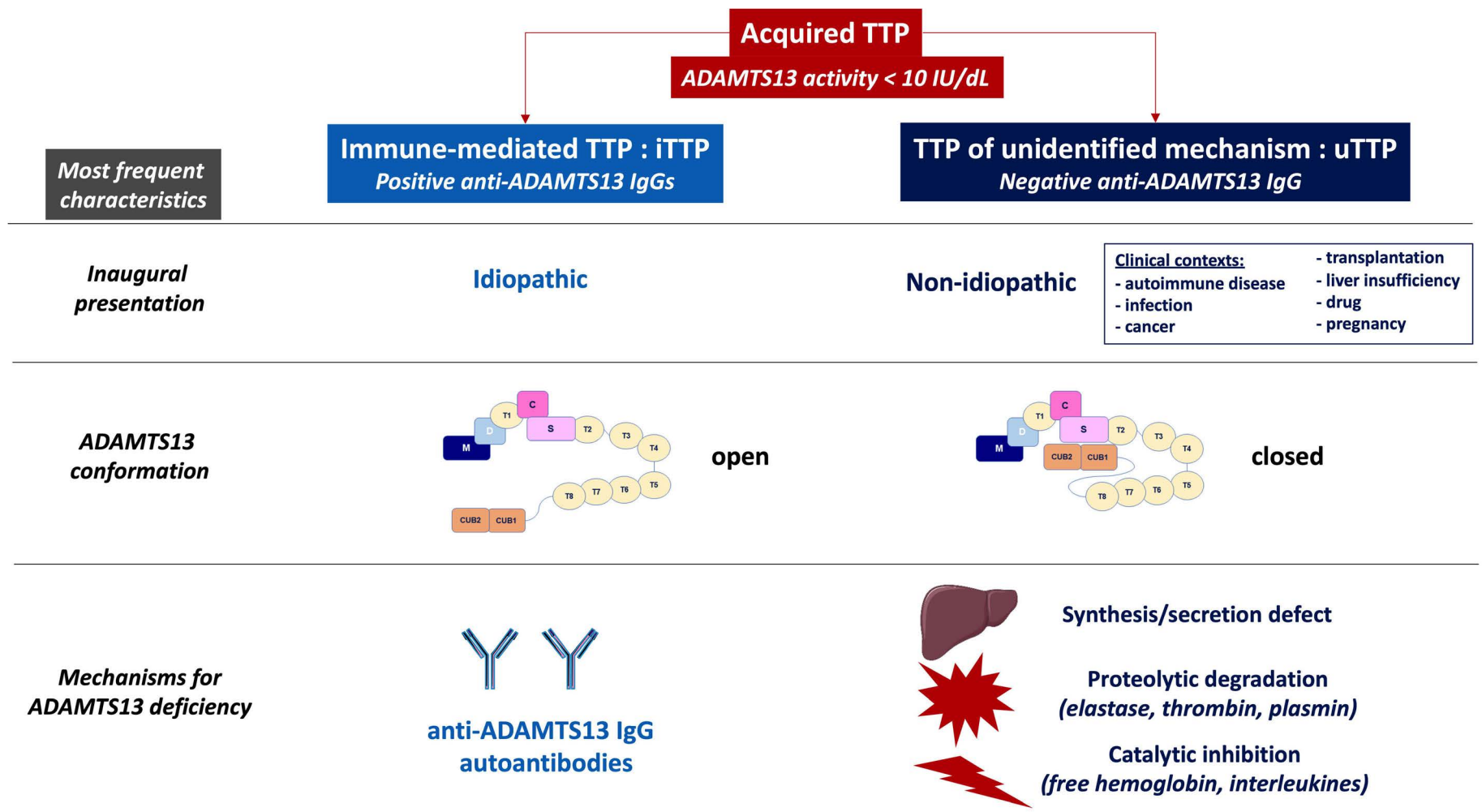

Figure 2. Pathophysiology and clinical presentation of the acquired forms of thrombotic thrombocytopenic purpura. ADAMTS13 is a multidomain protease, whose structure includes a metalloprotease domain (M), a disintegrin-like domain (D), eight thrombospondin type 1 repeats (T1-T8), a cysteine-rich domain (C), a spacer domain (S) and two CUB domains (CUB1, CUB2). Acquired severe deficiency of ADAMTS13 (activity <10 IU/dL) leading to the acquired forms of thrombotic thrombocytopenic purpura (TTP), may be either immune-mediated (iTTP) by anti-ADAMTS13 IgG autoantibodies or of unidentified pathophysiology (uTTP) when no anti-ADAMTS13 IgG autoantibodies can be detected. In most cases, iTTP is characterized by an idiopathic clinical presentation, an open ADAMTS13 conformation and ADAMTS13 IgG autoantibodies. In contrast, uTTP is associated with one or several clinical contexts at presentation, a closed ADAMTS13 conformation and miscellaneous speculated mechanisms for the severe deficiency of the ADAMTS13 (synthesis or secretion defect, proteolytic degradation, catalytic inhibition).

hereditary TTP and iTTP, there is a third form of TTP related to an acquired ADAMTS13 deficiency of unidentified mechanism (uTTP) in which ADAMTS13 IgG are not detectable and the ADAMTS13 conformation is closed. A wide international collaboration is needed to further define this entity as it may have important therapeutic implications.

Appendix

The members of the Reference Center for Thrombotic Microangiopathies (CNR-MAT) are: Augusto Jean-François (Service de Néphrologie, Dialyse et Transplantation; CHU Larrey, Angers); Azoulay Elie (Service de Réanimation Médicale, Hôpital Saint-Louis, Paris); Barbay Virginie (Laboratoire d'Hématologie, CHU Charles Nicolle, Rouen); Benhamou Ygal (Service de Médecine Interne, CHU Charles Nicolle, Rouen); Bouzid Raïda (Service d'Hématologie, Hôpital Saint-Antoine, Paris); Charasse Christophe (Service de Néphrologie, Centre Hospitalier de Saint-Brieuc); Charvet-Rumpler Anne (Service d'Hématologie, CHU de Dijon) ; Chauveau Dominique (Service de Néphrologie et Immunologie Clinique, CHU Rangueil, Toulouse); Choukroun Gabriel (Service de Néphrologie, Hôpital Sud, Amiens); Coindre Jean-Philippe (Service de Néphrologie, CH Le Mans); Coppo Paul (Service d'Hématologie, Hôpital Saint-Antoine, Paris); Corre Elise (Service d'Hématologie, Hôpital Saint-Antoine, Paris); Delmas Yahsou (Service de Néphrologie, CHU de Bordeaux); Jaccard Arnaud (Service d'Hématologie, Hôpital Dupuytren, Limoges); Kwon Theresa (Service de Néphrologie Pédiatrique, Hôpital Robert Debré, Paris); Dossier Antoine (Service de Néphrologie, Hôpital Bichat, Paris); Fain Olivier (Service de Médecine Interne, Hôpital Saint-Antoine, Paris); Frémeaux-Bacchi Véronique (Laboratoire d'Immunologie, Hôpital Européen Georges Pompidou, Paris); Galicier Lionel (Service d'Immunopathologie, Hôpital Saint-Louis, Paris); Grangé Steven (Service de Réanimation Médicale, CHU Charles Nicolle, Rouen); Guidet Bertrand (Service de Réanimation Médicale, Hôpital Saint-Antoine, Paris); Halimi Jean-Michel (Service de Néphrologie Pédiatrique, Hôpital Bretonneau, Tours); Hamidou Mohamed (Service de Médecine Interne, Hôtel-Dieu, Nantes); Herbrecht Raoul (Service d'Oncologie et d'Hématologie, Hôpital de Hautepierre, Strasbourg); Hié Miguel (Service de Médecine Interne, Groupe Hospitalier Pitié-Salpétrière, Paris); Jacobs Frédéric (Service de Réanimation Médicale, Hôpital Antoine Béclère, Clamart); Joly Bérangère (Service d'Hématologie Biologique, Hôpital

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Disclosures

AV and PC are members of the French advisory boards for caplacizumab (Sanofi) and for recombinant ADAMTS13 (Takeda). KV is a member of the advisory board of Takeda for recombinant ADAMTS13.

Contributions

BJ, ER, PC, KV and AV designed the research. BJ, AV and PC provided samples. ER performed experiments. BJ, ER, AV and KV analyzed data. BJ and ER wrote the manuscript. AV, KV and PC critically reviewed the manuscript. KV provided funding. All authors approved the final manuscript.

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Original data can be made available on reasonable request to the authors.

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