Received: 17 October 2023 | Revised: 9 November 2023 | Accepted: 16 November 2023

https://doi.org/10.1016/j.rpth.2023.102292

BRIEF REPORT

Hemophagocytic lymphohistiocytosis is associated with deficiency and closed conformation of ADAMTS-13

Amélie Launois[1,2] | Sandrine Valade[3] | Eric Mariotte[3] | Lionel Galicier[4] | Elie Azoulay[3] | Elien Roose[5] | Karen Vanhoorelbeke[5] | Agnès Veyradier[1,2] | Bérangère S. Joly[1,2]

[1]Service d'Hématologie biologique, Hôpital Lariboisière, Assistance Publique-Hôpitaux de Paris Nord, Université Paris Cité, Paris, France

[2]Equipe d'Accueil 3518, Institut de Recherche Saint-Louis, Hôpital Saint-Louis, Université Paris Cité, Paris, France

[3]Service de Réanimation médicale, Hôpital Saint-Louis, Assistance Publique-Hôpitaux de Paris Nord, Université Paris Cité, Paris, France

[4]Service d'Immunologie clinique, Hôpital Saint-Louis, Assistance Publique-Hôpitaux de Paris Nord, Université Paris Cité, Paris, France

[5]Laboratory for Thrombosis Research, Interdisciplinarity Research Facility Life Sciences, Katholieke Universiteit Leuven Campus Kulak Kortrijk, Kortrijk, Belgium

Correspondence
Bérangère S. Joly, Service d'Hématologie biologique, Hôpital Lariboisière, Assistance Publique-Hôpitaux de Paris Nord, Université Paris Cité, 2 Rue Ambroise Paré, 75010 Paris, France.
Email: berangere.joly@aphp.fr

Handling Editor: Prof Michael Makris

Abstract

Background: A disintegrin and metalloprotease with thrombospondin type 1 repeats, member 13 (ADAMTS-13) is the specific von Willebrand factor–cleaving protease and circulates in a closed and latent conformation due to a spacer/CUB1 domain interaction. ADAMTS-13 is allosterically activated after binding of its substrate or antibodies, inducing an open conformation. Recently, we suggested a potential role of plasmin (fibrinolysin) in hemostasis disorders reported in most patients with hemophagocytic lymphohistiocytosis (HLH), a rare and life-threatening condition related to a severe systemic inflammatory state. Most patients with HLH had a partial ADAMTS-13 deficiency, and plasmin could induce a truncation of the C-terminal part of ADAMTS-13 and thus an open conformation.

Objectives: To understand the effect of plasmin on ADAMTS-13, our study aimed to investigate ADAMTS-13 conformation in patients with HLH.

Methods: Forty-five critically ill patients with HLH were prospectively enrolled between April 2015 and December 2018. ADAMTS-13 activity was measured by fluorescent resonance energy transfer-VWF73 assay, ADAMTS-13 antigen, and conformation with our homemade 3H9-enzyme-linked immunosorbent assay and 1C4-enzyme-linked immunosorbent assay.

Results: ADAMTS-13 activity ranged from <10 to 65 IU/dL, and 41 of the 45 patients had a quantitative deficiency in ADAMTS-13 (activity <50 IU/dL). Twenty patients had a severe ADAMTS-13 deficiency (activity <20 IU/dL). ADAMTS-13 conformation was folded in all patients under normal conditions. Surprisingly, the switch of ADAMTS-13 conformation expected with the monoclonal antibody 17G2 (anti-CUB1) was disturbed in 6 patients (activity <20 IU/dL).

Conclusion: Our study reported that ADAMTS-13 conformation is closed in HLH and provides an indirect proof that plasmin is not able to massively degrade ADAMTS-13. Further studies on glycosylation and citrullination profiles of ADAMTS-13 are needed to understand their role in HLH.

KEYWORDS
ADAMTS-13 protein, fibrinolysin, hemophagocytic, hemophagocytic lymphohistiocytosis, lymphohistiocytosis, thrombocytopenia, thrombotic microangiopathies, von Willebrand factor

Essentials

- Severe hemophagocytic lymphohistiocytosis (HLH) is linked to coagulation disorders.
- ADAMTS-13 phenotype, including its conformation, was investigated in 45 patients with severe HLH.
- ADAMTS-13 conformation is closed in HLH, and plasmin is not able to massively degrade ADAMTS-13.
- Our data support an open conformation of ADAMTS-13 linked to anti–ADAMTS-13 autoantibodies.

1 | INTRODUCTION

A disintegrin and metalloprotease with thrombospondin type 1 repeats, member 13 (ADAMTS-13) is a blood enzyme that controls the hemostatic power of its substrate von Willebrand factor (VWF), a large multimeric glycoprotein crucial for platelet adhesion and aggregation at high shear rates of blood flow [1,2]. Hence, by cleaving VWF ultralarge multimers, ADAMTS-13 prevents the spontaneous binding of VWF to platelets and, consequently, the formation of platelet-rich microthrombi in the microvasculature. ADAMTS-13 is a multidomain heavily glycosylated protein consisting of a metalloprotease domain, a disintegrin-like domain, a first thrombospondin type-1 (TSP-1) repeat, a cysteine-rich domain, a spacer domain, 7 additional TSP-1 repeats, and 2 CUB domains [1,2]. Under physiological conditions, ADAMTS-13 circulates in a closed and latent conformation due to a self-interaction between its spacer and CUB1 domains [3–6]. When needed, ADAMTS-13 is allosterically activated after binding to the D4-CK domains of VWF, inducing a transitory physiological open conformation in which the spacer/CUB1 interaction is abrogated and a cryptic epitope located within ADAMTS-13 spacer domain is consequently exposed [3–6]. The normal range for ADAMTS-13 activity is >50 IU/dL [7].

ADAMTS-13 functional deficiencies of various degrees are associated with miscellaneous diseases. A severe functional deficiency of ADAMTS-13 (activity <10 IU/dL) is the cause of a unique and rare thrombotic microangiopathy (TMA) named thrombotic thrombocytopenic purpura (TTP) [7–9]. Because of the presence of platelet-rich microthrombi occluding the microvessels, patients with TTP suffer from hemolytic anemia, profound thrombocytopenia, and multivisceral ischemia [7–9]. In TTP, severe ADAMTS-13 deficiency is mostly due (∼80% of all TTP cases) to immunoglobulin G (IgG) autoantibodies against ADAMTS-13 causing immune TTP (iTTP), very rarely (∼5% of all TTP cases) due to *ADAMTS13* gene mutations causing congenital TTP, and sometimes (∼15% of all TTP cases) due to still ununderstood mechanisms causing TTP of unidentified pathophysiology (uTTP) [10,11]. Partial functional deficiencies of ADAMTS-13 (activity ranging from 11 to 49 IU/dL) with no clinical relevance are described as a bystander of many pathological conditions such as sepsis [12], disseminated intravascular coagulation (DIC) [13], COVID-19 infection [14], or hemophagocytic lymphohistiocytosis (HLH) [15]. In some cases, TMA syndrome complicates these conditions; however, there is no causal link with the partial ADAMTS-13 defect [12–18].

Beyond ADAMTS-13 activity, ADAMTS-13 conformation was recently shown to be an exciting biological marker for TTP. The distinction between the closed and open conformation of ADAMTS-13 in patients' plasma samples is performed thanks to an original enzyme-linked immunosorbent assay (ELISA), in which the capturing antibody is directed against a cryptic epitope of the spacer domain of ADAMTS-13 [19]. An open conformation of ADAMTS-13, very likely linked to the presence of ADAMTS-13 autoantibodies, was demonstrated to be a hallmark of both acute iTTP [19] and subclinical iTTP [20]. The strong link between ADAMTS-13 autoantibodies and the open conformation was corroborated by a closed conformation of ADAMTS-13 in congenital TTP [21], uTTP [11], or other conditions like sepsis [19] and COVID-19 infections [14].

In a recent study devoted to an exhaustive investigation of hemostasis in 45 critically ill patients with secondary HLH, a rare condition in relation with defects in cytotoxic lymphocytes, resulting in macrophage activation and a cytokine storm [22], we found a partial deficiency of ADAMTS-13 in 91% of cases (among which half of the patients had ADAMTS-13 activity levels <20 IU/dL) associated with decreased ADAMTS-13 antigen levels but not anti–ADAMTS-13 IgG antibodies [15]. None of our patients with HLH exhibited TMA syndrome, which underlines the absence of clinical relevance of ADAMTS-13 deficiency in this context [15]. However, the mechanism inducing ADAMTS-13 deficiency in HLH remains unexplained. As major hyperfibrinolysis was also found in almost all these patients, we suggested a potential role of plasmin in the degradation of ADAMTS-13, inducing its partial quantitative deficiency in HLH [15]. Indeed, ADAMTS-13 is established to be cleaved by plasmin at several sites, including 2 peptidyl bonds located after the spacer domain [23,24]. In HLH, we hypothesize that ADAMTS-13 degradation by plasmin could induce a truncation of its C-terminal part and, consequently, exposure of its spacer domain and, per se, an open conformation. To test this hypothesis, the current study aimed to investigate our patients with HLH by studying ADAMTS-13 conformation. An open conformation would imply that ADAMTS-13 is massively truncated by plasmin in HLH and, thus, that anti–ADAMTS-13 autoantibodies are not the only

factor able to lead to the exposure of the cryptic epitope of the spacer domain.

2 | METHODS

Patients were prospectively enrolled in Saint-Louis Hospital, Paris, France (inclusion period April 01, 2015, to December 31, 2018), if they met the following inclusion criteria: age >18 years, diagnosis of HLH [22], available medical records, citrated plasma samples, and informed consent [15]. The project (number 15-008) was approved by the Institutional Review Board (00006477v) of Hôpitaux Universitaires Paris Nord, Val-de-Seine, Paris 7 University, Assistance Publique - Hôpitaux de Paris.

ADAMTS-13 activity was measured using our homemade fluorescent resonance energy transfer-VWF73 assay (normal range, 50-150 IU/dL) [10]. Murine monoclonal anti–ADAMTS-13 antibodies (mAb) 3H9, 1C4, and opening 17G2 are directed against the metalloprotease, spacer, and CUB1 domains of human ADAMTS-13, respectively. ADAMTS-13 antigen (normal range, 0.930-1.350 μg/mL) and conformation were assessed by our homemade 3H9- and 1C4-based ELISA, as previously described [19]. ADAMTS-13 conformation was tested with or without the opening mAb 17G2, and a conformation index of >0.5 was defined as open ADAMTS-13.

Quantitative parameters were reported as median and IQR. Continuous variables were compared with the nonparametric Kruskal–Wallis test as appropriate (GraphPad Prism version 8.4.3). Statistical significance was set at $P < .05$.

3 | RESULTS AND DISCUSSION

Forty-five patients with HLH were included in the current study. Oncohematological malignancies (n = 35; mostly lymphomas), infections (n = 8), or autoimmune diseases (n = 2) were potential underlying triggers of HLH (Table). At diagnosis, median age was 53 ± 15 years (range, 27-77 years), and sex ratio (male-to-female) was 1.6. Median hemoglobin level was 7.9 g/dL (IQR, 6.9-8.7 g/dL), platelet count was 46 × 10^9/L (IQR, 22-65 × 10^9/L), and fibrinogen level was 2.71 g/L (IQR, 1.63-5.52 g/L) (Table). More detailed clinical and biological parameters have been reported previously [15].

In this study, we first investigated ADAMTS-13 phenotype. ADAMTS-13 activity ranged from <10 to 65 IU/dL, without any significant difference between the miscellaneous clinical contexts (P = .8617, not significant) (Figure 1A, Table). Interestingly, although none of our patients with HLH exhibited any symptoms of TMA, 41 (91%) had a quantitative deficiency in ADAMTS-13 (activity <50 IU/dL), including 20 (44%) with an activity lower than 20 IU/dL (Table). Interestingly, 7 (16%) patients even exhibited an ADAMTS-13 activity lower than 10 IU/dL, with a context of either oncohematological malignancy (6 patients) or infectious trigger (1 patient) (Table). Four of them had bilirubin levels over 100 μmol/L, potentially underestimating the ADAMTS-13 activity. Bilirubin in hyperbilirubinemic plasma samples may interfere with the fluorescent resonance energy transfer-VWF73 assay by acting as a quencher and decreasing ADAMTS-13 activity [25]. Two of them also had elevated alanine aminotransferase levels (levels exceeding 4 times the normal range), suggesting a deficiency in both synthesis and secretion of the protease. However, decreased ADAMTS-13 antigen levels were detectable in all patients (median, 0.415 μg/mL; IQR, 0.244-0.573 μg/mL), allowing the study of ADAMTS-13 conformation for all 45 patients in a second step (Table). In all patients, ADAMTS-13 conformation was closed under normal conditions (-mAb 17G2, conformation index <0.5) (Table, Figure 1B). Surprisingly, the switch of ADAMTS-13 conformation (from closed to open) expected after addition of mAb 17G2 was disturbed in 6 (13%) patients (4 patients with oncohematological malignancy and 2 patients with severe infection) (Figure 1C). Indeed, the activating mAb 17G2 was not able to open ADAMTS-13 conformation in the 6 patients whose common features were an ADAMTS-13 activity lower than 20 IU/dL and an ADAMTS-13 antigen lower than 0.25 μg/mL (Table).

As previously reported in other severe clinical conditions like sepsis/septic shock, DIC, or COVID-19 infection [12,14], we found that important quantitative defects of ADAMTS-13 also exist in HLH. In our patients, however, these defects were unable to induce any general TMA syndrome or specific TTP symptoms, in contrast to what can be observed in some extreme cases of septic shock complicated by DIC and TMA [12,13]. Hence, the latter complex clinical conditions may sometimes be complicated either by a TTP-like syndrome, an endotheliopathy with microvascular thrombosis usually occurring in critically ill patients with severe comorbidities (pathogen, cancer, transplant, or trauma) and associated with subnormal or normal ADAMTS-13 activities [26] or by uTTP associated with an ADAMTS-13 activity lower than 10 IU/dL and no anti–ADAMTS-13 IgG antibodies [11]. While none of our 45 patients with HLH had TMA, the potential association between HLH and TMA warrants consideration in some patients with HLH who develop disproportionate anemia, thrombocytopenia, and lactate dehydrogenase increase or have multiorgan failure [18]. More specifically, Minoia et al. [16] have recently suggested the possible coexistence of HLH and TMA in a cohort of 23 children with rheumatic diseases, while Steffen et al. [17] have reported a case of simultaneous HLH and TMA syndrome after parvovirus B19 infection in a kidney transplant recipient.

In spite of the absence of clinical relevance of ADAMTS-13 deficiency in our patients with HLH, elucidating the mechanism explaining ADAMTS-13 defect in such a rare and specific clinical condition remains of interest. Because hyperfibrinolysis was the main hemostatic abnormality observed in our patients with HLH [15], it made sense to focus our study on ADAMTS-13 degradation by plasmin. It is established that plasmin is able to cleave 3 peptidyl bonds in ADAMTS-13 between Arg257-Ala258 (metalloprotease domain), Arg888-Thr889, and Arg1176-Arg1177 (TSP-1 domains), resulting in a loss of ADAMTS-13 activity (Figure 2) [23,24]. Intramolecular disulfide bridges ensure covalent linkage of the fragments generated by cleavage at Arg257-Ala258 and Arg888-Thr889, while cleavage at Arg1176-Arg1177 results in removal of the CUB domains

TABLE Clinical and biological features of 45 patients with hemophagocytic lymphohistiocytosis.

Patient	Clinical context	Platelet count ($\times 10^9$/L)	Fibrinogen (g/L)	ADAMTS-13 activity (IU/dL)	ADAMTS-13 antigen (µg/mL)	CI (−17G2)	CI (+17G2)
HLH-01	Malignancy	60	3.70	54	0.625	<0.5	1.04
HLH-02	Infection	82	0.60	65	0.692	<0.5	1.11
HLH-03	Malignancy	56	3.55	48	0.654	<0.5	0.96
HLH-04	Malignancy	55	0.99	33	0.449	<0.5	1.00
HLH-05	Infection	58	7.32	11	0.197	<0.5	1.37
HLH-06	Malignancy	6	6.83	8	0.081	<0.5	<0.5
HLH-07	Malignancy	81	2.34	12	0.185	<0.5	0.92
HLH-08	Malignancy	12	4.45	25	0.346	<0.5	1.28
HLH-09	Malignancy	81	7.49	14	0.200	<0.5	0.67
HLH-10	Infection	32	5.64	11	0.202	<0.5	<0.5
HLH-11	Malignancy	43	3.21	9	0.171	<0.5	0.98
HLH-12	Malignancy	19	5.52	9	0.178	<0.5	0.70
HLH-13	Malignancy	27	2.49	38	0.560	<0.5	0.86
HLH-14	Malignancy	63	1.89	3	0.178	<0.5	0.55
HLH-15	Infection	35	3.12	5	0.167	<0.5	<0.5
HLH-16	Malignancy	73	1.10	30	0.461	<0.5	0.69
HLH-17	Malignancy	19	6.65	17	0.244	<0.5	<0.5
HLH-18	AI disease	115	1.86	57	0.723	<0.5	0.98
HLH-19	Malignancy	22	1.91	33	0.545	<0.5	0.75
HLH-20	Malignancy	53	4.83	21	0.370	<0.5	0.76
HLH-21	Malignancy	47	2.70	38	0.629	<0.5	0.69
HLH-22	Infection	74	1.75	26	0.421	<0.5	0.76
HLH-23	Malignancy	50	6.50	39	0.637	<0.5	0.69
HLH-24	Malignancy	21	2.14	29	0.455	<0.5	0.56
HLH-25	Malignancy	57	1.30	51	0.913	<0.5	0.72
HLH-26	Malignancy	18	7.03	22	0.321	<0.5	0.69
HLH-27	Malignancy	17	5.38	4	0.244	<0.5	<0.5
HLH-28	Malignancy	34	8.58	41	0.967	<0.5	0.96
HLH-29	Malignancy	16	3.74	22	0.490	<0.5	0.71
HLH-30	Malignancy	54	1.63	27	0.573	<0.5	0.97
HLH-31	Malignancy	2	1.12	28	0.600	<0.5	0.95
HLH-32	Infection	66	1.60	38	0.581	<0.5	1.10
HLH-33	Infection	39	1.58	19	0.415	<0.5	1.10
HLH-34	Malignancy	168	5.95	31	0.692	<0.5	0.90
HLH-35	Malignancy	10	2.71	16	0.372	<0.5	0.79
HLH-36	AI disease	28	0.80	10	0.271	<0.5	0.93
HLH-37	Malignancy	22	1.20	24	0.549	<0.5	0.89
HLH-38	Malignancy	44	0.99	10	0.277	<0.5	1.02
HLH-39	Malignancy	74	5.87	25	0.463	<0.5	1.14

(Continues)

TABLE (Continued)

Patient	Clinical context	Platelet count (× 10^9/L)	Fibrinogen (g/L)	ADAMTS-13 activity (IU/dL)	ADAMTS-13 antigen (µg/mL)	CI (−17G2)	CI (+17G2)
HLH-40	Malignancy	81	1.75	16	0.409	<0.5	1.19
HLH-41	Malignancy	29	1.59	32	0.489	<0.5	1.06
HLH-42	Malignancy	198	2.03	13	0.262	<0.5	1.05
HLH-43	Malignancy	44	2.94	5	0.143	<0.5	<0.5
HLH-44	Malignancy	46	6.15	12	0.371	<0.5	0.89
HLH-45	Infection	65	2.93	14	0.225	<0.5	1.04

AI, autoimmune; CI, conformation index; HLH, hemophagocytic lymphohistiocytosis; Malignancy, oncohematological malignancy.

[27]. Our hypothesis was that removal of the CUB domains after proteolytic inactivation of ADAMTS-13 by plasmin resulted in abrogating the spacer/CUB1 interaction and allowing the exposure of the cryptic epitope of the spacer domain. This sequence should theoretically lead to the recognition of this cryptic epitope by the mAb 1C4, translated as an open ADAMTS-13 conformation by ELISA. In contrast, all 45 patients with HLH exhibited a closed ADAMTS-13 conformation at baseline (before addition of the opening mAb 17G2), suggesting that the CUB domains had not been truncated. Another option is that a conformational change occurred in the spacer domain (after truncation of the CUB domains or another effect induced by plasmin) and modified the cryptic epitope, making it unrecognizable by the mAb 1C4. As ADAMTS-13 conformation was able to switch open after addition of mAb 17G2 (anti-CUB1 domain) in 39 of the 45 patients with HLH, the hypothesis of truncation of the C-terminal part of ADAMTS-13 by plasmin finally remains unlikely. Here, we suggest that the reduced activity of ADAMTS-13 observed in the patients with HLH is due to changes in the glycosylation or

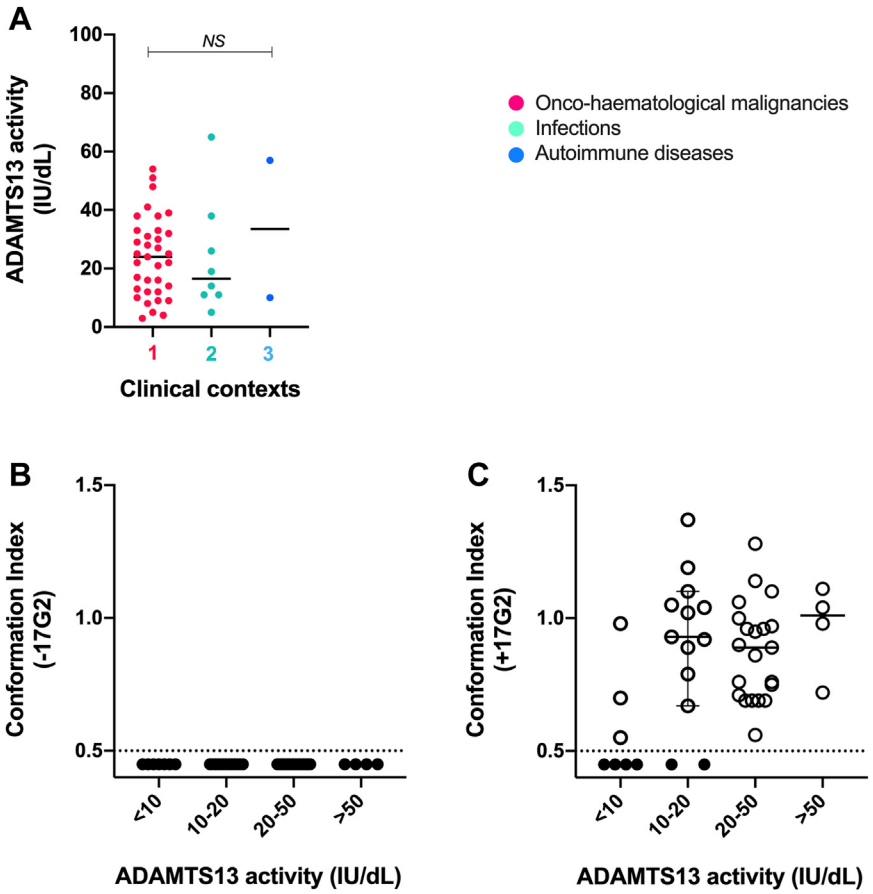

FIGURE 1 Investigations of ADAMTS-13 activity and conformation (without/with monoclonal antibody 17G2) in 45 patients with hemophagocytic lymphohistiocytosis. Each patient is represented by a dot. The continuous lines indicate median values. All parameters were measured in plasma samples of 45 patients with hemophagocytic lymphohistiocytosis (35 oncohematological malignancies, 8 infections, and 2 autoimmune diseases). (A) ADAMTS-13 activity was measured using the reference method fluorescent resonance energy transfer-VWF73. No significant differences were reported between groups. (B and C) ADAMTS-13 conformation was performed using 1C4-ELISA (B) without or (C) with the activating murine monoclonal anti–ADAMTS-13 antibody 17G2. A conformation index <0.5 corresponds to a closed ADAMTS-13 (shown as ● in the figures), while a conformation index >0.5 corresponds to an open ADAMTS-13 (shown as ○ in figures). The dotted line (conformation index = 0.5) indicates the cutoff value between closed and open ADAMTS-13. Hemophagocytic lymphohistiocytosis is associated with a folded ADAMTS-13 conformation under normal conditions (−17G2, conformation index <0.5). Surprisingly, ADAMTS-13 conformation remained folded after addition of the activating 17G2 in 6 patients.

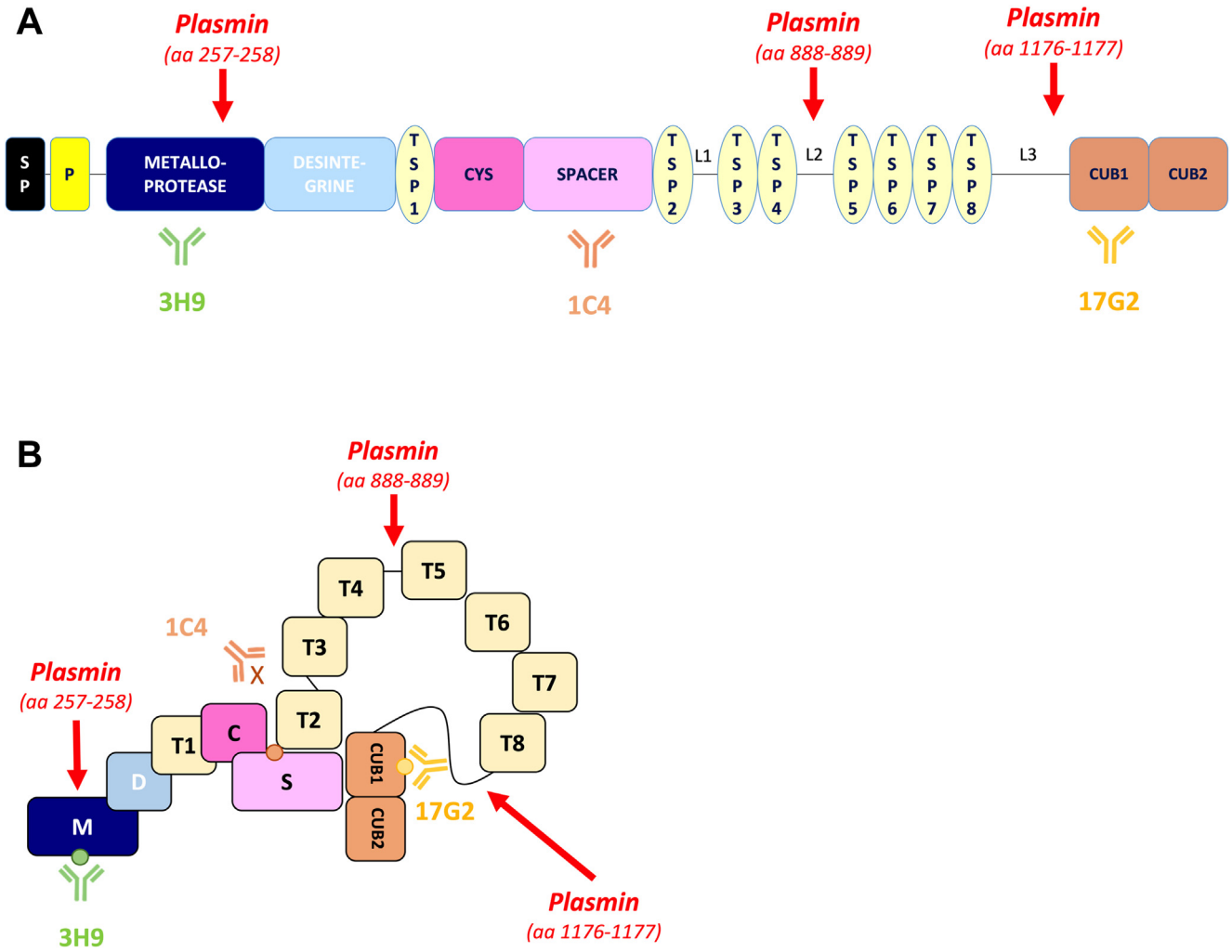

FIGURE 2 Cleavage sites of ADAMTS-13 by plasmin and targets of murine monoclonal anti–ADAMTS-13 antibodies: (A) unfolded and open ADAMTS-13, (B) folded and closed ADAMTS-13. ADAMTS-13 consists of several domains: a metalloprotease domain (M), a disintegrin-like domain (D), a first thrombospondin type (TSP)-1 repeat (T1), a cysteine-rich domain (C), a spacer domain (S), 7 additional thrombospondin type-1 repeats (T2-T8), and 2 CUB domains. The C-terminal part of ADAMTS-13 also contains 3 linker regions located between T2 and T3, T4 and T5, T8 and CUB1, which introduce a lot of flexibility in the protease. ADAMTS-13 normally circulates in a closed conformation where spacer and T8-CUB2 domains interact. The fibrinolytic enzyme plasmin is able to cleave 3 peptidyl bonds in ADAMTS-13 between Arg257-Ala258, Arg888-Thr889, and Arg1176-Arg117. Murine monoclonal anti–ADAMTS-13 antibodies 3H9, 1C4, and 17G2 are directed against the M, spacer (cryptic epitope accessible only in the open conformation), and CUB1 domains of ADAMTS-13, respectively.

citrullination profile of ADAMTS-13. First, glycosylation plays an important role in many processes, such as protein folding, function, or clearance. ADAMTS-13 is heavily glycosylated, and glycosylation of TSP1-2, TSP1-4, and CUB domains is a known modulator of ADAMTS-13 conformation [28]. Second, during citrullination, positively charged arginine residues are deiminated by peptidylarginine deiminase type IV to generate neutral citrulline [29,30]. Peptidylarginine deiminase type IV is known to reduce and modulate ADAMTS-13 activity [29,30]. Surprisingly, in 6 of the 45 patients, however, mAb 17G2 was unable to induce an opening of ADAMTS-13. The epitope structure of mAb 17G2 and the mechanism by which it regulates ADAMTS-13 activity are still unknown [19]. To understand why 17G2 was not able to open ADAMTS-13 conformation in 6 patients with HLH, we suggest either changes in the glycosylation or citrullination profile of ADAMTS-13 or a polymorphism in the 17G2 epitope in the CUB1 domain that could abrogate binding of 17G2. Thus, these biochemical and structural changes of plasma ADAMTS-13 could modify the epitope of the 17G2 and contribute to the interaction of ADAMTS-13 spacer/CUB domains. In order to demonstrate the involvement of glycosylation and citrullination in our 6 patients with a closed conformation of ADAMTS-13 despite the addition of mAb17G2, we are currently identifying the epitope of mAb17G2.

This study has 2 main strengths: the large number of patients with HLH enrolled and the innovative exploration of ADAMTS-13 conformation. However, some limitations of this study are based on the selection of patients who had mainly an oncohematological malignancy associated with HLH due to Saint-Louis Hospital specificity and on plasma sampling limited at the time of enrollment.

4 | CONCLUSION

To conclude, using the original tool of ADAMTS-13 conformation ELISA, our study shows that ADAMTS-13 conformation is mostly closed in HLH. First, this observation provides indirect proof that plasmin is unlikely to massively degrade ADAMTS-13 in HLH despite the important hyperfibrinolytic process present in this condition. Second, hypothetic HLH-induced change in the glycosylation/citrullination profile of ADAMTS-13 may modify ADAMTS-13 metabolism (reduced activity, defective synthesis, consumption by VWF, and accelerated clearance, among others) and be responsible for quantitative deficiency. Further investigations of the ADAMTS-13 glycosylation profile and citrullination are warranted to clarify their role in HLH-related ADAMTS-13 deficiency and to potentially identify new therapeutic targets. Last but not least, this study reinforces the position of ADAMTS-13 open conformation as a witness of the presence of ADAMTS-13 autoantibodies and as an early and pathognomonic marker for iTTP [31].

ACKNOWLEDGMENTS

The authors thank Sandrine Benghezal, Sylvaine Savigny, and Sophie Capdenat for expert technical assistance. The authors thank Dr Issa Kalidi for the collection of plasma samples.

FUNDING

The authors received no funding for this study.

ETHICS STATEMENT

The project (number 15-008) was approved by the Institutional Review Board (00006477v) of Hôpitaux Universitaires Paris Nord, Val-de-Seine, Paris 7 University, Assistance Publique - Hôpitaux de Paris. Patients were prospectively enrolled if they provided informed consent.

AUTHOR CONTRIBUTIONS

A.L. analyzed and interpreted data and critically reviewed the manuscript. B.S.J. and A.V. supervised research, analyzed data, and wrote the manuscript. S.V., E.M., L.G., and E.A. enrolled patients, collected clinical and laboratory information, and critically reviewed the manuscript. E.R. did the phenotypic analysis, interpreted data, and critically reviewed the manuscript for scientific content. K.V. provided monoclonal antibodies, analyzed data, and reviewed the manuscript for scientific content. The final version of the manuscript was read and approved by all authors.

RELATIONSHIP DISCLOSURE

E.A. reports grants or contracts from Merck Sharp & Dohme Avenir Foundation (money to St. Louis Hospital) and Alexion IIR on long term outcomes in patients with thrombotic microangiopathy (money to St. Louis Hospital) and payment or honoraria for lectures from Alexion (2021-2023), Sanofi (2021-2022), and Mindray (2023). A.V. is a member of the French advisory boards for Sanofi and Takeda and received speaker fees from Sanofi, Octapharma, Laboratoire Français du Fractionnement et des Biotechnologies-Biomédicaments, and Takeda. B.S.J. received speaker fees for lectures and presentations from Laboratoire Français du Fractionnement et des Biotechnologies-Biomédicaments, Sanofi, and Takeda; support for attending congresses from Sanofi and Takeda; and travel support from Takeda. The other authors declare no conflict of interest.

REFERENCES

[1] Zheng X, Chung D, Takayama TK, Majerus EM, Sadler JE, Fujikawa K. Structure of von Willebrand factor-cleaving protease (ADAMTS13), a metalloprotease involved in thrombotic thrombocytopenic purpura. *J Biol Chem*. 2001;276:41059–63.

[2] Levy GG, Nichols WC, Lian EC, Foroud T, McClintick JN, McGee BM, et al. Mutations in a member of the ADAMTS gene family cause thrombotic thrombocytopenic purpura. *Nature*. 2001;413:488–94.

[3] South K, Luken BM, Crawley JTB, Phillips R, Thomas M, Collins RF, et al. Conformational activation of ADAMTS13. *Proc Natl Acad Sci U S A*. 2014;111:18578–83.

[4] Muia J, Zhu J, Gupta G, Haberichter SL, Friedman KD, Feys HB, et al. Allosteric activation of ADAMTS13 by von Willebrand factor. *Proc Natl Acad Sci U S A*. 2014;111:18584–9.

[5] Petri A, Kim HJ, Xu Y, de Groot R, Li C, Vandenbulcke A, et al. Crystal structure and substrate-induced activation of ADAMTS13. *Nat Commun*. 2019;10:3781. https://doi.org/10.1038/s41467-019-11474-5

[6] Kim HJ, Xu Y, Petri A, Vanhoorelbeke K, Crawley JTB, Emsley J. Crystal structure of ADAMTS13 CUB domains reveals their role in global latency. *Sci Adv*. 2021;7:eabg4403. https://doi.org/10.1126/sciadv.abg4403

[7] Joly BS, Coppo P, Veyradier A. Thrombotic thrombocytopenic purpura. *Blood*. 2017;129:2836–46.

[8] Furlan M, Robles R, Galbusera M, Remuzzi G, Kyrle PA, Brenner B, et al. von Willebrand factor-cleaving protease in thrombotic thrombocytopenic purpura and the hemolytic-uremic syndrome. *N Engl J Med*. 1998;339:1578–84.

[9] Tsai HM, Lian EC. Antibodies to von Willebrand factor-cleaving protease in acute thrombotic thrombocytopenic purpura. *N Engl J Med*. 1998;339:1585–94.

[10] Mariotte E, Azoulay E, Galicier L, Rondeau E, Zouiti F, Boisseau P, 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:e237–45.

[11] 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;108:638–44.

[12] Peigne V, Azoulay E, Coquet I, Mariotte E, Darmon M, Legendre P, et al. The prognostic value of ADAMTS13 (a disintegrin and metalloprotease with thrombospondin type 1 repeats, member 13) deficiency in septic shock patients involves interleukin-6 and is not dependent on disseminated intravascular coagulation. *Crit Care*. 2013;17:R273. https://doi.org/10.1186/cc13115

[13] Levi M, Scully M, Singer M. The role of ADAMTS-13 in the coagulopathy of sepsis. *J Thromb Haemost*. 2018;16:646–51.

[14] Joly BS, Darmon M, Dekimpe C, Dupont T, Dumas G, Yvin E, et al. Imbalance of von Willebrand factor and ADAMTS13 axis is rather a biomarker of strong inflammation and endothelial damage than a cause of thrombotic process in critically ill COVID-19 patients. *J Thromb Haemost*. 2021;19:2193–8.

[15] Valade S, Joly BS, Veyradier A, Fadlallah J, Zafrani L, Lemiale V, et al. Coagulation disorders in patients with severe hemophagocytic lymphohistiocytosis. *PLoS One*. 2021;16:e0251216. https://doi.org/10.1371/journal.pone.0251216

[16] Minoia F, Tibaldi J, Muratore V, Gallizzi R, Bracaglia C, Arduini A, et al. Thrombotic microangiopathy associated with macrophage activation syndrome: a multinational study of 23 patients. *J Pediatr*. 2021;235:196–202.

[17] Steffen CJ, Koch N, Eckardt KU, Amann K, Seelow E, Schreiber A. Hemophagocytic lymphohistiocytosis and thrombotic microangiopathy after parvovirus B19 infection and renal transplantation: a case report. *BMC Nephrol*. 2021;22:337. https://doi.org/10.1186/s12882-021-02538-0

[18] Gloude NJ, Dandoy CE, Davies SM, Myers KC, Jordan MB, Marsh RA, et al. Thinking beyond HLH: clinical features of patients with concurrent presentation of hemophagocytic lymphohistiocytosis and thrombotic microangiopathy. *J Clin Immunol*. 2020;40:699–707.

[19] Roose E, Schelpe AS, Joly BS, Peetermans M, Verhamme P, Voorberg J, et al. An open conformation of ADAMTS-13 is a hallmark of acute acquired thrombotic thrombocytopenic purpura. *J Thromb Haemost*. 2018;16:378–88.

[20] Roose E, Schelpe AS, Tellier E, Sinkovits G, Joly BS, Dekimpe C, et al. Open ADAMTS13, induced by antibodies, is a biomarker for subclinical immune-mediated thrombotic thrombocytopenic purpura. *Blood*. 2020;136:353–61.

[21] Joly BS, Boisseau P, Roose E, Stepanian A, Biebuyck N, Hogan J, et al. ADAMTS13 gene mutations influence ADAMTS13 conformation and disease age-onset in the French cohort of Upshaw-Schulman Syndrome. *Thromb Haemost*. 2018;118:1902–17.

[22] Henter JI, Horne A, Aricó M, Egeler RM, Filipovich AH, Imashuku S, et al. HLH-2004: diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. *Pediatr Blood Cancer*. 2007;48:124–31.

[23] Shin Y, Miyake H, Togashi K, Hiratsuka R, Endou-Ohnishi K, Imamura Y. Proteolytic inactivation of ADAMTS13 by plasmin in human plasma: risk of thrombotic thrombocytopenic purpura. *J Biochem*. 2018;163:381–9.

[24] Clark CC, Mebius MM, de Maat S, Tielens AGM, de Groot PG, Urbanus RT, et al. Truncation of ADAMTS13 by plasmin enhances its activity in plasma. *Thromb Haemost*. 2018;118:471–9.

[25] Meyer SC, Sulzer I, Lämmle B, Kremer Hovinga JA. Hyperbilirubinemia interferes with ADAMTS-13 activity measurement by FRETS-VWF73 assay: diagnostic relevance in patients suffering from acute thrombotic microangiopathies. *J Thromb Haemost*. 2007;5:866–7.

[26] Chang JC. TTP-like syndrome: novel concept and molecular pathogenesis of endotheliopathy-associated vascular microthrombotic disease. *Thromb J*. 2018;16:20. https://doi.org/10.1186/s12959-018-0174-4

[27] Feys HB, Vandeputte N, Palla R, Peyvandi F, Peerlinck K, Deckmyn H, et al. Inactivation of ADAMTS13 by plasmin as a potential cause of thrombotic thrombocytopenic purpura. *J Thromb Haemost*. 2010;8:2053–62.

[28] Nowak AA, O'Brien HER, Henne P, Doerr A, Vanhoorelbeke K, Laffan MA, et al. ADAMTS-13 glycans and conformation-dependent activity. *J Thromb Haemost*. 2017;15:1155–66.

[29] Sorvillo N, Mizurini DM, Coxon C, Martinod K, Tilvawala R, Cherpokova D, et al. Plasma peptidylarginine deiminase IV promotes VWF-platelet string formation and accelerates thrombosis after vessel injury. *Circ Res*. 2019;125:507–19.

[30] Ercig B, Arfman T, Hrdinova J, Wichapong K, Reutelingsperger CPM, Vanhoorelbeke K, et al. Conformational plasticity of ADAMTS13 in hemostasis and autoimmunity. *J Biol Chem*. 2021;297:101132. https://doi.org/10.1016/j.jbc.2021.101132

[31] Halkidis K, Zheng XL. ADAMTS13 conformations and mechanism of inhibition in immune thrombotic thrombocytopenic purpura. *J Thromb Haemost*. 2022;20:2197–203.