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ORIGINAL ARTICLE

Autoantibodies enhance ADAMTS-13 clearance in patients with immune thrombotic thrombocytopenic purpura

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Abstract

Background: Severe deficiency in ADAMTS-13 (<10%) and the loss of von Willebrand factor–cleaving function can precipitate microvascular thrombosis associated with thrombotic thrombocytopenic purpura (TTP). Patients with immune-mediated TTP (iTTP) have anti-ADAMTS-13 immunoglobulin G antibodies that inhibit ADAMTS-13 function and/or increase ADAMTS-13 clearance. Patients with iTTP are treated primarily by plasma exchange (PEX), often in combination with adjunct therapies that target either the von Willebrand factor-dependent microvascular thrombotic processes (caplacizumab) or the autoimmune components (steroids or rituximab) of the disease.

Objectives: To investigate the contributions of autoantibody-mediated ADAMTS-13 clearance and inhibition in patients with iTTP at presentation and through the course of the PEX therapy.

Patients/Methods: Anti-ADAMTS-13 immunoglobulin G antibodies, ADAMTS-13 antigen, and activity were measured before and after each PEX in 17 patients with iTTP and 20 acute TTP episodes.

Results: At presentation, 14 out of 15 patients with iTTP had ADAMTS-13 antigen levels of <10%, suggesting a major contribution of ADAMTS-13 clearance to the deficiency state. After the first PEX, both ADAMTS-13 antigen and activity levels increased similarly, and the anti-ADAMTS-13 autoantibody titer decreased in all patients, revealing ADAMTS-13 inhibition to be a modest modifier of the ADAMTS-13 function in iTTP. Analysis of ADAMTS-13 antigen levels between consecutive PEX treatments revealed that the rate of ADAMTS-13 clearance in 9 out of 14 patients analyzed was 4- to 10-fold faster than the estimated normal rate of clearance.

Conclusion: These data reveal, both at presentation and during PEX treatment, that antibody-mediated clearance of ADAMTS-13 is the major pathogenic mechanism that causes ADAMTS-13 deficiency in iTTP. Understanding the kinetics of ADAMTS-13 clearance in iTTP may now enable further optimization of treatment of patients with iTTP.

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KEYWORDS
ADAMTS-13, autoantibodies, autoimmune disease, purpura, thrombotic thrombocytopenic, thrombosis

1 | INTRODUCTION

ADAMTS-13 regulates the multimeric size of von Willebrand factor (VWF) in a shear-dependent manner [1]. In this way, ADAMTS-13 controls the platelet-tethering function of VWF. Severe deficiency of ADAMTS-13 (<10 IU/dL) leads to the presence of ultralarge VWF within the circulation, which can spontaneously unravel under conditions of elevated shear stress such as those present in the microvasculature. This can lead to microvascular occlusion and therefore to the development of thrombotic thrombocytopenic purpura (TTP), which is characterized by microangiopathic haemolytic anemia, schistocytes, and end organ damage [2–5]. Patients with TTP either have a congenital deficiency (ie, mutations in *ADAMTS-13*) or an acquired, immune-mediated ADAMTS-13 deficiency due to anti-ADAMTS-13 autoantibodies [6,7]. Immune-mediated TTP (iTTP) accounts for ~95% of the TTP cases, with patients varying in clinical severity [8]. Currently, the mainstay of treatment is plasma exchange (PEX), which provides a source of ADAMTS-13, removes UL-VWF, and may reduce anti-ADAMTS-13 immunoglobulin G (IgG) plasma levels. Immunosuppressive therapeutics such as high-dose steroids and rituximab are used to target the autoimmune component of the disease and also to diminish the rate of relapse [8]. These treatments facilitate remission in most patients with iTTP, but exacerbation and relapse remain a significant problem, requiring long-term follow-up. More recently, caplacizumab, an anti–VWF A1 domain antibody that blocks VWF-platelet binding, has been demonstrated to be effective in ameliorating TTP symptoms [9]. Although this alleviates the incidence of VWF-dependent microvascular thrombosis in patients with iTTP, it does not treat the cause of the disease (ie, ADAMTS-13 deficiency). Recombinant ADAMTS-13 has been successfully trialed for the treatment of congenital TTP as it efficiently replaces the deficient enzyme [10]. To date, there have been no trials for its use in iTTP; the reason for this is questions over its effectiveness in individuals with anti-ADAMTS-13 autoantibodies.

In iTTP, a polyclonal, predominantly IgG, immune response develops toward ADAMTS-13 [11–15]. Although polyclonal in nature, autoantibodies most frequently recognize epitopes in the ADAMTS-13 spacer domain. Due to the functional importance of the spacer domain in VWF recognition, antibodies directed toward the spacer domain have the potential to inhibit, or diminish, the proteolysis of VWF by ADAMTS-13 [11]. However, in many patients, inhibition of ADAMTS-13 activity alone is insufficient to account for the severe ADAMTS-13 deficiency that is characteristic in patients with iTTP at presentation [11]. Instead, autoantibodies frequently appear to promote ADAMTS-13 clearance [11,16–18]. This probably occurs due to the formation of immune complexes between ADAMTS-13 and anti-ADAMTS-13 IgG [19]. These complexes are likely preferentially cleared from the circulation, thereby reducing ADAMTS-13 antigen levels.

Essentials

- At presentation, all patients with immune-mediated thrombotic thrombocytopenic purpura (iTTP) exhibited evidence of antibody-mediated ADAMTS-13 clearance.
- Plasma exchange treatment in iTTP rapidly reduces anti–ADAMTS-13 autoantibody titer.
- During the early plasma exchange treatments, antibody-mediated ADAMTS-13 clearance is the major pathogenic mechanism promoting ADAMTS-13 deficiency.
- Autoantibodies in iTTP enhance ADAMTS-13 clearance by 4- to 10-fold.

Antibody-mediated clearance is an important disease process as ADAMTS-13 antigen levels are significantly lower in patients who die from a TTP episode than in those who survive [11,20]. This suggests that the levels of anti-ADAMTS-13 antibodies that actively promote ADAMTS-13 clearance and the rate at which clearance occurs may directly influence or compromise the efficacy of the treatment. However, the mechanism(s) of clearance of these IgG or ADAMTS-13 immune complexes remain(s) unclear. Although both ADAMTS-13 inhibition and clearance are important pathogenic mechanisms, the relative contribution of each has not yet been investigated. Therefore, we analyzed both ADAMTS-13 clearance and inhibition in patients with iTTP during PEX treatment.

2 | METHODS

2.1 | Patients with immune-mediated thrombotic thrombocytopenic purpura

Citrated plasma samples from patients with iTTP were used for analysis. Consent was obtained from all patients (Multicentre Research Ethics Committee; approval numbers: 08/H0810/54, 08/H0716/72). Acute iTTP was diagnosed as described previously [21]. Anti-ADAMTS-13 IgG antibody titers were measured using an in-house assay as described previously [22]. In brief, binding of patient IgG in a 1/100 plasma dilution to immobilized recombinant ADAMTS-13 was measured and expressed as a percentage of anti-ADAMTS-13 IgG measured in an index case (standard). The TTP standard plasma, diluted to produce a standard curve for IgG quantification, came from an index patient with high IgG levels, and these levels were arbitrarily

set to 100% and levels reported relative to this. Anti-ADAMTS-13 IgG is estimated to be present in ~4% of healthy individuals [12,20,23]; therefore, plasma from 49 healthy donors was used to assign a cut-off for positivity (a larger number of healthy controls than in the original paper describing the assay) [20,22]. The cut-off value (6%) for assigning positivity was determined using the 95% of the IgG levels measured in these controls (note that the 6% positivity limit does not reflect the sensitivity of the assay, which we estimate to be sensitive to anti-ADAMTS-13 IgG levels of >2%). ADAMTS-13 activity and antigen were measured as previously described [20] (detection limit activity, 5%; normal range, 64%-134%; antigen detection limit, 0.5%; normal range, 74%-134%). The same pooled normal plasma was used to make standard curves to quantify ADAMTS-13 antigen and activity and was set to 100%; samples of patients with iTTP are presented as percent relative to this. Following diagnosis, all patients with iTTP received corticosteroids and rituximab in addition to PEX, as described previously [24]. Four patient episodes received caplacizumab (patients 1, 4b, 11, and 17b) as part of the HERCULES trial [9]. Baseline presentation samples were taken before PEX and immunosuppression. Pre-PEX samples were taken immediately prior to PEX, and post-PEX samples were taken within 30 minutes of PEX completion. For longitudinal analyses, 17 patients with iTTP encompassing 20 iTTP episodes were analyzed before, during, and after PEX therapy. In some instances, both pre-PEX and post-PEX samples were not available for analysis. These samples are as follows: i. TTP-2: no post-PEX5; ii. TTP-4a: no post-PEX5; iii. TTP-6b: no post-PEX1 or PEX7, PEX2, and PEX3 were given back-to-back so post-PEX2 = pre-PEX3; iv. TTP-8: no post-PEX1 or pre-PEX6; and v. TTP-11: no post-PEX1 or PEX5.

3 | RESULTS

3.1 | Patients with immune-mediated thrombotic thrombocytopenic purpura at presentation

In this study, we monitored 17 patients with iTTP during 20 acute episodes. Each patient is represented by a number (1–17). Those patients who experienced more than one episode during this study (patients 4, 6, and 17) are further denoted by a and b. At presentation, all patients with iTTP had thrombocytopenia (platelet count < 150×10^9/L) and severely deficient ADAMTS-13 activity (<10%) (Table, Figures 1 and 2). The median age at presentation was 53 (range: 17-67), 12 out of 17 patients were female, and 13 out of 17 patients presented with TTP for the first time (patients 5, 10, 16, and 17 had iTTP episodes prior to their first episode in this study).

Interestingly, 14 out of 15 patients with iTTP (16 of 18 episodes) presented with ADAMTS-13 antigen levels of <10%. In patients 1 to 10 and 12 to 16, therefore (presentation data for patient 11 with iTTP were not available), the entire deficiency state could potentially be explained by the severe reduction in ADAMTS-13 antigen. In patient 17, consisting of 2 episodes (TTP-17a and TTP-17b), ADAMTS-13 antigen levels were appreciably (but not severely) reduced (28% and 58%, respectively). Despite this, ADAMTS-13 activity levels were <10%, suggesting the presence of inhibitory antibodies in this patient, in addition to those promoting clearance.

Patient 6a relapsed during treatment for the first episode as observed by the re-emergence of anti-ADAMTS-13 IgG, severe reduction in ADAMTS-13 antigen and activity, and drop in platelet count (Figure 1G). Follow-on treatment is shown in episode 6b initiated 5 days after PEX treatment for episode 6a (Figure 1H). Similarly, patient 17 relapsed 37 days after the completion of PEX treatment for episode 17a, and follow-on treatment is shown in 17b (Figure 2).

3.2 | Influence of plasma exchange upon anti-ADAMTS-13 immunoglobulin G titer

At presentation, all patients with iTTP involved in this study had anti-ADAMTS-13 IgG antibodies. Anti-ADAMTS-13 IgG titer varied appreciably at the point of diagnosis (range: 4%-83%, Table), similar to previous studies [12,20,23]. Immediately after the first PEX, the plasma levels of anti-ADAMTS-13 antibodies dropped markedly, and in some cases to levels below the detection threshold (Figures 1 and 2). Rituximab treatment is associated with B cell depletion over a median of 3 days [25] (although the effect of this upon circulating levels of IgG will take appreciably longer), highlighting a major benefit of PEX in removing/markedly diminishing the accumulated plasma anti-ADAMTS-13 IgG. Patients were also given steroids, which could have only minimally contributed to this decrease within this timeframe. In patients 1, 2, 6a, 10, 11, and 13 all with iTTP, ADAMTS-13 IgG levels dropped immediately after the first PEX, but increased again prior to the subsequent PEX. This likely reflects a combination of the continued production of anti-ADAMTS-13 IgG as well as the diffusion of extravascular anti-ADAMTS-13 IgG into the blood; IgG is approximately evenly distributed between the intravascular and extravascular space and can diffuse freely between these locations [26]. Anti-ADAMTS-13 IgG in these patients tended to fluctuate in this way over the course of the first few PEX. Over the entire course of PEX treatments, anti-ADAMTS-13 IgG levels reduced with successive treatments or persisted at very low levels. In patient 11, IgG levels increased between PEX 4 and PEX 6, which was associated with a drop in ADAMTS-13 activity and antigen, suggesting exacerbation during treatment.

3.3 | Influence of anti-ADAMTS-13 immunoglobulin G on ADAMTS-13 antigen and activity after the first plasma exchange

At presentation, it was clear that most patients with iTTP had severely reduced ADAMTS-13 antigen, suggestive of the presence of anti-ADAMTS-13 IgG that promoted ADAMTS-13 clearance. As a result, it was not possible to ascertain at presentation whether the anti-ADAMTS-13 IgG in these individuals was also inhibitory (except in patient 17 – Table). Analysis of the ADAMTS-13 antigen and activity immediately after the first PEX enabled us to gauge the inhibitory

TABLE Presenting ADAMTS-13-related parameters and platelet count in 20 iTTP episodes.

Patient	ADAMTS-13 activity (% PNP)	ADAMTS-13 antigen (% PNP)	Anti-ADAMTS-13 IgG (% standard)	Platelet count ($\times 10^9$/L)	Relapse?	Age at episode (years)	Sex	Cap	Outcome
1	<5	1	83	7	No	63	F	Yes	Relapse-free[a]
2	<5	6	36	18	No	67	F	No	Relapse-free
3	<5	3	26	16	No	48	F	No	Relapse
4a	<5	n/a	n/a	6	No	30	F	No	3 relapses (including 4b)
4b	6	3	9	38	Yes	30	F	Yes	2 relapses
5	<5	1	6	17	Yes	34	M	No	Relapse
6a	<5	2	28	5	No	45	M	No	Exacerbation (6b)
6b	<5	2	12	16	Yes	45	M	No	Relapse-free
7	<5	2	23	10	No	54	F	No	Relapse-free
8	<5	2	18	5	No	56	M	No	Relapse-free
9	<5	1	8	11	No	33	M	No	Relapse-free
10	<5	4	36	41	Yes	64	F	No	Relapse-free
11	<5	n/a	n/a	n/a	No	39	F	Yes	Relapse-free
12	<5	1	16	15	No	61	F	No	Death (not TTP-related)
13	<5	1	70	8	No	63	F	No	Relapse-free
14	<5	2	4	15	No	17	M	No	Relapse-free
15	<5	2	28	10	No	57	F	No	Relapse-free
16	<5	3	6	19	Yes	52	F	No	Subclinical relapse
17a	<5	28	15	12	Yes	54	M	No	Exacerbation (17b)
17b	9	58	13	26	Yes	54	M	Yes	Relapse-free

ADAMTS-13 activity and antigen levels, anti-ADAMTS-13 IgG levels, and platelet count at presentation in patients are shown. n/a indicates data that was not available from the presentation sample.

Cap, caplacizumab; TTP, thrombotic thrombocytopenic purpura.

[a] At 30 months, lost to follow-up.

potential of the anti-ADAMTS-13 IgG remaining in circulation. This revealed in all cases that there was an increase in ADAMTS-13 antigen to between 25% and 75%, consistent with the provision of plasma (Figures 1 and 2). Interestingly, the ADAMTS-13 antigen and activity levels after the first PEX were frequently in close agreement. In patients 6, 8, and 17, there was evidence of inhibitory antibodies. In patient 6a, ADAMTS-13 activity dropped appreciably below the ADAMTS-13 antigen level (54% ADAMTS-13 antigen vs 32% ADAMTS-13 activity), suggesting the presence of antibodies capable of inhibiting ADAMTS-13 function (Figure 1G). Patient 17 had inhibitory antibodies at presentation (Table) and immediately after the first PEX in episode 17a (Figure 2I, 32% ADAMTS-13 activity vs 44% ADAMTS-13 antigen). In patient 8, immediately prior to the second PEX, the ADAMTS-13 antigen was 37% vs 8% ADAMTS-13 activity, suggesting that inhibitory antibody titer may have been reduced after the first PEX, but re-emergence of inhibitory antibodies (from extravascular site, or de novo B cell production) may have occurred prior to the second PEX (Figure 1J). Intriguingly, in patients 9, 12, 13, and 14, the ADAMTS-13 activity was markedly higher that the antigen levels (Figure 2A, D–F). Although being counter-intuitive, this may reflect the ability of certain anti-ADAMTS-13 IgGs to induce opening of ADAMTS-13, which can augment the proteolysis of short VWF A2 fragment substrates [27].

3.4 | Anti-ADAMTS-13 autoantibodies enhance the rate of clearance of ADAMTS-13

To investigate the influence of anti-ADAMTS-13 IgG upon ADAMTS-13 clearance, we examined the change in ADAMTS-13 antigen levels between samples taken immediately after the second PEX and those taken immediately before the third PEX (Figure 3). Some patients received the first and second PEXs in comparatively quick succession, and for this reason, samples before and after these PEXs were not always available. Therefore, we assessed samples between PEX2 and PEX3 as these were the earliest and most frequently available. TTP 3, 4b 6a, 7, 15 and 17a did not have samples from consecutive PEX,

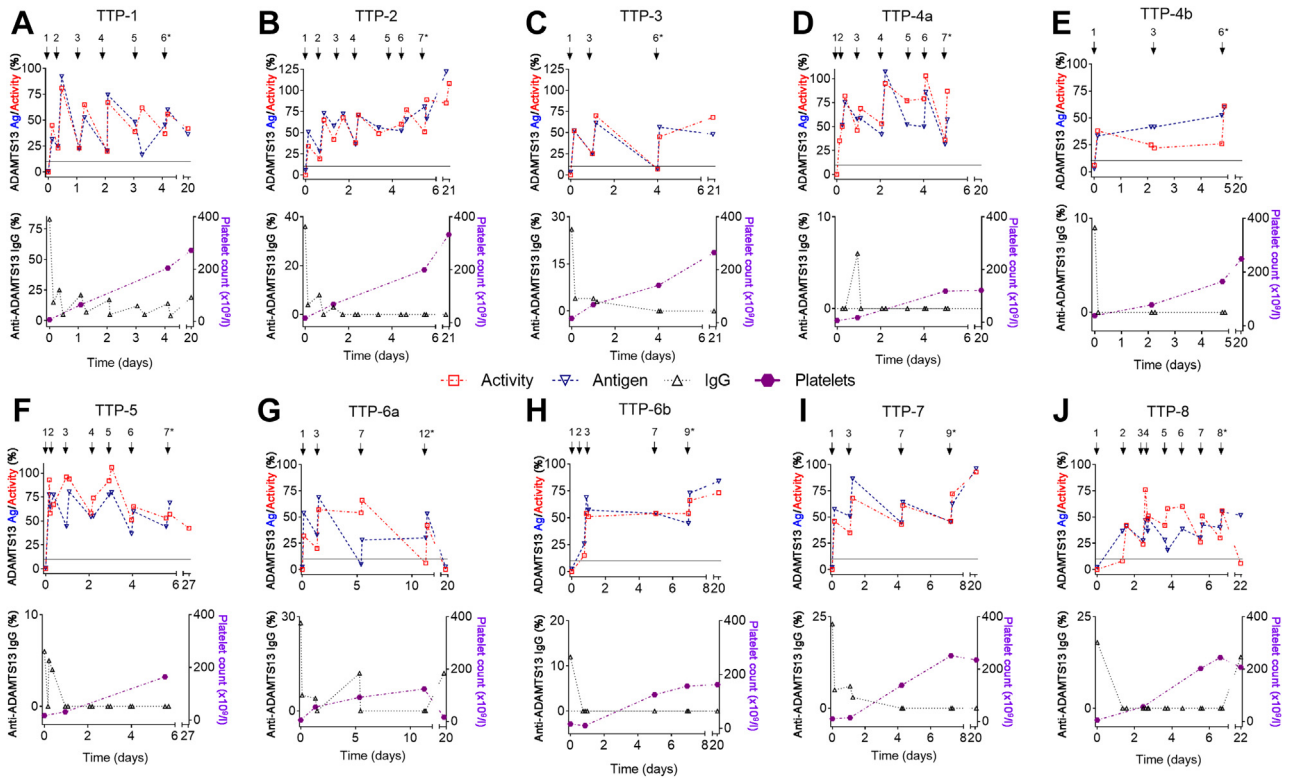

FIGURE 1 ADAMTS-13 antigen, its activity, anti-ADAMTS-13 IgG levels, and platelet counts during PEX treatment in patients with iTTP. Plasma levels of ADAMTS-13 antigen (blue) and activity (red) were measured by ELISA and using FRETS-VWF73, respectively. Data are shown as % of normal pooled plasma. Levels were measured at presentation and before and after PEX treatment. Exceptions were as follows: i. TTP-2: no post-PEX5; ii. TTP-4a: no post-PEX5; iii. TTP-6b: no post-PEX1 or PEX7, PEX2, and PEX3 were given back-to-back so post-PEX2 = pre-PEX3; and iv. TTP-8: no post-PEX1 or pre-PEX6. The 10% cut-off for reduced ADAMTS-13 activity is indicated by the black line. PEX treatment numbering where samples were taken are denoted by numbered arrows. Anti-ADAMTS-13 IgG levels (black) were measured by ELISA and presented as a percentage of an iTTP standard (with previously characterized high titer antibodies). Platelet counts (purple) when measured are included. *Last PEX. IgG, immunoglobulin G; iTTP, immune-mediated thrombotic thrombocytopenic purpura; PEX, plasma exchange.

which prevented their inclusion in this analysis. For patients 13, 16, and 17b with TTP, sampling between PEX 2 and PEX 3 was not available. For these patients, the samples presented are between PEX 3 and PEX 4 (TTP 13 and TTP 17b) or PEX 7 and PEX 8 (TTP 16).

ADAMTS-13 antigen levels post-PEX in these samples ranged from 28% to 92%. In order to normalize data, the ADAMTS-13 antigen level after PEX completion is presented as 1 for each individual patient and the concentration prior to the next PEX presented relative to this. As the precise timing between sampling was recorded, this enabled us to estimate the relative rates of ADAMTS-13 clearance in these individuals (Figure 3). To provide context, the rate of ADAMTS-13 clearance in congenital patients with TTP receiving plasma infusion is presented in red, which represents the normal rate at which ADAMTS-13 is cleared from circulation (median, 130 hours; range, 89-189 hours) (Figure 3) [28]. Nine out of the 14 patients analyzed in this way exhibited evidence of a markedly increased rate of disappearance of ADAMTS-13 from circulation with a half-life that we estimated to be between 12 and 30 hours, which is approximately 4- to 10-fold faster than ADAMTS-13 clearance in patients with congenital TTP (Figure 3).

Many of the patients' ADAMTS-13 activity and antigen levels increased in response to PEX and decreased prior to the next treatment, revealing a "sawtooth" effect, the extent of which diminished as the treatment progressed. Of these individuals, TTP 1, 2, 4a, 5, 10, and 11 all had detectable anti-ADAMTS-13 IgG levels at the time of sampling. However, TTP 6b, 8, and 9 had anti-ADAMTS-13 IgG levels below the detection threshold during treatment. Despite this, increased ADAMTS-13 clearance was evident (Figures 1 and 2). This suggests that comparatively low quantities of anti-ADAMTS-13 IgG may be sufficient to promote antigen clearance. The ability of trace amounts of anti-ADAMTS-13 IgG to exert clearance effects can be seen in many of the patients with iTTP during the early PEX treatments.

In 5 out 14 patients with iTTP, the ADAMTS-13 antigen levels did not drop appreciably between the presented PEX treatments (Figure 3), suggesting that the anti-ADAMTS-13 IgG in these patients did not enhance ADAMTS-13 clearance at the time of sampling. Of these patients, patient 17 was the only patient who did not have severe ADAMTS-13 antigen deficiency at presentation, consistent with the anti-ADAMTS-13 IgG in this patient not promoting clearance to

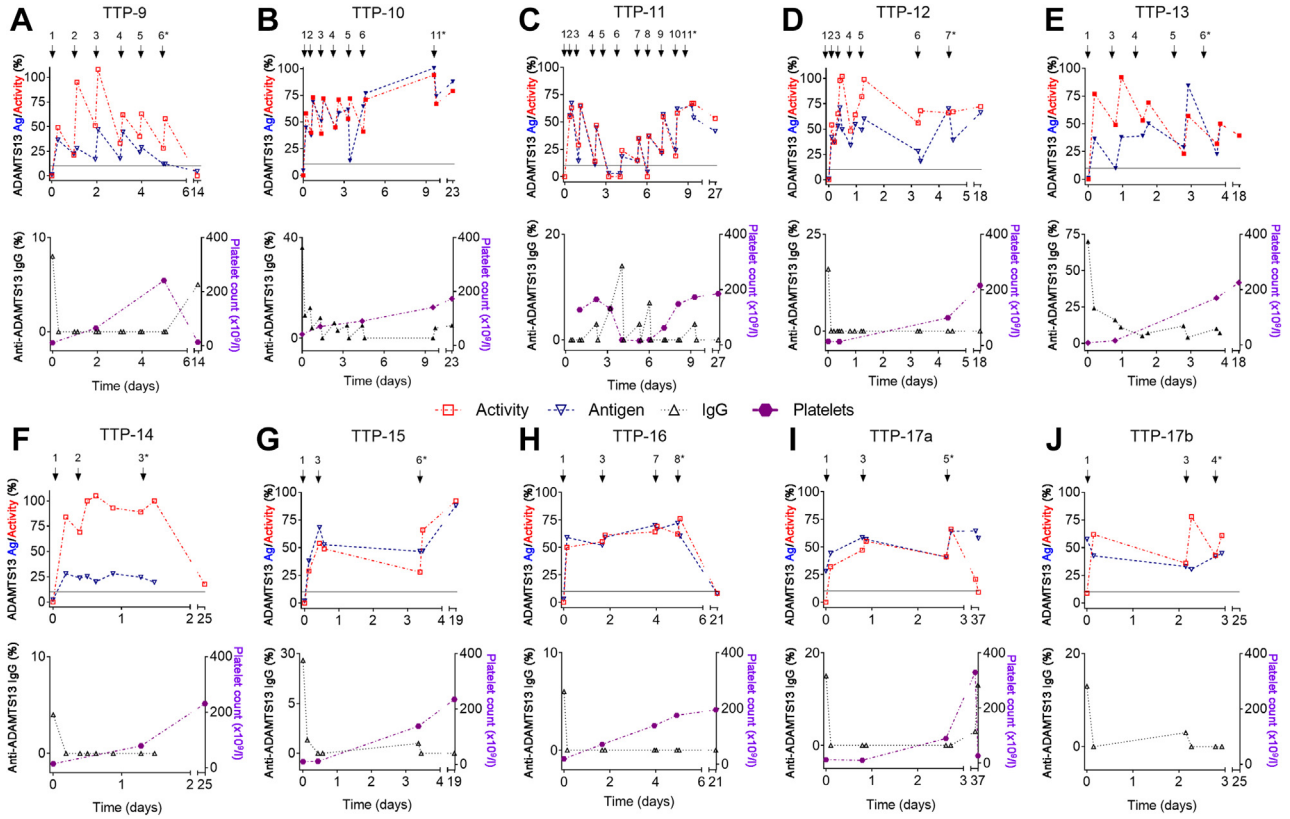

FIGURE 2 ADAMTS-13 antigen, its activity, anti-ADAMTS-13 IgG levels, and platelet counts during PEX treatment in patients with iTTP. Plasma levels of ADAMTS-13 antigen (blue) and activity (red) were measured by ELISA and using FRETS-VWF73, respectively. Data are shown as percentage of normal pooled plasma. Levels were measured at presentation and before and after PEX treatment, except for TTP-11: no post-PEX1 or PEX5. The 10% cut-off for reduced ADAMTS-13 activity is indicated by the black line. PEX treatment numbering where samples were taken are denoted by numbered arrows. Anti-ADAMTS-13 IgG levels (black) were measured by ELISA and presented as a percentage of an iTTP standard (with previously characterized high titer antibodies). Platelet counts (purple) when measured are included. *Last PEX. IgG, immunoglobulin G; iTTP, immune-mediated thrombotic thrombocytopenic purpura; PEX, plasma exchange.

the same extent as other patients with iTTP. The other patients all had severe ADAMTS-13 antigen deficiency at presentation, suggesting that the early PEX treatments (and potentially steroids) in these individuals had reduced the anti-ADAMTS-13 titer sufficiently to suppress this process.

Through the course of the PEX treatments of the presented 17 patients with iTTP, the major effect of PEX was 2-fold. First, the anti-ADAMTS-13 IgG level was appreciably and rapidly reduced, and second, the provision of ADAMTS-13 in the plasma enabled increases in plasma ADAMTS-13 antigen and activity of >10% (Figures 1 and 2). Maintaining an ADAMTS-13 level of >10% is considered the threshold, above which ADAMTS-13 can protect against VWF-mediated microvascular thrombosis [8]. Consistent with this, all patients with iTTP exhibited increases in platelet count suggestive of amelioration of the microvascular thrombosis.

Of the 17 patients presented, 13 remained relapse-free at this time. There was a drop in ADAMTS-13 activity for patients 14 and 16 on days 25 and 21 once PEX was stopped. Despite this, both patients continued to have a normal platelet count with no exacerbation or relapse. Platelet count is the primary determinant of the clinical response to TTP treatment [21]. PEX is commonly ended once the platelet count normalizes (>150 × 10^9/L) for 2 days [4]. It is not uncommon that patients with iTTP may still exhibit reduced ADAMTS-13 activity at the end of PEX treatment. ADAMTS-13 activity also often drops after PEX completion as levels are no longer "supplemented" with exogenous ADAMTS-13, but these levels gradually rise as immunosuppression continues and its effects manifest, with most patients with iTTP going on to achieve complete ADAMTS-13 remission with the ADAMTS-13 activity normalizing [21,29]. For patient 16, ADAMTS-13 activity levels gradually rose after PEX completion (day 30: ADAMTS-13 activity, 11%, day 60: ADAMTS-13 activity, 56%). Anti-ADAMTS-13 IgG levels were measured on day 21 and were undetectable. The anti-ADAMTS-13 IgG present in patient 16 may likely have been low titer and/or complexed with ADAMTS-13 and removed from the circulation. Anti-ADAMTS-13 IgG levels are not available for patient 14 after the completion of PEX, but ADAMTS-13 activity rose steadily with further immunosuppression, slowly normalizing by day 120 with ongoing clinical remission.

There was no discernible difference in clearance rates between those patients who relapsed when compared with the rates of those who did not: both enhanced and moderate rates of ADAMTS-13 clearance were observed during PEX. Although a small number of

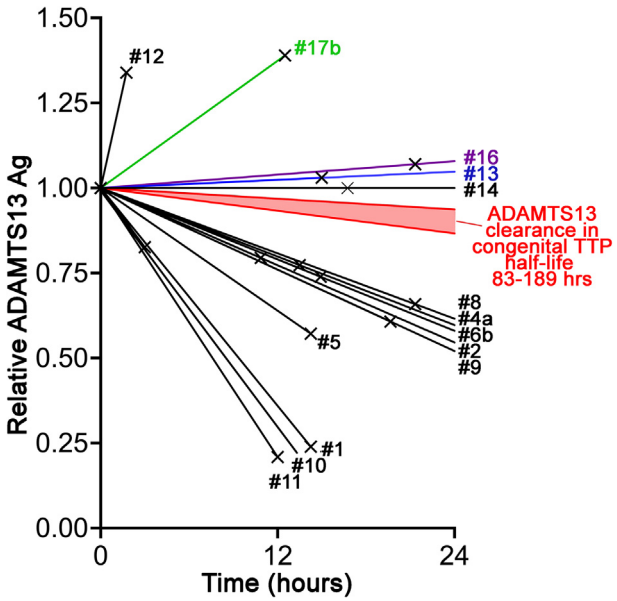

FIGURE 3 Rate of changes in plasma ADAMTS-13 antigen levels in patients with iTTP treated with PEX. Plasma levels of ADAMTS-13 antigen were measured by ELISA in patients with iTTP immediately after PEX and then again immediately before the next PEX. All data shown are after the second PEX and before the third PEX, except for patients #13 and #17b (PEX 3 and 4) and #16 (PEX 7 and 8) with iTTP. For all data presented, the initial ADAMTS-13 antigen concentration was set to 1, and the second samples concentration is plotted relative to this to enable comparison in the relative change in ADAMTS-13 concentration over time. The area shaded in red represents the reported clearance rate of ADAMTS-13 in patients with congenital TTP receiving plasma infusion (median, 130 hours; range, 83-189 hours). IgG, immunoglobulin G; iTTP, immune-mediated thrombotic thrombocytopenic purpura; PEX, plasma exchange.

patients received caplacizumab (n = 4), differences in the rate of ADAMTS-13 clearance in those receiving caplacizumab compared with those that did not were not evident.

4 | DISCUSSION

In this study, we investigated the effect of PEX upon anti-ADAMTS-13 IgG and the influence of these antibodies on both the inhibition and clearance of ADAMTS-13. In a previous study, Thomas et al. [11] revealed that, at presentation, the deficiency state of the majority of patients with iTTP could be explained by reduced ADAMTS-13 antigen. However, this does not mean that inhibition of ADAMTS-13 does not contribute to the disease. Indeed, it is likely that antibodies that induce ADAMTS-13 clearance may also inhibit the activity of the enzyme [16]. However, this effect on ADAMTS-13 activity is masked if the ADAMTS-13 antigen is also severely diminished in patient plasma. With this in mind, this study investigated ADAMTS-13 activity, antigen, and anti-ADAMTS-13 titer through the duration of PEX treatments.

In all cases, the first PEX treatment resulted in increases in both ADAMTS-13 antigen and activity to between 25% and 75% (Figures 1 and 2; normal ADAMTS-13 antigen range, 64%-134%; activity range, 74%-134%). At these higher levels, the degree of antibody inhibition can be assessed in patient plasma by comparing ADAMTS-13 antigen and activity values. In most cases, the ADAMTS-13 antigen and activity were in close agreement, with few patients showing evidence of partial (but not complete) inhibition. At presentation, antibody inhibition was evident in patient 17. In addition to this patient, patient 6a had ADAMTS-13 activity levels (32%) appreciably below antigen levels (54%), potentially reflecting the influence of inhibitory anti-ADAMTS-13 IgG. Patient 8 exhibited evidence of inhibitory antibodies prior to the second PEX, and ADAMTS-13 activity (8%) was appreciably below antigen levels (37%). The potentially surprising finding that after PEX there was little evidence of inhibitory antibodies suggests that either most of these patients do not have autoantibodies that appreciably compromise ADAMTS-13 function or the first PEX (in addition to immunosuppression) appreciably reduces the circulating titer of inhibitory antibodies to a level that does not appreciably inhibit ADAMTS-13.

The comparatively low incidence of inhibitory anti-ADAMTS-13 antibodies among the patients with iTTP in this study highlights the important pathogenic mechanism associated with antibody-mediated ADAMTS-13 clearance. At presentation, 14 out of 15 patients with iTTP had severely reduced (<10%) ADAMTS-13 antigen levels, with the remaining patients having moderately reduced ADAMTS-13 antigen levels, highlighting that clearance of ADAMTS-13 likely represents the primary mechanism by which ADAMTS-13 deficiency is manifest in iTTP. Following PEX, despite a reduction in autoantibody titer, the increased rate of ADAMTS-13 clearance remained in the primary pathogenic mechanism. A recent estimation of the half-life of ADAMTS-13 following plasma infusion in patients with congenital TTP revealed a median half-life of 130 hours (5.4 days) [28]. In contrast, 9 out of 14 patients with iTTP analyzed in this study exhibited ADAMTS-13 half-lives of ~12 to 30 hours. We did not measure other immunoglobulin isotypes, such as IgM and IgA, which may also contribute to ADAMTS-13 clearance. These classes have been measured in some patients with TTP [12,19,30], but IgG anti-ADAMTS-13 appears to be the most relevant [31]. With respect to IgG subclasses, we did not measure the proportions of anti-ADAMTS-13 IgG1-4. IgG1 and IgG4 are reportedly the most frequent IgG subclasses detected in iTTP [32,33]. These subclasses differ in effector function, and IgG1 and IgG3 have an increased affinity toward activating Fcγ receptors compared to IgG2 and IgG4 [34,35]. Immune complexes of these subclasses, ie, IgG1 and IgG3, may play a bigger role in the removal of the antigen they bind to. Furthermore, IgG4 antibodies form smaller immune complexes as they undergo Fab arm exchange [36,37], and so, they may produce less inflammatory immune complexes. Although, IgG4 is less likely to be directly involved in promoting clearance, the presence of IgG1-3 even at low titer may be sufficient for the formation of immune complexes that could be more readily cleared.

For congenital patients with TTP, simple provision of ADAMTS-13 in the form of plasma infusion or more recently with recombinant ADAMTS-13 suffices. In iTTP, this approach is hampered by the actions of the anti-ADAMTS-13 autoantibodies, which are frequently perceived to inhibit the enzyme, potentially making this approach less efficacious. However, this may now warrant some reconsideration. The data presented herein suggest that the inhibition of ADAMTS-13 likely only contributes modestly to the overall deficiency state. Although the anti-ADAMTS-13 IgG clearly exerts a pathogenic effect, these antibodies do not drive aggressive clearance of ADAMTS-13, which consequently provides a therapeutic window in which ADAMTS-13 can still function. These findings have potentially important implications for the treatment of patients with iTTP using recombinant ADAMTS-13.

It is clear that a single PEX is insufficient to prevent iTTP relapse. The reason for this is likely the continual production of anti-ADAMTS-13 autoantibodies and their diffusion from tissues back into the blood, thereby enabling them to exert their pathogenic effects. The data that we present herein highlight the particular importance of the first/early PEX treatments. Not only does this provide a source of ADAMTS-13, but it also markedly reduces the anti-ADAMTS-13 titer (in combination with immunosuppression). This latter feature of PEX is likely of particular importance in the response of patients with iTTP. Therefore, recombinant ADAMTS-13 as an adjunctive therapy to PEX may be efficacious. Following initial PEX treatments, one might be able to reduce the dependence on PEX and instead intermittently substitute with replacement therapy. Trials to formally test the efficacy of this approach, as well as the benefit of reducing iTTP patient time on PEX, could have appreciable therapeutic advantages for both the patient and the health care systems.

In conclusion, we have demonstrated for the first time the enhanced rate of ADAMTS-13 clearance in patients with iTTP and this appears to be the major mechanism by which reduced ADAMTS-13 activity is manifest during PEX therapy, with few patients showing evidence of antibody-mediated inhibition. Further studies are required to investigate how anti-ADAMTS-13 antibodies facilitate the removal of ADAMTS-13 immune complexes from the circulation.

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AUTHOR CONTRIBUTIONS

M.I.U. designed the experiments, analyzed the data, prepared the figures, and wrote the manuscript. F.A. designed and performed the experiments, analyzed the data, and wrote the manuscript. M.R.T. designed the experiments, analyzed the data, and wrote the manuscript. M.A.S. designed the experiments, analyzed the data, and wrote the manuscript. J.T.B.C. designed the experiments, analyzed the data, prepared the figures, and wrote the manuscript.

DECLARATION OF COMPETING INTERESTS

There are no competing interests to disclose.

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