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Delayed normalisation of ADAMTS13 activity in acute Thrombotic Thrombocytopenic Purpura in the caplacizumab era

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Abstract:
The benefits of caplacizumab in acute immune mediated TTP (iTTP) are well established. We identified a delay in normalisation of ADAMTS13 activity (>30%) in a subgroup of caplacizumab treated patients which was not evident in the pre-caplacizumab era. Caplacizumab treated patients (n=64) achieved ADAMTS13 activity >30% at median 31 days post PEX, compared to 11.5 days in the non-caplacizumab group (n=50, p=0.0004). 18/64 (28%) caplacizumab treated patients had an ADAMTS13 activity <10% at the time of stopping caplacizumab with a longer time to ADAMTS13 activity >30% (median 139 days after completing PEX). 18/64 (28%) of patients receiving extended caplacizumab (31-58 days) failed to achieve ADAMTS13 activity >30% at time of stopping caplacizumab compared to 4/47 (8.5%) of historical controls at similar timepoint (30+28 days, p<0.0001). Failure to achieve ADAMTS13 activity >30% within 30+28 days was 6 times more likely in caplacizumab treated patients (OR 6.3, p=0.0006). ADAMTS13 antigen level <30% at caplacizumab cessation was associated with increased iTTP recurrence (4/10 vs 0/9 in patients with ADAMTS13 antigen ≥30%). Admission anti-ADAMTS13 IgG antibody level did not predict recurrence. Anti-ADAMTS13 IgG antibody levels, immunosuppression and ethnicity did not account for differences in ADAMTS13 activity response. ADAMTS13 antigen levels ≥30% may be useful to guide stopping caplacizumab therapy after extended use in patients with ADAMTS13 activity <10%, but patients require close monitoring. The reason for delayed ADAMTS13 normalisation is unclear and requires further investigation.

Conflict of interest: COI declared - see note

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Key points:

1. 28% of caplacizumab treated patients had persistent ADAMTS13 activity <30% within 30+28 days post PEX (OR 6.3)
2. In patients with ADAMTS13 activity <10% on stopping caplacizumab, an ADAMTS13 antigen level<30% had higher risk of recurrence

Abstract

The benefits of caplacizumab in acute immune mediated TTP (iTTP) are well established. We identified a delay in normalisation of ADAMTS13 activity (>30%) in a subgroup of caplacizumab treated patients which was not evident in the pre-caplacizumab era. Caplacizumab treated patients (n=64) achieved ADAMTS13 activity >30% at median 31 days post PEX, compared to 11.5 days in the non-caplacizumab group (n=50, p=0.0004). 18/64 (28%) caplacizumab treated patients had an ADAMTS13 activity <10% at the time of stopping caplacizumab with a longer time to ADAMTS13 activity >30% (median 139 days after completing PEX). 18/64 (28%) of patients receiving extended caplacizumab (31-58 days) failed to achieve ADAMTS13 activity >30% at time of stopping caplacizumab compared to 4/47 (8.5%) of historical controls at similar timepoint (30+28 days, p<0.0001). Failure to achieve ADAMTS13 activity >30% within 30+28 days was 6 times more likely in caplacizumab treated patients (OR 6.3, p=0.0006). ADAMTS13 antigen level <30% at caplacizumab cessation was associated with increased iTTP recurrence (4/10 vs 0/9 in patients with ADAMTS13 antigen ≥30%). Admission anti-ADAMTS13 IgG antibody level did not predict recurrence. Anti-ADAMTS13 IgG antibody levels, immunosuppression and ethnicity did not account for differences in ADAMTS13 activity response. ADAMTS13 antigen levels ≥30% may be useful to guide stopping caplacizumab therapy after extended use in patients with ADAMTS13 activity <10%, but patients require close monitoring. The reason for delayed ADAMTS13 normalisation is unclear and requires further investigation.

Introduction

Thrombotic thrombocytopenic purpura (TTP) is a rare microangiopathic haemolytic anaemia (MAHA) associated with microvascular thrombosis and thrombocytopenia,

with potential for end organ damage. It is confirmed by a severe deficiency of the von Willebrand factor (VWF) cleaving protease called ADAMTS13 (A disintegrin and metalloprotease with thrombospondin type 1 motif, member 13) to less than 10%. The presence of autoantibodies, usually IgG, against ADAMTS13 distinguishes immune from congenital TTP. Current established management of immune TTP consists of daily plasma exchange (PEX) at 1.5 plasma volume initially followed by a reduction to 1.0 plasma volume with an increasing platelet count. This has reduced untreated mortality from 90% to 10-20%[1,2]. PEX aims to replenish ADAMTS13 as well as reduce the autoantibodies. Immunosuppression is key in the management of immune TTP (iTTP) and primarily consists of steroids and rituximab.[3,4] In recent years, caplacizumab has become an integral part of the acute management of iTTP.[5,6]

Caplacizumab (Cablivi®) is an anti-von Willebrand factor (VWF) humanized single domain immunoglobulin which binds with high affinity to the A1 domain of VWF, thereby preventing interaction with the platelet GPIb-IX-V receptor. The single blind placebo-controlled phase 2 TITAN study, and the double-blind randomised controlled phase 3 HERCULES trial have shown therapeutic efficacy of caplacizumab[5–7]. Approval from European Medicines Agency (EMA)[8] and Food and Drug Administration (FDA)[9] were granted in 2018 and 2019 respectively. In 2020, caplacizumab received NICE approval for patients greater than 12 years old with acute TTP, to be used in conjunction with PEX and immunosuppression for at least 30 days after completion of PEX, or longer if required until ADAMTS13 normalises.[10]

Using FRETS VWF73 method[11] we investigated time to achieve ADAMTS13 activity >30% in patients receiving caplacizumab therapy compared to patients in the pre-caplacizumab era and factors which may contribute to this. International TTP working group recommendation is to continue caplacizumab until ADAMTS13 activity>20%, but in the UK we initially opted for confirmed ADAMTS13 activity levels >30% as a guide to stopping caplacizumab. This provided time to ensure adequate control on autoimmunity is achieved and to reduce risk of recurrence associated with lower ADAMTS13 activities. This protocol was initiated before the international working group suggested a threshold of 20% to safely stop caplacizumab[12]. We also

investigated the role of ADAMTS13 antigen levels to guide stopping caplacizumab in those who have ongoing severely deficient ADAMTS13 activity.

Methods

Patient population

64 patients who received caplacizumab in conjunction with PEX and immunosuppression for a confirmed diagnosis of acute iTTP between January 2016 and October 2021 at a single TTP referral centre were investigated (Medical Research Ethics Committee Numbers 08/H0810/54 and 08/H0716/72).

The HERCULES trial protocol[6] allowed use of caplacizumab for 30 days post PEX, and then a further 28 days if there is ongoing severe ADAMTS13 deficiency (<10%), hence termed '30+28' days. Caplacizumab use is NICE (National Institute for Health and Care Excellence) approved in the UK for 30 days post completion of PEX, with the option to extend treatment further guided by risk factors for recurrence of TTP such as persistent ADAMTS13 deficiency.

Citrated plasma samples for ADAMTS13 analysis were taken at presentation, the time of stopping caplacizumab and the closest timepoint to 30+28 days.

For comparison, data from 50 sequential iTTP patients who did not receive caplacizumab as part of the management for acute TTP between January 2013 and December 2015 were analysed from presentation and 30+28 days following completion of PEX. All patients received standard therapy: PEX, steroids (either methylprednisolone and/or high dose prednisolone) and rituximab 375mg/m^2 x 4 infusions.

Median number of days of IV and/or PO steroids, and days taken to wean PO steroids have been analysed. Due to wide weight range in patients, we have not given a particular steroid dose threshold to signify high dose steroids. Switching from intravenous (IV) to oral (PO) steroids, as well as duration of wean for oral steroids were determined by clinical and laboratory markers. Further immunosuppression was given depending on laboratory parameters, specifically platelet counts and ADAMTS13 activity, aiming for normalisation of levels.

Informed consent was received from the patients.

ADAMTS13 assays

In-house FRETS VWF73 method was used to measure ADAMTS13 activity (normal range (NR) 60% to 146%)[11]. Previously described in-house ELISAs were used to quantify ADAMTS13 antigen levels (NR 74-134%) and anti-ADAMTS13 IgG antibody (NR<6.1%)[11].

Treatment definitions

Time to achieve a normal platelet count ≥150 X 10^9/L was calculated in days following admission for treatment of acute TTP. Time to ADAMTS13 activity >30% was defined as a sustained rise in ADAMTS13 activity >30% with no subsequent drop in activity. This ADAMTS13 activity level is used to stop caplacizumab in the UK. Increasing familiarity in using caplacizumab following completion of HERCULES trial, and subsequent routine ADAMTS13 antigen analysis in these patients allowed us to adapt our practice further. ADAMTS13 antigen levels, in addition to ADAMTS13 activity level and anti-ADAMTS13 IgG antibody level at time of stopping caplacizumab is taken into consideration to determine when to stop caplacizumab. ADAMTS13 activity at the 30+28 day timepoint[6] was taken as the result available 58 days after completion of PEX or the closest date available.

The International Working Group consensus report on redefining outcomes in immune TTP updated definitions to take into account ADAMTS13 activity and the effects of caplacizumab on platelet count[12]. Clinical remission is defined as sustained clinical response (platelet count ≥150 X 10^9/L and lactate dehydrogenase (LDH) <1.5 X upper limit of normal) with either no PEX and no anti-VWF therapy for ≥30 days, or ADAMTS13 remission – partial remission is defined as ADAMTS13 activity ≥20%, and complete remission ADAMTS13 activity above the lower limit of the normal laboratory range. Exacerbation is defined as a fall in platelet count following clinical response within 30 days of stopping PEX or anti-VWF therapy, whilst a fall in platelet count following clinical remission is defined as relapse.
In this analysis, any recurrence (exacerbation/relapse) has been defined in terms of days after completion of PEX and/or days after stopping caplacizumab therapy.

Recommencement of caplacizumab treatment due to exacerbation or relapse after previously stopping was analysed as a separate episode of caplacizumab use. Relapses within one year of the acute iTTP episode were assessed.

Statistical analysis

Statistical analysis was performed using Graphpad Prism 9. Continuous data was summarised as median and range/interquartile range (IQR) with use of the Mann Whitney test to compare ranks across 2 groups and Kruskal-Wallis test for more than 2 groups. Gaussian distribution was not assumed for comparison between groups. Number and percentage were used to summarise categorical data. A Chi-squared/Fishers exact test was used to assess statistical significance in categorical variables. The odds ratio (OR) and 95% confidence interval (CI) was estimated using logistic regression models. A p-value<0.05 was considered statistically significant.

Data Sharing Statement

Contact Dr N Prasannan (n.prasannan@nhs.net) for data sharing.

Results

Demographics

Patient demographics were analysed (table 1). Patient groups were matched for age however there was a slight preponderance for females in the non-caplacizumab patients.

64 patients received caplacizumab as part of their management for iTTP. 10 received caplacizumab as part of the HERCULES trial and 54 received the drug as compassionate use and subsequently following UK regulatory approval.

Patients in whom caplacizumab therapy was stopped early for clinical reasons (eg. development of venous thromboembolism) and those who started caplacizumab later than standard practice (>48 hours from admission) were not included in the analysis.

Clinical and ADAMTS13 response in caplacizumab and non-caplacizumab treated patients.

Caplacizumab was used for a median of 35 days (range 15-130 days), corresponding to a median of 29.5 days (range 9-121 days) post completion of PEX. Caplacizumab treated patients achieved a sustained normal platelet count after a median of 4 days (range 2-16 days) following initiation of acute treatment, compared to median of 9 days (range 2-39 days, $p<0.0001$) in non-caplacizumab treated patients. Patients receiving caplacizumab required fewer days of PEX with a median of 6 days (range 3-17 days) compared to median 12.5 days (range 5-36 days, $p<0.0001$) in non-caplacizumab treated patients.

A confirmed ADAMTS13 activity >30% was achieved at median 31 days (IQR 14-83 days) following completion of PEX in caplacizumab treated patients compared to 11.5 days (IQR 7.25-27 days, $p=0.0004$) in non-caplacizumab treated patients. Race (Asian/Black/Caucasian did not affect time taken to achieve an ADAMTS13 activity >30% in the caplacizumab and non-caplacizumab treated patients ($p=0.6$, $p=0.4$ respectively).

ADAMTS13 activity at caplacizumab discontinuation

ADAMTS13 activity at time of stopping caplacizumab was analysed against days of caplacizumab used following completion of PEX (Figure 1). The proportion of patients failing to achieve an ADAMTS13 activity >30% as well as those who remained severely deficient in ADAMTS13 activity are shown. 35/64 (55%) patients stopped caplacizumab within 30 days following completion of PEX, median 24 days (range 9-30 days). Two patients stopped caplacizumab with an ADAMTS13 activity <10% due to widespread maculopapular rash attributable to caplacizumab, which resolved promptly on cessation of the drug.

24/64 (38%) patients received caplacizumab up to a further 28 days after completion of the initial 30 day post PEX course (day 31 to day 58), stopping a median of 46.5 days (range 31-58 days). Following extended therapy (30+28 days), 13 cases had ongoing severe deficiency in ADAMTS13 activity.

A further five patients received caplacizumab beyond 30 plus 28 days (>58 days) post PEX, range 62-121 days. 3 patients had persistent severe ADAMTS13 deficiency at 30+28 days.

For comparison, the proportion of non-caplacizumab treated patients failing to achieve an ADAMTS13 activity >30% at 30 and 30+28 days are shown in figure 1c. 3/50 patients in this group did not survive the acute admission. A smaller number of patients remained severely deficient in ADAMTS13 activity at 30 days and 30+28 days. Patients with an ADAMTS13 activity <30% at 30+28 days took a median of 84 days (range 33-135 days) to achieve an ADAMTS13 activity >30%.

In summary, following extended use of caplacizumab (31 to 58 days), 18/64 (28%) failed to achieve an ADAMTS13 activity >30% at the time of stopping caplacizumab. This was significantly lower at 4/47 (8.5%) at 30+28 days following PEX in the non-caplacizumab group ($p<0.0001$). The risk of failing to achieve an ADAMTS13 activity >30% within 2 months post PEX was 6 times more likely in patients treated with caplacizumab (OR 6.3, 95% CI 2.15-17.94, $p=0.0006$).

ADAMTS13 activity <10% at time of caplacizumab discontinuation

18/64 patients (28%) had an ADAMTS13 activity <10% at time of stopping caplacizumab taking a median of 139 days (IQR 83.5-213 days) after completing PEX to achieve a sustained ADAMTS13 activity >30%. In contrast, in the remaining 46/64 responders who had rising ADAMTS13 activity levels at time of caplacizumab discontinuation, it took a median of 22 days (IQR 12-33 days, $p<0.0001$). The delay in achieving an ADAMTS13 activity >30% in the caplacizumab cohort prompted further analysis of ADAMTS13 parameters in this sub-group in relation to subsequent clinical outcomes.

Anti-ADAMTS13 IgG antibody and ADAMTS13 antigen at caplacizumab discontinuation

The potential role of anti-ADAMTS13 IgG antibody level and ADAMTS13 antigen in predicting delayed normalisation of ADAMTS13 activity was investigated in patients with an ADAMTS13 activity <10% at the time of stopping caplacizumab (n=18), compared to those cases in whom ADAMTS13 activity increased (n=46). There was no difference in presenting anti-ADAMTS13 IgG antibody level (at time of acute TTP diagnosis), median 41% (IQR 19-83.5%) vs 39% (14.5-72.25%, $p=0.95$) or

presenting ADAMTS13 antigen, median 2% (IQR 1-4.75%) vs 4% (IQR 2-13%, p=0.08).

Anti-ADAMTS13 IgG antibody and ADAMTS13 antigen levels at time of stopping caplacizumab were significantly different as expected, with higher anti-ADAMTS13 IgG levels and lower ADAMTS13 antigen levels in patients who were severely deficient in ADAMTS13 activity on stopping caplacizumab (table 2). Patients who were severely deficient in ADAMTS13 activity at time of stopping caplacizumab were also more likely to have a rising anti-ADAMTS13 IgG antibody level from the time of diagnosis to when they stopped caplacizumab, compared to responders. Conversely the majority of responders had a falling anti-ADAMTS13 IgG level at cessation of caplacizumab (39/46 vs 4/18 in patients with ADAMTS13 activity <10% at time of stopping caplacizumab). Race did not influence time taken to achieve an ADAMTS13 activity >30% in these groups either (p=0.6).

Non-caplacizumab group ADAMTS13 assays

In the 4/47 patients who had an ADAMTS13 activity <10% at 30 days post PEX, the median anti-ADAMTS13 IgG antibody levels at presentation and 30+28 days were 44% (IQR 27.5-86%) and comparable to the caplacizumab cohort, and 40% (IQR 34.5-86) respectively. In these 4 cases, the IgG antibody level increased in two patients, decreased in another and was stable in the third patient.

TTP recurrence

Three patients experienced exacerbation(s) in the caplacizumab cohort. Two patients had an exacerbation 7 days after stopping caplacizumab (42 days after completion of PEX) and 10 days after stopping caplacizumab (60 days after completion of PEX). Both had an ADAMTS13 activity <10% at the time of exacerbation. A 3rd patient had two exacerbations occurring 14 and 11 days after stopping caplacizumab respectively. ADAMTS13 activities were <10% for both exacerbations. The second exacerbation in patient 3 was treated with caplacizumab and further immunosuppression (2 additional rituximab and MMF) alone without PEX due to mild thrombocytopenia (platelet count 139 x 10^9/L) and therefore not included in the analysis. There were no deaths in the caplacizumab treated group.

ADAMTS13 activity, anti-ADAMTS13 IgG antibody and ADAMTS13 antigen levels in TTP exacerbation and non-exacerbation cases are shown in figure 2/table 3.

In the non-caplacizumab patient cohort, 4 patients had an exacerbation within 30 days of stopping PEX and had an ADAMTS13 activity <10%. 1 patient had a clinical relapse within 1 year and this occurred at 37 days following previous exacerbation of TTP following a non-sustained partial rise in ADAMTS13 activity.

ADAMTS13 antigen level as a predictor for recurrence and to guide safe discontinuation of caplacizumab in patients with ADAMTS13 activity <10%

ADAMTS13 antigen thresholds were investigated in patients who were severely deficient in ADAMTS13 activity at the time of stopping caplacizumab (table S1). A higher recurrence rate was observed in patients with an antigen level <30%. ADAMTS13 antigen levels above 30% in this cohort did not experience a recurrence despite high IgG antibody levels. Even at high levels, anti ADAMTS 13 IgG antibody did not predict recurrence.

Immunosuppression used during acute TTP

To exclude confounders to the delayed normalisation seen in caplacizumab treated patients, immunosuppression/immunomodulatory therapy including steroid use and anti-CD20 treatment with rituximab was analysed (Table 4). More doses of rituximab were required in the caplacizumab cohort and although not statistically significant, more cases relatively required additional immunosuppression such as mycophenolate (MMF) or bortezomib.

Discussion

Caplacizumab therapy is now an integral part of the management of patients with acute iTTP. The benefits of this additional agent to standard of care has been shown via the TITAN[5] and HERCULES[6] clinical trials which focused on time to platelet normalisation as the primary outcome. Our analysis of caplacizumab treated patients confirms the shorter time to platelet normalisation (4 vs 9 days for non-caplacizumab group, $p<0.0001$) and associated fewer days of PEX (6 versus 13 days for non-caplacizumab group, $p<0.0001$) comparable with other real world data reported.[13,14]

Volker et al[15] reported a median of 3 days to achieve a normal platelet count after starting caplacizumab, with a median 9 days of PEX required to achieve this. However, caplacizumab was started with a median of 3 days after diagnosis of TTP in their patients, whereas our patients receive caplacizumab once the diagnosis is confirmed within 24 hours of presentation and this is likely to explain this observed difference.

With increasing use and familiarity of caplacizumab, it became apparent that in caplacizumab treated patients, there is a delay in a proportion of patients achieving an ADAMTS13 activity >30%. Coppo et al[14] reported a median of 28 days to achieve ADAMTS13 activity ≥20% in patients treated with the triplet regime (PEX, rituximab and steroids, and caplacizumab). We evaluated patients treated with PEX, steroids and rituximab in the pre-caplacizumab era, as a comparator to show the delayed normalisation in a subgroup of patients in the caplacizumab era. In our cohort, the time taken to achieve an ADAMTS13 activity >30% from completion of PEX was nearly 3 times longer when analysing all caplacizumab treated patients compared to historical non caplacizumab treated acute TTP cases. Furthermore, nearly a third of our cohort had ongoing severe ADAMTS13 activity deficiency at the time of stopping caplacizumab, despite extended use. In this subgroup of patients, a median of 139 days was required to achieve an ADAMTS13 activity >30%. Significantly more caplacizumab treated patients failed to achieve an ADAMTS13 activity >30% at 30+28 days (2 months post PEX) than in the pre-caplacizumab era.

Presenting anti-ADAMTS13 IgG levels were not predictive of the delayed normalisation of ADAMTS13 activity, yet a rise in antibody level from diagnosis to the time of stopping caplacizumab does appear relevant. Median ADAMTS13 antigen level was significantly lower at the time of stopping caplacizumab in patients with severe ADAMTS13 activity levels. Concurrent ADAMTS13 antigen levels <30% at time of caplacizumab discontinuation were associated with a greater risk of recurrence. However, raised anti-ADAMTS13 IgG levels was not predictive of TTP recurrence.

Whilst the TITAN trial showed that early relapses occurred in a subgroup of patients with ADAMTS13 activity <10% when caplacizumab was stopped, the HERCULES

trial design was adapted to allow continuation of caplacizumab if ADAMTS13 activity remained severely deficient. These changes led to fewer relapses in the HERCULES trial, and the relapses that occurred were in patients who still had ADAMTS13 activity <10%. Of the 120 patients who had ADAMTS13 activity levels available at the time of stopping caplacizumab/placebo (60 at the end of the period of double-blind administration of caplacizumab, 34 at the end of the period of double-blind administration of placebo, and 26 at the end of the period of open-label administration of caplacizumab), 29 patients (24%) had an ADAMTS13 activity <10%. 9 patients from this group had a relapse, of which 3 received open label caplacizumab. We identified 28% of caplacizumab treated patients had severely deficient ADAMTS13 activity at the time of stopping caplacizumab in our cohort.

In the HERCULES trial ADAMTS13 antigen levels were not examined. ADAMTS13 antigen levels have been shown to be variable at iTTP presentation despite an ADAMTS13 activity <10%[16–18] and to have prognostic significance.[18,19] Here, we show the use of ADAMTS13 antigen levels to guide stopping caplacizumab therapy.

Volker et al[20] have suggested that an ADAMTS13 guided approach can be used to guide caplacizumab therapy and prevent overtreatment and undertreatment. Whilst anti-ADAMTS13 IgG antibody and low ADAMTS13 activity levels were associated with greater risk of TTP recurrence, a severe deficiency of ADAMTS13 antigen was not[21]. As suggested by the authors, this may be explained by the small sample size. Our analysis importantly adds further insight to guide stopping caplacizumab as well as highlighting risk of exacerbations/relapses in those patients with ongoing severe ADAMTS13 deficiency. In patients with ADAMTS13 activity <10%, a severely deficient ADAMTS13 antigen level <30% was predictive of a higher risk of recurrence.

Steroid use was investigated as a possible confounder for the delayed normalisation of ADAMTS13 activity in caplacizumab treated patients. No difference in overall dose of IV methylprednisolone or prednisolone use was demonstrated. However, there was an increased use of rituximab and other immunosuppression (MMF and bortezomib) since the introduction of caplacizumab reflecting the delayed normalisation of ADAMTS13 activity levels, a finding significantly different to the pre-

caplacizumab era. Chaturvedi et al[22] have recently shown that race affects response to rituximab in iTTP. They showed that black race was associated with shorter relapse free survival and whilst addition of rituximab to steroids improved relapse free survival in white patients, this was not evident in patients of black race. Furthermore, they showed that there was no effect of race related to ADAMTS13 activity recovery at 90 days. Similarly, our findings suggest that race does not influence time to achieving ADAMTS13 activity >30%.

Ongoing analysis of ADAMTS13 assays in future cases of recurrence would help to support the association, given this was a retrospective analysis. A prospective analysis to confirm that stopping caplacizumab once an ADAMTS13 antigen level>30% has been achieved in patients who remains severely deficient in ADAMTS13 activity would support our finding. Whether the smaller number of PEX procedures caplacizumab treated patients require play a role in the delayed normalisation of ADAMTS13 activity requires investigating too.

In summary, the use of caplacizumab in acute TTP has had a very positive impact in patient care. In a large cohort of caplacizumab treated iTTP patients, a delay in ADAMTS13 activity level increment (>30%) in 28% of cases was associated with increasing anti-ADAMTS13 IgG antibody levels and the need for further immunosuppressive therapy, when compared to consecutive cases in the pre-caplacizumab era. The reason for this observation is not clear and does not appear clearly attributable to differences in early immunosuppression, although patients receiving caplacizumab had fewer PEX procedures. However, the timing of PEX in relation to analysis of ADAMTS13 activity levels (30 days or 30+28 days) would make this less likely to be contributory. Our data suggests that ADAMTS13 antigen levels may provide a further laboratory marker to guide when caplacizumab therapy can be safely stopped. The precise mechanism causing delayed normalisation of ADAMTS13 activity in a proportion of patients treated in the caplacizumab era remains to be characterised and requires further investigation.

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Authorship Contributions

N.P. designed the research, conducted laboratory testing, collected data, analysed data and wrote the manuscript; M.T. designed research and reviewed the manuscript; M.S. collected data and reviewed the manuscript; J-P.W. reviewed the manuscript; R.dG. reviewed the manuscript; D.S. conducted laboratory testing and reviewed the manuscript; M.S. is senior author, conception of research, designed the research and reviewed the manuscript.

Conflict-of-interest disclosures

M.S. has received speaker fees and has served on advisory boards for Alexion, Novartis, Takeda, Sanofi, and Octapharma and has received research grants from Shire and Alexion. M.T. received speaker fees/honoraria/served on advisory boards for Sanofi, Bayer and Anthos. The remaining authors declare no competing financial interests. J-P.W. received speaker fees and has served on advisory boards for Sanofi.

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Table 1. Demographics of patients treated with and without caplacizumab.

	Caplacizumab	Non-caplacizumab
Number analysed	64	50

Male: Female (%)	22/64:42/64 (34:66%)	13/50:37/50 (26:74%)
Median age (range)	47 (14-78)	45 (15-89)
Ethnicity - Asian - Black - White	 7 (11%) 26 (41%) 31 (48%)	 9 (18%) 13 (26%) 28 (56%)
De novo: Recurrence	59:5	46:4
Mortality (%)	0%	3/50 (6%)

Table 2. Median anti-ADAMTS13 IgG antibody and ADAMTS13 antigen levels in patients who remained severely deficient in ADAMTS13 activity at time of stopping caplacizumab compared to patients who showed an increase in ADAMTS13 activity >10IU/dL.

Levels at time of stopping caplacizumab	Severely deficient in ADAMTS13 activity (n=18)	Responders: ADAMTS13 activity >10IU/dL (n=46)	P value
Median anti-ADAMTS13 IgG antibody (IQR)	72% (32-107%)	6.5% (3.75-18%)	<0.0001
Rising anti-ADAMTS13 IgG antibody	12/18 (67%)	3/46 (6.5%)	<0.0001
Median ADAMTS13 antigen (IQR)	31% (10.75-51%)	77.5% (46.5-99%)	<0.0001

Table 3. Differences in ADAMTS13 activity, anti-ADAMTS13 IgG antibody and ADAMTS13 antigen levels between patients who experienced exacerbation and no exacerbation.

	No exacerbation (n=61)	Exacerbation (n=4)
Median ADAMTS13 activity (IQR)	50IU/dL (9.35-80IU/dL)	<10IU/dL
Median Anti-ADAMTS13 IgG antibody (IQR)	11% (4-40.5%)	80% (33.25-125.3%)
Median ADAMTS13 antigen (IQR)	63% (37.5-97%)	2% (1-3.75%)

Table 4. Comparison of immunosuppression and anti-CD20 treatment in caplacizumab and non-caplacizumab cohort.

	Caplacizumab	Non-caplacizumab	P value
Median days of IV methylprednisolone only (IQR)	2 (1-2 days)	2 (2-3.5 days)	0.1
Median dose of IV methylprednisolone (IQR)	1.5g (0.875-2g)	2g (1.25-2.25g)	0.2
Number of patients receiving steroids*	62/64	47/50	0.65
Days of prednisolone wean (IQR)	13 days (9-19 days)	16 days (10-18 days)	0.4
Number of patients receiving rituximab	61/64	43/50**	0.1
Median number of rituximab (range)	6 (4-8)	4 (1-8)	0.05
Number of patients receiving additional immunosuppression***	21/64 (33%)	12/50 (24%)	0.5

*Methylprednisolone and/or prednisolone

**Includes 2 patients who died (1 patient received 2 doses and another patient received 1 dose).5 patients had antiretroviral disease, 1 patient had viral encephalitis and clinical information was lacking in 1 patient.

***Caplacizumab patients: received MMF and 2 patients additionally received bortezomib. Non caplacizumab patients: majority received MMF except 3 patients who received bortezomib

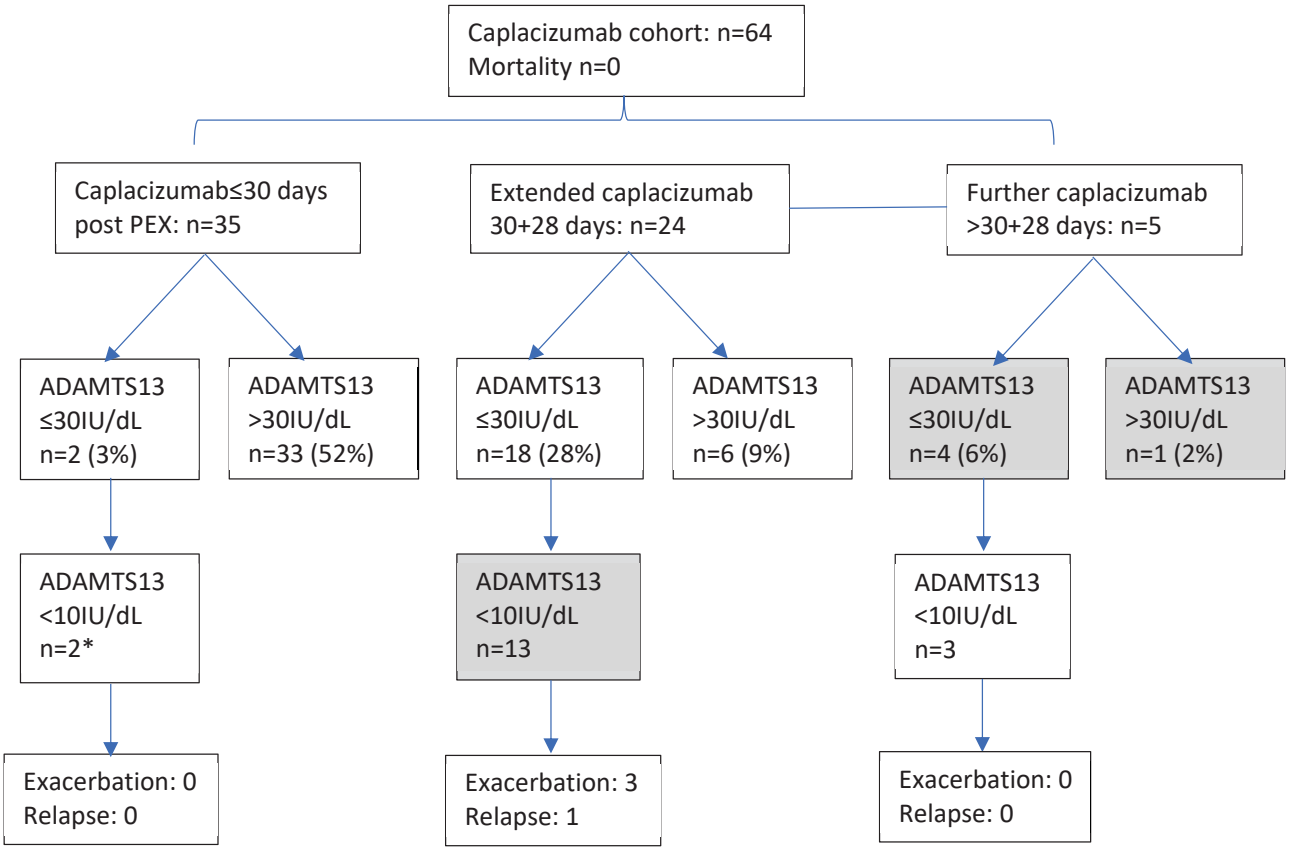
Legends: Figure 1 a) Duration of caplacizumab use after completion of PEX, ADAMTS13 activity at point of stopping caplacizumab and clinical outcomes. Failure to achieve an ADAMTS13 activity>30IU/dL was 6 times more likely in caplacizumab treated patients (OR 6.3, 95% CI 2.15-17.94, p=0.0006). This was calculated based on the 18 patients with an ADAMTS13 activity≤30IU/dL in those who received extended caplacizumab, and additionally 5 patients who continued to receive capalcizumab after 30+28 days due to ongoing ADAMTS13 activity<30IU/dL. This was compared to the 33 patients who received ≤30 days of caplacizumab and had an ADAMTS13 activity>30IU/dL at time of stopping caplacizumab, in addition to the 6 patients with an ADAMTS13 activity>30IU/dL in those who received extended caplacizumab. The 2 patients with early caplacizumab cessation were not included in this analysis.

b) ADAMTS13 activity at time of stopping caplacizumab and duration of caplacizumab used post completion of PEX.

c) ADAMTS13 activity at 30 days and 30+28 days in non-caplacizumab cohort and clinical outcomes.

Figure 2. Graphic representation of comparison of ADAMTS13 activity IgG antibody and ADAMTS13 antigen levels at time of stopping caplacizumab in patients with exacerbation and no exacerbation. ADAMTS13 activity<10IU/dL has been depicted at a level of 5IU/dL for clarity on figure 2. Patients with exacerbation of TTP had a lower ADAMTS13 activity, higher anti-ADAMTS13 antibody level and lower ADAMTS13 antigen level.

Figure 1a



* early drug cessation due to widespread rash secondary to caplacizumab.

Indicates number of patients who remained severely deficient in ADAMTS13 activity at 30+28 days.

Figure 1b

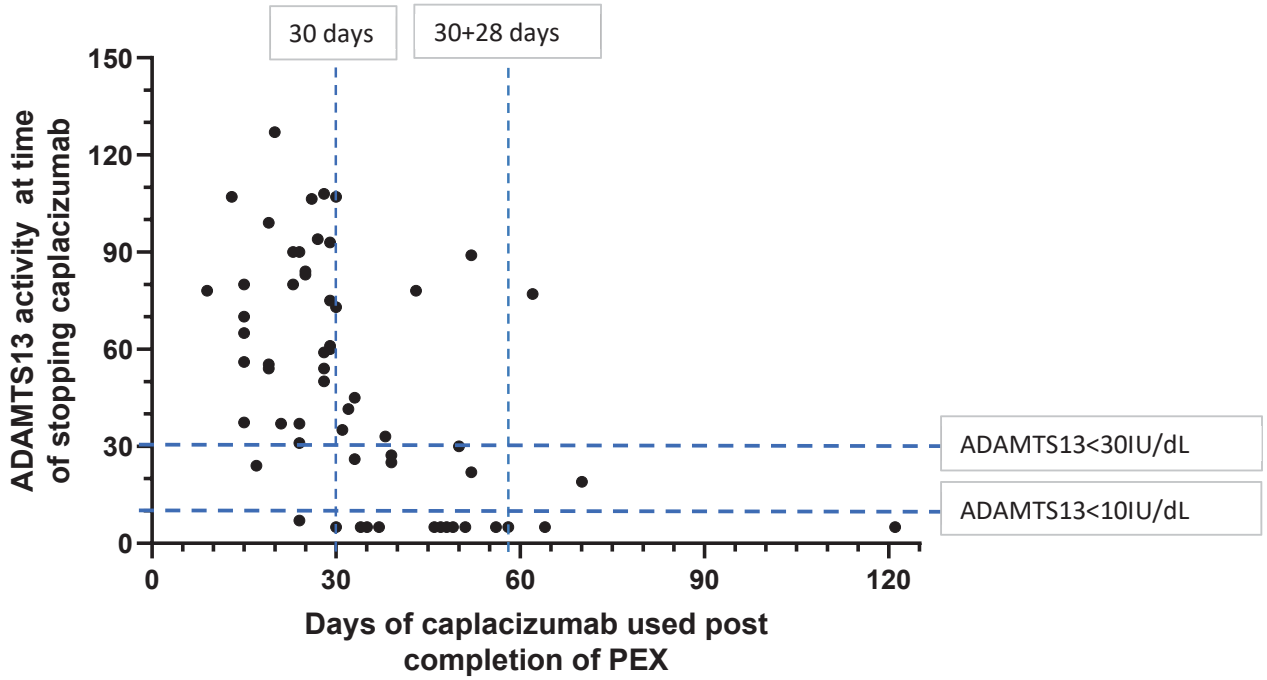


Figure 1c

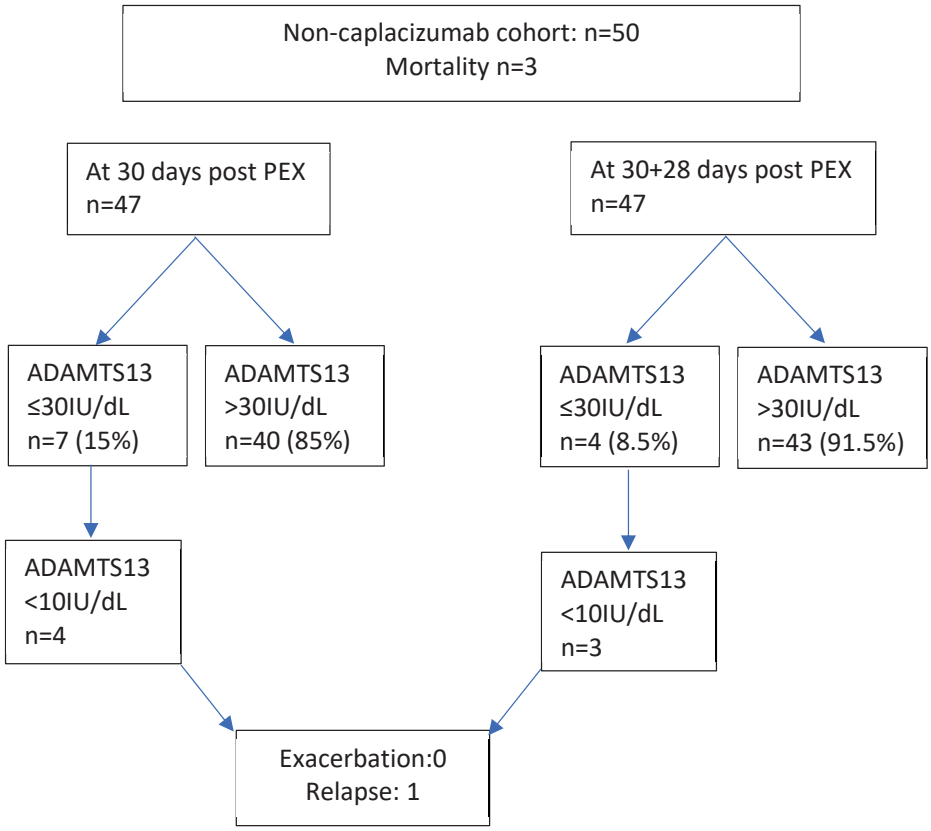
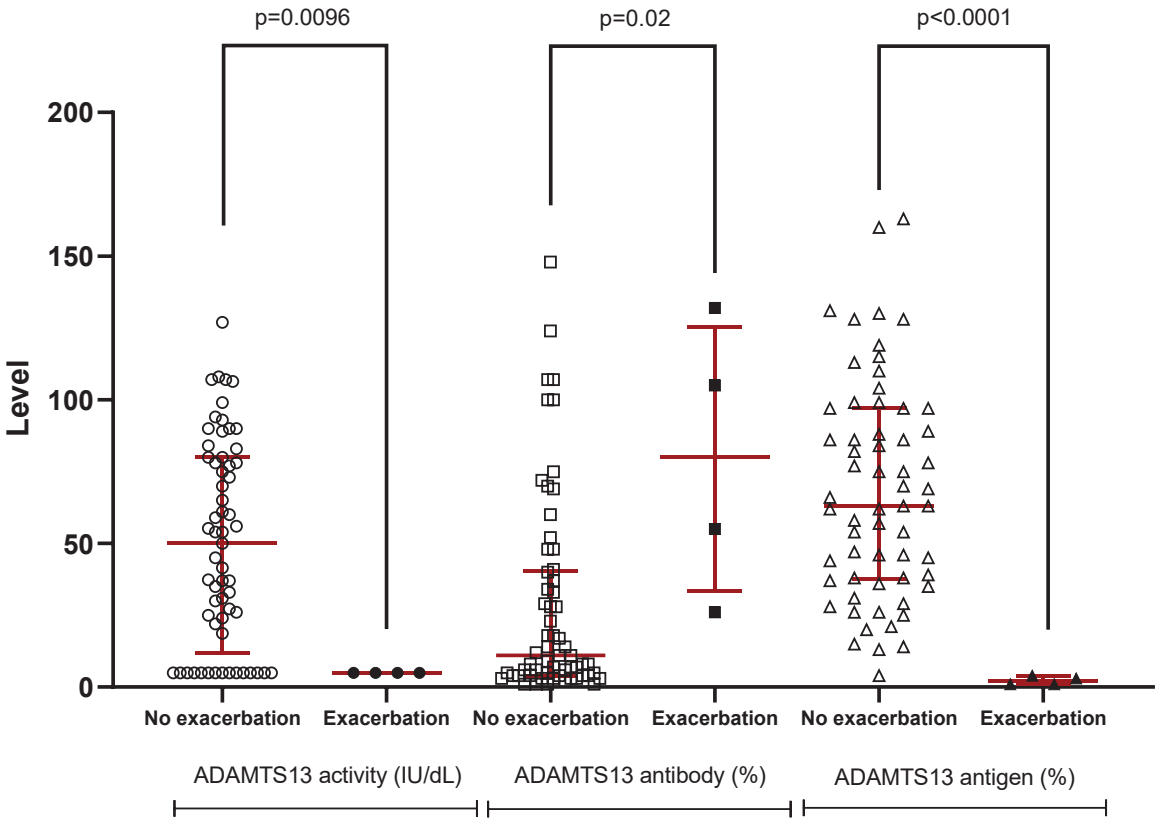


Figure 2


p=0.0096
p=0.02
p<0.0001
200
150
100
50
0
Level
No exacerbation
Exacerbation
No exacerbation
Exacerbation
No exacerbation
Exacerbation
ADAMTS13 activity (IU/dL)
ADAMTS13 antibody (%)
ADAMTS13 antigen (%)