Anti-ADAMTS13 autoantibody profiling in patients with immune-mediated thrombotic thrombocytopenic purpura

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Key Points

- The anti-ADAMTS13 autoantibody profile comprising only anti-CS autoantibodies is the most prevalent during both acute phase and remission.
- Anti-CS autoantibodies seem to persist or reappear during remission, underscoring the relevance of targeted anti-CS autoantibody therapy.

Anti-A Disintegrin and Metalloproteinase with a Thrombospondin type 1 motif, member 13 (ADAMTS13) autoantibodies cause a severe ADAMTS13 deficiency in immune-mediated thrombotic thrombocytopenic purpura (iTTP). ADAMTS13 consists of a metalloprotease (M), a disintegrin-like (D) domain, 8 thrombospondin type 1 repeats (T1-T8), a cysteine-rich (C), a spacer (S), and 2 CUB domains (CUB1-2). We recently developed a high-throughput epitope mapping assay based on small, nonoverlapping ADAMTS13 fragments (M, DT, CS, T2-T5, T6-T8, CUB1-2). With this assay, we performed a comprehensive epitope mapping using 131 acute-phase samples and for the first time a large group of remission samples (n = 50). Next, samples were stratified according to their immunoprofiles, a field that is largely unexplored in iTTP. Three dominant immunoprofiles were found in acute-phase samples: profile 1: only anti-CS autoantibodies (26.7%); profile 2: both anti-CS and anti-CUB1-2 autoantibodies (12.2%); and profile 3: anti-DT, anti-CS, anti-T2-T5, anti-T6-T8, and anti-CUB1-2 autoantibodies (8.4%). Interestingly, profile 1 was the only dominant immunoprofile in remission samples (52.0%). Clinical data were available for a relatively small number of patients with acute iTTP (>68), and no correlation was found between immunoprofiles and disease severity. Nevertheless, profile 1 was linked with younger and anti-T2-T5 autoantibodies with older age and the absence of anti-CUB1-2 autoantibodies with cerebral involvement. In conclusion, identifying acute phase and remission immunoprofiles in iTTP revealed that anti-CS autoantibodies seem to persist or reappear during remission providing further support for the clinical development of a targeted anti-CS autoantibody therapy. A large cohort study with acute iTTP samples will validate possible links between immunoprofiles or anti-domain autoantibodies and clinical data.
Introduction

The life-threatening disease, immune-mediated thrombotic thrombocytopenic purpura (iTTP), is caused by autoantibodies targeting the von Willebrand factor (VWF)-cleaving protease A Disintegrin and Metalloproteinase with a Thrombospondin type 1 motif, member 13 (ADAMTS13). ADAMTS13 is a multidomain metalloprotease consisting of a metalloprotease (M) domain, a disintegrin-like (D) domain, a first thrombospondin type 1 (T) repeat, a cysteine-rich (C) domain, and a spacer (S) domain; 7 additional thrombospondin type 1 repeats (T2-T8); and 2 CUB domains (CUB1-2). During the acute phase of iTTP, anti-ADAMTS13 autoantibodies inhibit and/or clear ADAMTS13 activity. Therefore, we developed an ELISA allowing a high-throughput screening of iTTP patient plasma or serum samples paving the way for novel epitope mapping enzyme-linked immunosorbent assay (ELISA) based strategies in iTTP. Studying links between ADAMTS13 autoantibodies and disease severity, exacerbation, and relapse might lead to a better insight into the diversity of the immune response and into the changes of the immune response during disease progression (acute phase, remission, and relapses). Unraveling the immunoprofiles not only in acute phase but also in remission could contribute to a rational design of targeted anti-ADAMTS13 autoantibody strategies in iTTP. Studying links between immunoprofiles and disease severity, exacerbation, and relapse might also improve the management of patients with iTTP as has been demonstrated for other autoimmune diseases, whereas an immunoprofile might help to predict response to specific therapies.

Therefore, in this study, we aimed to identify immunoprofiles in patients with iTTP based on the presence or absence of anti-M, anti-DT, anti-CS, anti-T2-T5, anti-T6-T8, and anti-CUB1-2 autoantibodies in both acute-phase and remission samples. Our large multicenter cohort consisted of 213 iTTP patients, from whom we had 159 acute iTTP samples and 206 remission samples. Detection of anti-M, anti-DT, anti-CS, anti-T2-T5, anti-T6-T8, and anti-CUB1-2 autoantibodies was performed using a recently developed novel assay. Samples were then stratified according to their anti-ADAMTS13 autoantibody profiles. Next, immunoprofiles were compared between acute and remission phases and were studied in follow-up samples. Finally, we investigated whether certain immunoprofiles or certain domain-specific autoantibodies were linked with disease severity.

Materials and methods

iTTP patient samples

In total, 365 samples from 213 patients with iTTP were available for this study. The citrated plasma or serum samples from 30 patients with iTTP from Budapest Semmelweis University; 77 patients with iTTP from the Center for Thrombosis and Hemostasis, University Medical Center Mainz; and 106 patients with iTTP from the French Reference Center for Thrombotic Microangiopathies were investigated. Written informed consent in accordance with the Declaration of Helsinki was obtained from each patient. The study protocol had been approved by Ethics Committee on Human Clinical Research (263/PI/2011 [8361-1/2011-ECU]; Budapest, Hungary), Ethik-Kommission, Landesärztekammer Rheinland-Pfalz (837.506.15 [10274]; Mainz, Germany), and the Ethics Committee of the Hospital Pitié-Salpêtrière and the Hospital Saint-Antoine (NTC00426686; Paris, France).

The acute phase of iTTP is defined by microangiopathic hemolytic anemia, severe thrombocytopenia and organ ischemia, a severely deficient ADAMTS13 activity (<10 IU/dL), and the presence of anti-ADAMTS13 autoantibodies. In remission, the clinical parameters were in the normal range (no active clinical signs); however, in some cases, the ADAMTS13 activity remained severely deficient. The samples were taken during the acute phase (n = 159) or in remission (n = 206). Samples from the French cohort have been previously used to investigate ADAMTS13 conformations, anti-idiotypic antibodies and reactivity against ADAMTS13 variants, samples from the German cohort for ADAMTS13 conformation, and samples from the Hungarian cohort for autoantibody subclass distribution. Medical records of 86 patients with iTTP from the French cohort were extensively reviewed to collect biological and clinical data (eg, platelet count, lactate dehydrogenase [LDH] level, age, cerebral involvement, administered treatment).

Immunoprofiling based on epitope mapping of anti-M, anti-DT, anti-CS, anti-T2-T5, anti-T6-T8, and anti-CUB1-2 autoantibodies from iTTP patient samples

Epitope mapping of patient samples was performed as described previously, using the following ADAMTS13 fragments with the N-terminal albumin domain 1 (AD1) as a fusion partner with AD1-ADAMTS13, AD1-M, AD1-DT, AD1-CS, AD1-T2-T5, AD1-T6-T8, and AD1-CUB1-2. As a control, AD1 was used. All fragments were transiently expressed in Chinese hamster ovary (CHO)-derived cell line CHOEBNALT85 in association with pQMCF vectors (USPTO patent no. 7 790 446), and purified from the culture media using immobilized metal ion affinity chromatography. Briefly, the 96-well plates were coated at 5 μg/mL with AD1-ADAMTS13, AD1-M, AD1-DT, AD1-CS, AD1-T2-T5, AD1-T6-T8, AD1-CUB1-2, or with AD1. AD1 was coated in triplicate for each patient sample. After blocking, the patient plasma or serum samples were added in a 1-in-40 dilution. Bound immunoglobulin G (IgG) was detected by horseradish peroxidase-labeled goat anti-human IgG (Fc specific) antibodies (1/10 000; Sigma-Aldrich, St Louis, MO). Colorimetric development was performed by 3,3′,5,5′-tetramethylbenzidine VII solution (Biopana Diagnostics, Belfast, UK). The reaction was stopped using 0.5 M H2SO4, and
absorbance was measured at 450 nm. On each plate, human monoclonal antibody II-1 (0.0156 μg/mL) binding to AD1-ADAMTS13 was used as a reference. The optical density (OD) value corresponding to II-1 binding to AD1-ADAMTS13 was set as 1 to calculate the relative OD values. The mean OD value (+3× standard deviations) of each patient’s IgG binding to AD1 was subtracted from the OD value of the IgG binding to the respective fragment. A residual OD value >0.2 was considered positive as determined in our previous study.14

Next, patients were stratified according to their immunoprofile.

Statistical analysis
Differences between groups were assessed by χ² or Fisher’s exact test for categorical variables, and Mann-Whitney or Kruskal-Wallis test followed by post hoc Dunn’s multiple comparisons test for continuous variables in Prism v6.0.1 (GraphPad Software, La Jolla, CA).

Results

Percentages of acute-phase and remission samples with anti-M, anti-DT, anti-CS, anti-T2-T5, anti-T6-T8, and anti-CUB1-2 autoantibodies

We aimed to identify immunoprofiles based on the presence or absence of anti-M, anti-DT, anti-CS, anti-T2-T5, anti-T6-T8, and anti-CUB1-2 autoantibodies in a large cohort of patients with iTTP both in acute phase and remission. Therefore, 365 samples from 213

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**Figure 1.** Fine mapping of anti-ADAMTS13 autoantibodies in acute-phase and remission samples. (A) Flow chart representing an overview of the number of patients and their corresponding plasma or serum samples analyzed in this study. A total of 365 plasma or serum samples from 213 patients were selected and screened for the presence of anti-ADAMTS13 autoantibodies. More than 1 sample was available for 92 patients. Samples were both from acute phase (orange) and remission (blue). Positive samples were further screened for the presence or absence of anti-M, anti-DT, anti-CS, anti-T2-T5, anti-T6-T8, and anti-CUB1-2 autoantibodies. From the positive samples, more than 1 sample was available for 27 patients. These samples can be identified in supplemental Table 1 as samples with the same patient identification number but with a different sample identification number. (B) Comparison of the percentage of samples with detectable anti-M, anti-DT, anti-CS, anti-T2-T5, anti-T6-T8, and anti-CUB1-2 autoantibodies between acute-phase samples (n = 131) and remission samples (n = 50). Fisher’s exact test; *P < .05; ***P < .001; ****P < .0001.
patients with iTTP were selected for this study, of which 159 samples were taken during acute phase before plasma exchange and 206 during remission (Figure 1A). Of the 365 selected samples, 230 had detectable anti-ADAMTS13 autoantibodies. As expected, the majority of the samples without detectable anti-ADAMTS13 autoantibodies were remission samples (n = 129). The 230 samples with detectable anti-ADAMTS13 autoantibodies were further analyzed for the presence or absence of anti-M, anti-DT, anti-CS, anti-T2-T5, anti-T6-T8, and anti-CUB1-2 autoantibodies. In 131 of the acute-phase samples and in 50 of the remission samples, anti-ADAMTS13 autoantibodies could be detected against at least 1 of the analyzed fragments (Figure 1A). In the remaining 49 samples, overall anti-ADAMTS13 autoantibody titers were too low to detect autoantibodies against small fragments, or the autoantibodies did not recognize the small ADAMTS13 fragments.

Before stratifying the samples according to their immunoprofile, we first analyzed the percentage of samples that contained anti-M, anti-DT, anti-CS, anti-T2-T5, anti-T6-T8, or anti-CUB1-2 autoantibodies in the acute-phase and remission samples as has been done in previous epitope mapping studies using acute-phase plasma samples. These data allowed us for the first time to compare percentages of samples which were positive for anti-ADAMTS13 domain-specific antibodies between acute phase and remission. Our data show that anti-M autoantibodies were present in 9.2% of the acute-phase samples, but absent in the remission samples (P = .0387; Figure 1B). Detectable anti-DT autoantibodies were found in 25.2% of the acute-phase samples, and only in 4.0% of the remission samples (P = .0007; Figure 1B). In contrast, there was no difference between the percentages of acute-phase and remission samples having anti-CS autoantibodies (74.8% and 66.0%, respectively; Figure 1B). A slight difference was found in the presence of the anti-T2-T5 autoantibodies (35.1% and 20.0%, respectively; Figure 1B), and in the presence of the anti-T6-T8 autoantibodies (38.2% and 22.0%, respectively; Figure 1B), although the difference was statistically not significant. Finally, anti-CUB1-2 autoantibodies were more prevalent (P < .0001) in acute phase (56.5%) than in remission samples (22.0%; Figure 1B).

Interestingly, 84.6% (33/39) of the patients in remission with an ADAMTS13 activity <10% had anti-CS autoantibodies, whereas 100% of the patients in remission (n = 11) with an ADAMTS13 activity >10% had no anti-CS autoantibodies (Figure 2).

In conclusion, we show that anti-CS autoantibodies are not only the dominant autoantibodies in acute phase but also in remission. However, other anti-ADAMTS13 domain-specific autoantibodies are more prevalent in acute phase than in remission.

**Immunoprofiles based on the presence or absence of anti-M, anti-DT, anti-CS, anti-T2-T5, anti-T6-T8, and anti-CUB1-2 autoantibodies in acute-phase and remission samples**

We next stratified the samples according to their immunoprofile to identify which domain-specific autoantibody combinations were most prevalent. We identified 33 domain specific autoantibody combinations, and arranged these immunoprofiles according to their prevalence in the acute-phase samples (Figure 3). An overview of the individual immunoprofiles of all analyzed samples can be found in supplemental Table 1. In acute phase, 31 of the 33 immunoprofiles were present, whereas in remission only 12 of the 33 immunoprofiles were identified (Figure 3). Three dominant immunoprofiles (each in >8%) in acute-phase samples could be identified that covered 47.3% of the analyzed acute-phase samples: profile 1, which consists of only detectable anti-CS autoantibodies (in 26.7% of the acute plasma samples); profile 2, in which both detectable anti-CS and anti-CUB1-2 autoantibodies were present (in 12.2% of the acute plasma samples); and profile 3, in which anti-DT, anti-CS, anti-T2-T5, anti-T6-T8, and anti-CUB1-2 autoantibodies were present (in 8.4% of the acute plasma samples). In contrast, only 1 dominant immunoprofile could be identified in the remission samples, profile 1 (only anti-CS autoantibodies), which was present in 52.0% of the remission samples (Figure 3).

In conclusion, acute-phase samples are characterized by 3 dominant immunoprofiles, whereas only 1 dominant immunoprofile was detected in remission samples (profile 1, only anti-CS autoantibodies).

**Immunoprofiles during disease course**

Because epitope spreading is known during disease course in iTTP,6 we studied the immunoprofile alteration in the follow-up samples from 15 patients available in our cohort (supplemental Figure 1). The immunoprofile in the first acute phase or remission differed from those in the later acute relapse phase(s), as well as immunoprofiles between the first acute/relapse phases and remission changed (supplemental Figure 1). Studying these follow-up samples also allowed us to follow the changes in autoantibody titers (expressed as relative OD; supplemental Figure 1). In most of the follow-up samples, a disappearance of previously detectable antibodies was observed, which is also in agreement with the overall decreasing antibody titers. However, in some cases, new domain-specific anti-ADAMTS13 autoantibodies were detected as well (supplemental Figure 1 B,D,J-L), which could be explained by an acute episode or a relapse.
Immunoprofiles and their link with severity of the acute iTTP episodes

We next studied the link between the immunoprofiles and disease severity, as assessed by the Benhamou score (n = 68) or its individual components (ie, age at sampling [n = 76], LDH level [n = 68], and cerebral involvement [n = 76]) in the samples of the French cohort because only for those samples was information on clinical data available, and the samples were from first acute phase from patients with idiopathic iTTP. We also studied the relation between platelet counts (n = 75) and immunoprofiles because platelet counts have been shown to be inversely correlated with anti-T2-T8 and/or anti-CUB autoantibodies, although no correlation was found in the most recent epitope mapping study.6

The Benhamou score (either 1, 2, 3, or 4) helps to identify patients with a worse prognosis at diagnosis and is based on 3 parameters: age, LDH level, and cerebral involvement.31 A score ≥3 has a positive predictive value for mortality and a score <3 has a negative predictive value. There was no link between the Benhamou score and the 3 most common immunoprofiles (profiles 1-3; Figure 4A). In addition, we also investigated whether the presence or absence of anti-M, anti-DT, anti-CS, anti-T2-T5, anti-T6-T8, and anti-CUB1-2 autoantibodies was linked with the Benhamou score, and also there no link was found (data not shown). Next, we looked at the individual parameters that determined the Benhamou score: age, LDH level, and cerebral involvement. The first parameter, age, was linked with the most common immunoprofile (profile 1; Figure 4B), where younger patients tended to have only anti-CS autoantibodies (P = .0033). Moreover, an older age was linked with the presence of anti-T2-T5 autoantibodies (P = .0012; Figure 4C). No further correlations were observed between age and other domain-specific autoantibodies (data not shown). There was no correlation between the second parameter, LDH level, and the 3 most common immunoprofiles (profiles 1, 2 and 3), nor with any of the domain-specific autoantibodies (data not shown). Interestingly, although the 3 most common immunoprofiles (profiles 1-3) were not linked with the presence of the third parameter, cerebral involvement (defined as presence of aphasia, confusion, headache, convulsion, disorder of language, seizure, transient focal defect, stroke, coma, and/or blindness; data not shown), was linked with the absence of anti-CUB1-2 autoantibodies (P = .0117; Figure 4D). No other correlations were found between cerebral involvement and any of the other domain-specific autoantibodies (data not shown).

Similarly to the study of Thomas et al,6 we also found no correlation between low platelet count and the 3 most common immunoprofiles (profiles 1-3) nor between the presence of domain-specific autoantibodies (data not shown).

Discussion

In this study, we determined anti-ADAMTS13 immunoprofiles based on the presence or absence of anti-M, anti-DT, anti-CS, anti-T2-T5, anti-T6-T8, and anti-CUB1-2 autoantibodies, in a large multicenter cohort of both acute-phase and remission iTTP plasma or serum samples. We stratified the iTTP patient samples according to their
Figure 4. Link between immunoprofile and domain-specific anti-ADAMTS13 autoantibodies and Benhamou score, age, and cerebral involvement. (A) Benhamou score according to the 3 most prevalent immunoprofiles. The Benhamou score is a biomarker of iTTP that is a surrogate marker for the presence of anti-ADAMTS13 autoantibodies. 

Interestingly, we found that anti-CS autoantibodies were not only the most prevalent in acute-phase samples, but also in remission samples. However, anti-CS autoantibody titers (expressed as relative OD; supplemental Figure 2) were not significantly different (Mann-Whitney test, \( P = 0.7091 \)) between acute-phase and remission samples. To the best of our knowledge, anti-ADAMTS13 autoantibody epitopes have not been studied in a large set of remission samples before. Of note, using our epitope mapping assay, we detected anti-CS autoantibodies in 74.8% of the acute-phase samples, whereas other studies reported anti-CS autoantibodies in up to 98% of the samples. 

To confirm our findings, we verified our data by screening the same samples on an ADAMTS13 variant where the ADAMTS13 spacer domain was swapped with an ADAMTS1 spacer domain. These data showed that the majority of the samples without anti-CS autoantibodies also did not have detectable anti-S autoantibodies. Whether differences in the percentage of patients with iTTP with detectable anti-CS autoantibodies in the previous 2 largest epitope-mapping studies might be due to the different types of assays used because we demonstrated direct binding of anti-CS autoantibodies to the coated CS fragment, whereas in the previous 2 largest epitope-mapping studies, the presence of anti-CS autoantibodies was deduced from data of autoantibodies binding to MDTCS fragments (ie, negative for binding to MDT\(^{11}\) or MD/MDTCS\(^6\) and positive for binding to MDTCS). Whether differences in the percentage of patients with iTTP with detectable anti-CS autoantibodies in the different studies could be related to diverse ethnicity could not be investigated because information on ethnicity was not available for our patient cohort. Of note, no correlation between ethnicity and anti-ADAMTS13 autoantibody epitopes was described in the previously reported 2 largest epitope-mapping studies. 

In addition, an ADAMTS13 activity <10% in remission was associated with the presence of anti-CS autoantibodies, whereas none of

Figure 4. (continued) score (\( \geq 3 \) [1-2]; \( \leq 3 \) [3-4]) was calculated based on age, cerebral involvement, and lactate dehydrogenase level. \( \chi^2 \) test; \( P = 0.4833 \). (B) Age of patients according to the 3 most prevalent immunoprofiles. Kruskal-Wallis test followed by Dunn’s multiple comparisons test; \( P = 0.0012 \). (C) Age of patients according to the presence and absence of anti-T2-T5 autoantibodies. Mann-Whitney test; \( P = 0.0333 \). (D) Cerebral involvement according to the presence and absence of anti-CUB1-2 autoantibodies. Fisher’s exact test; \( P = 0.0177 \).
the patients in remission with an ADAMTS13 activity >10% had detectable anti-CS autoantibodies. This further underscores the pathogenic nature of the anti-CS autoantibodies. Whether the appearance of anti-CS autoantibodies, like ADAMTS13 activity <10%, is another biomarker to predict relapse, remains to be determined.

Immunoprofiling iTTP patients according to the presence or absence of anti-M, anti-DT, anti-CS, anti-T2-T5, anti-T6-T8, and anti-CUB1-2 autoantibodies allowed us to unravel the diversity of the immune response between individual iTPP patients and this both in acute phase and remission. Although the diversity of immunoprofiles was relatively high, 3 main profiles were identified: profile 1 (only anti-CS autoantibodies), profile 2 (anti-CS and anti-CUB1-2 autoantibodies), and profile 3 (anti-DT, anti-CS, anti-T2-T5, anti-T6-T8, and anti-CUB1-2 autoantibodies). Interestingly, profile 1 was the dominant immunoprofile in both acute-phase (26.7%; 35/131) and remission samples (52.0%; 26/50). It is interesting to observe that profile 1 (only anti-CS antibodies) was the main profile in the remission samples, and it seems that anti-CS autoantibodies are the first to reappear or are the ones that persist during remission and that the other domain-specific autoantibodies are mainly appearing during acute phase.

Several research groups have invested in the development of targeted autoantibody therapies. ADAMTS13 variants have been designed with mutations in the spacer domain that prevent autoantibodies from binding and that are (partly) active or even overactive. Our group has developed anti-idiotypic antibodies that target anti-S autoantibodies in patients with iTPP. However, these strategies were based on the knowledge of autoantibody epitopes in acute phase. Because it was known that the immune response in acute phase is polyclonal, but with the majority of the iTPP patients having anti-CS autoantibodies, it was not clear whether iTPP patients with other anti-ADAMTS13 domain autoantibodies besides the anti-CS autoantibodies, would also benefit from these therapies. Our immunoprofiling results in remission samples now show that the majority of the iTPP remission samples (52.0%) have only anti-CS autoantibodies, making anti-CS autoantibodies very good targets in a personalized targeted therapy with a recombinant ADAMTS13 (rADAMTS13) variant that escapes the anti-CS autoantibodies because of the modified spacer domain. Therefore, the knowledge on immunoprofiles might support the improvement of targeted therapies for a better iTPP patient management. By knowing the anti-ADAMTS13 autoantibody repertoire in each patient with iTPP, the administered treatment could be adjusted to the knowledge of the immunoprofile. For example, if a patient has only anti-S autoantibodies, an rADAMTS13 variant could be used that has been mutated in the S domain to escape binding of anti-S autoantibodies. This kind of personalized therapy could be possible in the future, when (1) an easy-to-perform anti-ADAMTS13 autoantibody epitope mapping assay is available in the hospitals and (2) the radAMTS13 mutants are available as therapeutics for patients with TTP. Furthermore, the immunoprofiling could be used to monitor the development of anti-ADAMTS13 autoantibodies in both patients with iTPP and congenital TTP during the treatment of rADAMTS13. The administered radAMTS13 might trigger an immune response that could be easily detected using the epitope mapping ELISA.

We had follow-up samples available from 27 patients in our cohort to study changes in immunoprofile during disease course, and we observed a change in the immunoprofile in 15 patients (56%). In these 15 patients, we could identify changes in immunoprofiles between acute phase and remission, between acute phase and relapse, and between remission and relapse. Our results are in line with previous work in which alteration in the domain specificity of anti-ADAMTS13 autoantibodies during disease course has also been described. Future studies on large numbers of follow-up samples are needed, however, to get a deeper insight into changes in immunoprofiles during disease course in iTPP.

Autoantibody profiling is a promising tool to predict disease severity and outcome in autoimmune diseases. From 76 acute-phase samples, we had clinical data available to study a link between the immunoprofiles and disease severity. We found no correlation between immunoprofiles and disease severity. However, because our sample size in this part of the study was relatively small, a study using a large cohort of patient samples is needed to confirm our findings. In contrast, we did find that profile 1 was more prevalent in younger patients and anti-T2-T5 autoantibodies were more prevalent in older patients, as well as the absence of anti-CUB1-2 autoantibodies was correlated with cerebral involvement. However, Thomas et al could not link domain specificity of anti-ADAMTS13 autoantibodies with disease severity. Also, here it would be important to perform a large cohort study to validate these findings. In addition, it would be interesting to study whether immunoprofiles in combination with other biomarkers (e.g., ADAMTS13 activity, ADAMTS13 antigen, troponin, Benhamou score) are predictive for disease outcome and relapse.

In conclusion, we identified immunoprofiles in patients with iTPP from acute phase and for the first time also in a large cohort of patients with iTPP in remission. The most prevalent immunoprofile was the 1 in which patients had only anti-CS autoantibodies in both acute-phase (26.7%) and remission samples (52.0%). Having demonstrated that patients mainly have only anti-CS autoantibodies in remission underscores the clinical relevance for the development of targeted anti-CS autoantibody therapy. Because the number of follow-up samples and samples with biological investigations and clinical data were rather low in our cohort, a large cohort study is needed for an in-depth study on changes in immunoprofiles during disease course and for a study to identify a possible link between immunoprofiles and disease severity. In addition, investigating the link between immunoprofiles and a risk for relapse would also be interesting and might help improve the management of patients with iTPP.

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Authorship
Contribution: K.K. performed experiments, analyzed and interpreted the data, and wrote the manuscript; E.R., S.F.D.M., and A.M. interpreted data; J.V. provided monoclonal antibodies and interpreted data; B.S.J., P.C., A.V., G.S., Z.P., T.F., C.v.A., H.R., and B. provided plasma and/or serum samples; K.V. designed experiments, interpreted...
data, wrote the manuscript, and provided funding; and all authors critically reviewed the manuscript.

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