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ADAMTS13 conformation and immunoprofiles in Japanese patients with immune-mediated thrombotic thrombocytopenic purpura

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Key Points

- Open ADAMTS13 conformation is confirmed as a hallmark of acute iTTP in Japanese patients.
- The immunoprofile with only anti–cysteine-rich/spacer domain autoantibodies is the most dominant profile in Japanese patients with iTTP.

Immune-mediated thrombotic thrombocytopenic purpura (iTTP) is an ultrarare thrombotic disease caused by autoantibody-induced ADAMTS13 deficiency. Open ADAMST13 conformation, induced by autoantibodies, was identified as a novel biomarker for iTTP. Determining immunoprofiles in patients with iTTP has been shown to guide the development of novel targeted therapies. However, these studies were done in mainly Caucasian iTTP cohorts. To validate those findings across other ethnic cohorts, we investigated 195 acute TTP plasma samples from the Japanese iTTP registry. Seventy-six of the 195 samples had detectable ADAMTS13 antigen levels, of which 94.7% were shown to have an open ADAMTS13 conformation. A positive correlation was observed between ADAMTS13 inhibitor titers (a diagnostic parameter in Japan) and anti-ADAMTS13 immunoglobulin G autoantibody titers. Studying anti-M, anti-DT, anti-CS, anti-T2-T5, anti-T6-T8, anti–CUB1-2 autoantibodies and the corresponding immunoprofile showed that 73% of the patients had anti-CS autoantibodies and 25.8% had anti-M autoantibodies, with the latter being higher than in Caucasians. Stratifying patients according to their immunoprofiles revealed that the profile with only anti-CS autoantibodies was the most common immunoprofile similar to that in Caucasians (28.9%). Although this profile did not affect the 1-year TTP-related mortality rate, patients with autoantibodies against all 6 ADAMTS13 fragments had a higher risk for TTP-related death than other patients (P = .02). We here validated open ADAMTS13 as a novel biomarker for acute iTTP and determined the dominant immunoprofiling in the Japanese cohort, contributing to setting up the diagnosis and managing guidelines across different ethnic cohorts and developing ADAMTS13 variants that do not bind to the anti-CS autoantibodies.

Introduction

Thrombotic thrombocytopenic purpura (TTP) is an ultrarare and fatal thrombotic microangiopathy caused by ADAMTS13 deficiency, leading to severe thrombocytopenia, microangiopathic hemolytic anemia, and systemic microvascular thrombi.[1-3] ADAMTS13 is a metalloprotease consisting of

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a metalloprotease domain (M), a disintegrin-like (D) domain, a first thrombospondin type 1 repeat domain (T1), a cysteine-rich domain (C), a spacer domain (S), 7 more T domains (T2-T8), and 2 CUB domains (CUB1-2). More than 95% of patients with TTP develop anti-ADAMTS13 autoantibodies,[4,5] which is classified as immune-mediated TTP (iTTP).[6] Therapeutic plasma exchange (TPE) using fresh frozen plasma and corticosteroids is standard therapy for iTTP.[7,8] In addition, the anti-CD20 monoclonal antibody, rituximab, is often used to prevent TTP relapse by targeting ADAMTS13-specific B cells.[9,10]

Laboratory diagnosis of acute iTTP is based on an ADAMTS13 activity <10% and the presence of anti-ADAMTS13 autoantibodies. However, when ADAMTS13 activity is between 10% and 20%, the diagnosis of acute iTTP is less clear, and other thrombotic microangiopathies could be a differential diagnosis. Recently, we identified ADAMTS13 with an open conformation (open ADAMTS13) as a novel biomarker for acute iTTP and subclinical disease in remission.[11,12] Open ADAMTS13 has attracted clinical attention to correctly diagnose acute iTTP, especially when ADAMTS13 activity is between 10% and 20%.

Studying epitopes of anti-ADAMTS13 autoantibodies revealed that most patients have anti-CS autoantibodies but also autoantibodies against other ADAMTS13 domains.[13-15] Recently, we determined the immunoprofile in patients with iTTP and demonstrated that 3 dominant immunoprofiles could be identified: profile 1 with only anti-CS autoantibodies (26.7%); profile 2 with both anti-CS and anti–CUB1-2 autoantibodies (12.2%); and profile 3 with anti-DT, anti-CS, anti-T2-T5, anti-T6-T8, and anti–CUB1-2 autoantibodies (8.4%).[13] Knowing the immunoprofiles of anti-ADAMTS13 autoantibodies in patients with iTTP is essential for developing targeted anti-ADAMTS13 autoantibody therapies. Indeed, several groups have developed ADAMTS13 variants to which the anti-CS autoantibodies do not bind.[16,17] In addition, links between anti-ADAMTS13 autoantibody profiles and clinical presentations have been investigated to better predict outcomes in iTTP. High levels of anti-ADAMTS13 autoantibodies and IgG_4 subclass have been linked with a higher risk of relapse, and a link between the absence of anti–CUB1-2 autoantibodies and cerebral involvement was demonstrated by our group.[13,18]

Importantly, all these studies were mainly done on Caucasian cohorts, and little information is available for other ethnic cohorts, which is, however, crucial when developing diagnostic guidelines, risk factors for relapse, and novel treatment strategies that are applicable (or not) for the different ethnic cohorts worldwide.

Here, we used the unique, large Japanese iTTP cohort to study the conformation of ADAMTS13, total anti-ADAMTS13 autoantibody titers (not only inhibitors), and immunoglobulin G (IgG) subclasses of the anti-ADAMTS13 autoantibodies during the acute phase, and to identify the immunoprofiles of the anti-ADAMTS13 autoantibodies, information that is not available for this cohort yet.[19] We also investigated whether specific IgG subclasses, immunoprofiles, or domain-specific autoantibodies were linked with disease severity. Hence, this is the first study to explore well-known ADAMTS13 parameters in a Japanese cohort.

Methods

The Japanese iTTP cohort

The department of blood transfusion medicine at Nara Medical University established the only nationwide reference center for TTP in Japan in 1998. This Japanese TTP reference center harbors one of the largest patient cohorts worldwide.[19] For this study, 195 iTTP patient plasma samples taken during the acute phase (between 2004 and 2020) were selected. Based on the 2017 diagnostic and treatment guidelines for TTP in Japan,[20] iTTP was diagnosed when patients with severe thrombocytopenia and microangiopathic hemolytic anemia presented with a severe deficiency in ADAMTS13 activity (below 10% of healthy individuals) and with a detectable ADAMTS13 inhibitor. The sensitive chromogenic activity enzyme-linked immunosorbent essay (ELISA)[21] (detection limit of 0.5% of healthy individuals, Kainos, Japan) was used to measure ADAMTS13 activity. A plasma mixing assay was used to measure ADAMTS13 inhibitor titers (the cutoff level: 0.5 Bethesda units per mL). Two patients had undetectable ADAMTS13 inhibitor and for those, anti-ADAMTS13 IgG autoantibodies ELISA (TECHNOZYM ADAMTS13 INH ELISA Kit, Technoclone, Austria) was performed to detect total ADAMTS13 autoantibodies titers against ADAMTS13. Hence, ADAMTS13 activity and ADAMTS13 inhibitor titers were available before the start of this study.

This study was approved by the Ethics Committee of Nara Medical University and conducted under the tenets of the Declaration of Helsinki. Patients were considered to have provided informed consent if they did not opt out on a specified website. Those who opted out were excluded.

ADAMTS13 antigen ELISA

ADAMTS13 antigen levels were measured using our in-house developed ELISA as previously described.[22] Detailed information is found in the supplemental information.

ADAMTS13 conformation ELISA (1C4-ELISA)

ADAMTS13 conformation (open or closed ADMTS13) in plasma samples from patients with iTTP was determined using the previously described ADAMTS13 conformation ELISA (1C4-ELISA).[11,12] Detailed information is found in the supplemental information.

Anti-ADAMTS13 IgG antibody ELISA

Our in-house ELISA was used to determine total anti-ADAMTS13 IgG autoantibody levels as previously described.[23] Detailed information is found in the supplemental information.

IgG subclass ELISA

IgG subclasses (IgG_1, IgG_2, IgG_3, and IgG_4) of the patient anti-ADAMTS13 autoantibodies were determined as previously described.[18] Detailed information is found in the supplemental information.

Immunoprofiling ELISA

Immunoprofiling of patient anti-ADAMTS13 autoantibodies was done as previously described[13,24] using nonoverlapping ADAMTS13 fragments containing an N-terminal albumin domain 1

(AD1) as a fusion partner to enhance the protein expression. Detailed information is found in the supplemental information.

Statistical analysis

Fisher exact test was used to determine a significant association between 2 categorical variables. Clinical laboratory parameters between 2 and more than 3 independent groups were compared with Mann-Whitney U test and Kruskal-Wallis test, respectively. Gray test was used for the cumulative incidence of TTP-related deaths; the other cause of death was treated as a competing risk event. The final visit date and the date of clinical relapse were regarded as censors. Correlations between 2 variables were evaluated by Spearman analysis. All tests were 2-tailed, and a P value of $<.05$ was considered statistically significant. GraphPad Prism v9.3.1 (GraphPad Software, San Diego, CA) and EZR software version 1.55 (Saitama Medical center, Jichi Medical University, Saitama, Japan) were used for statistical analysis.

Results

Clinical information of the Japanese patients with iTTP included in the study

For this study, 195 acute iTTP plasma samples were selected, corresponding to 195 different Japanese patients with iTTP. Supplemental Table 1 describes (1) demographic information, (2) information on the occurrence of primary or secondary iTTP, (3) laboratory data including ADAMTS13 activity and ADAMTS13 inhibitor titers, (4) treatment, and (5) outcome. The median age was 55, and the female/male ratio was 111/84. Patients with iTTP were classified as having primary TTP (n = 166) or secondary iTTP, that is, iTTP linked with autoimmune disease (n = 25), pregnancy (n = 2), and others (n = 2). Both severe thrombocytopenia (median platelet count: 10.5 × 10^9/L) and hemolytic anemia (median hemoglobin level: 7.8 g/dL, median lactate dehydrogenase [LDH] level: 992 U/L, and median total bilirubin level: 3.0 mg/dL) were present in most patients. During the acute phase of iTTP, 167 patients received a combination regimen of TPE and corticosteroids, 24 patients received only steroids, and for 4 patients, no information was available. Additional immune-suppressive agents were administered to several patients as follows: rituximab in 66 patients, cyclophosphamide in 10 patients, cyclosporin A in 3 patients, and vincristine in 3 patients. The median follow-up period was 455 days (interquartile range; 57.5-1443). During follow-up, 20 patients died of iTTP episodes in the acute phase and 9 died of other causes (3 of sepsis, 3 of pneumonia, 1 of lung cancer, 1 of chronic liver failure, and 1 of subcortical hemorrhage).

ADAMTS13 conformation in the Japanese iTTP cohort

We first determined ADAMTS13 antigen levels in our Japanese iTTP cohort as ADAMTS13 antigen levels need to be >0.02 μg/mL to determine ADAMTS13 conformation.[11] As shown in Figure 1, ADAMTS13 antigen levels ranged from below 0.02 to 0.642 μg/mL. Of the 195 acute phase plasma samples, 81 were eligible for ADAMTS13 conformation determination (ADAMTS13 antigen levels were >0.02 μg/mL) (Figure 1; supplemental Table 2). Open ADAMTS13 was detected in 72 samples and closed ADAMTS13 in 4 samples. ADAMTS13 conformation could not be determined in the remaining 5 samples as the optical density values of the samples with and without mAb17G2 were below the OD

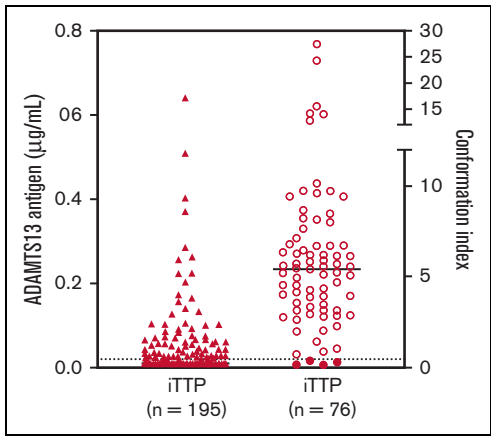

Figure 1. ADAMTS13 antigen and conformation in the Japanese iTTP cohort. ADAMTS13 antigen (triangle, n = 81) was determined using ELISA by capturing plasma ADAMTS13 with anti-ADAMTS13 mAb 3H9 and detecting captured ADAMTS13 with biotinylated anti-ADAMTS13 mAbs 19H4 and 17G2. ADAMTS13 conformation (open conformation, open circle, and closed conformation, closed circle; n = 76) was determined using ELISA where open ADAMTS13 was captured by anti-ADAMTS13 mAb 1C4 and bound ADAMTS13 detected with biotinylated anti-ADAMTS13 mAb 3H9. mAb1C4 does not capture closed ADAMTS13. OD492nm values corrected for ADAMTS13 Ag levels resulted in the CI. Only samples with ADAMTS13 antigen levels >0.02 μg/mL could be used to determine the ADAMTS13 conformation as shown in right panel (n = 76). The detection limit of the ADAMTS13 antigen ELISA is 0.02 μg/mL (dotted line) and the cutoff for determination of open-closed ADAMTS13 is a CI of 0.5 with CI < 0.5 closed ADAMTS13 and CI > 0.5 open ADAMTS13 (dotted line). CI, conformation index; mAb, monoclonal antibody.

corresponding to the detection limit in 1C4 ELISA. In summary, 94.7% (72/76) of the investigated Japanese patients in the acute phase had an open ADAMTS13 conformation, which is in agreement with our previous reports where mainly Caucasian patients were studied. Hence, we validated that open ADAMTS13 is a hallmark of iTTP independent of ethnicity (Figure 1).

Total anti-ADAMTS13 autoantibody IgG levels in the Japanese iTTP cohort

In Japan, the presence of anti-ADAMTS13 autoantibodies in patients with iTTP is routinely confirmed by determining the presence of an ADAMTS13 inhibitor using a plasma mixing assay. These data were available before the start of the study (Figure 2; supplemental Table 3). In contrast, in Western countries, total anti-ADAMTS13 autoantibody IgG levels (clearing autoantibodies and inhibitory antibodies) are generally determined by ELISA, where antibody binding to coated recombinant ADAMTS13 (rADAMTS13) is studied. We therefore determined the total anti-ADAMTS13 autoantibody IgG levels in our cohort of Japanese patients with iTTP. The median level of anti-ADAMTS13 IgG autoantibodies was 68.9% TTP03-A equivalents (interquartile range: 29.8-139.3, detection limit: 12%) (Figure 2; supplemental Table 3).[23] The positive control TTP03-A had a high titer of anti-ADAMTS13 IgG autoantibodies (110 U/mL in TECHNOZYM® ADAMTS13 INH ELISA Kit). For the 2 patients with undetectable ADAMTS13 inhibitors (patient codes 2014-23 and 2018-14; see also patient cohort information), total anti-ADAMTS13 autoantibody IgG levels could be detected (supplemental Table 3). As expected, there was a positive correlation between total

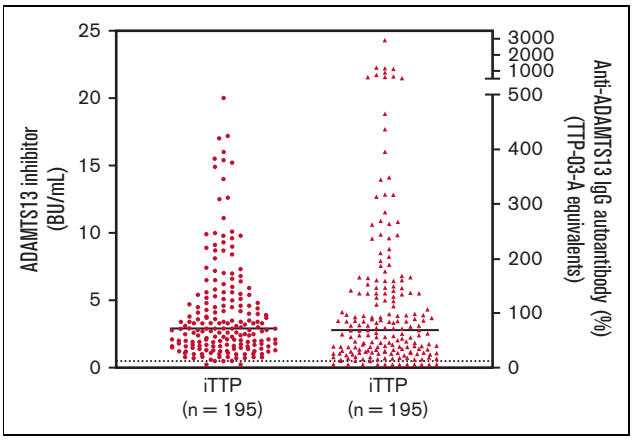

Figure 2. ADAMTS13 inhibitor and anti-ADAMTS13 IgG autoantibody titers in the Japanese iTTP cohort. ADAMTS13 inhibitor titers (filled circle, n = 195) were determined in a mixing assay using the chromogenic activity ELISA based on the GST-VWF73 fragment and detection of cleaved VWF substrate by the anti-N10 mAb. Total anti-ADAMTS13 IgG autoantibody titers (triangle, n = 195) was determined using ELISA where rADAMTS13 was coated and bound anti-ADAMTS13 autoantibodies were detected. The dotted line indicates cutoff values for ADAMTS13 inhibitor titers and anti-ADAMTS13 IgG autoantibody titers (0.5 BU/mL and 12% TTP-03-A equivalents respectively). mAb, monoclonal antibody.

anti-ADAMTS13 IgG autoantibody levels and ADAMTS13 inhibitor ($r = 0.432$, $P < .0001$, supplemental Figure 1).

The IgG subclass of anti-ADAMTS13 IgG autoantibodies in the Japanese iTTP cohort

We next determined the IgG subclass of the anti-ADAMTS13 IgG autoantibodies in the 183 patients who had detectable anti-ADAMTS13 IgG autoantibodies in their plasma (Figure 2; supplemental Table 3, % TTP03-A equivalents). Figure 3A shows that IgG_1, IgG_2, IgG_3, and IgG_4 were present in 103 (56.3%), 68 (37.2%), 153 (83.6%), and 158 (86.3%) samples, respectively. When studying the combinations of these IgG subclasses, the combination of all 4 subclasses was most prevalent (23.5%), followed by the combination of IgG_3 and IgG_4 (21.3%), the combination of IgG_1, IgG_3, and IgG_4 (19.7%), and only IgG_4 (10.4%) (Figure 3B). All other combinations were present in less than 10% of the patients.

Immunoprofiling of anti-ADAMTS13 IgG autoantibodies in the Japanese iTTP cohort

We next determined the immunoprofile of the anti-ADAMTS13 IgG autoantibodies in our Japanese patients with iTTP by checking their binding to the following ADAMTS13 fragments expressed as fusion proteins to AD1: M, DT, CS, T2-T5, T6-T8, and CUB1-2 as previously described for mainly Caucasian samples.[13,24] Binding to AD1-ADAMTS13 was used as a reference, and binding to AD1 as a negative control. In 159 of the 195 samples, we could detect anti-ADAMTS13 autoantibody binding to 1 or more of the ADAMTS13 fragments (M, DT, CS, T2-T5, T6-T8, and CUB1-2) and could hence determine the immunoprofile (supplemental Table 3). In the remaining 36 samples, anti-ADAMTS13 autoantibody titers were probably too low to detect antibody binding (supplemental Table 3).

Before grouping the samples according to their immunoprofile, we first analyzed the percentage of patient samples that contained anti-M, anti-DT, anti-CS, anti-T2-T5, anti-T6-T8, or anti–CUB1-2 autoantibodies (Figure 4). Of these, 73% of patients had anti-CS autoantibodies, 53.5% anti-T2-T5 autoantibodies, 51.6% anti–CUB1-2 autoantibodies, 45.9% anti-T6-T8 autoantibodies,

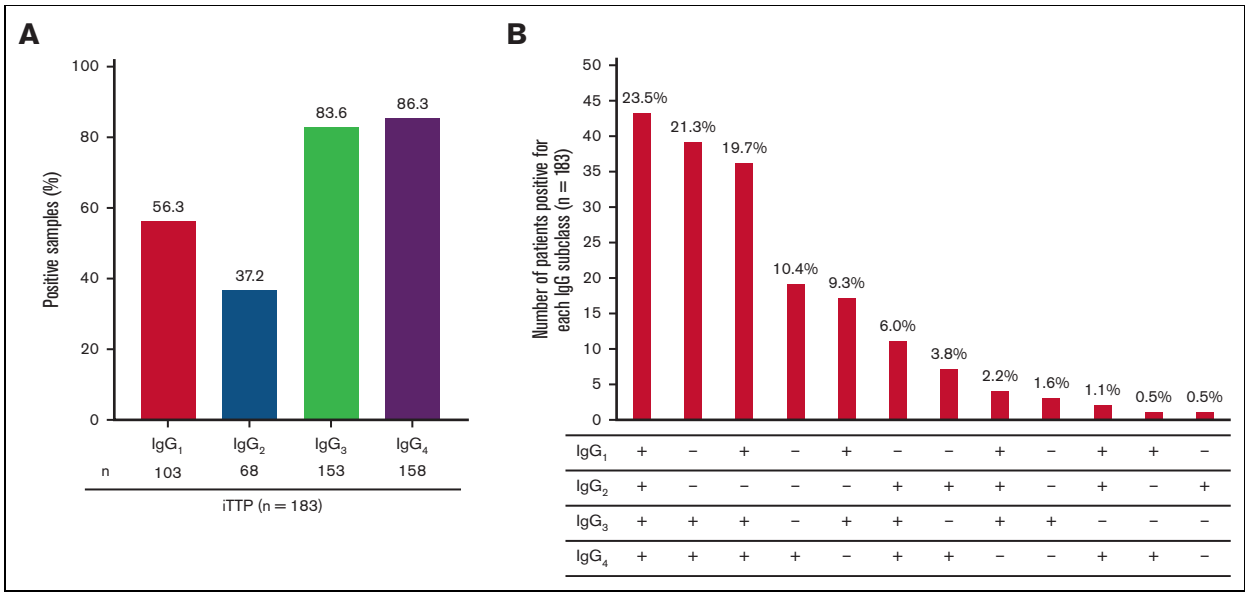

Figure 3. Presence of IgG subclasses of anti-ADAMTS13 IgG autoantibodies in the Japanese iTTP cohort. IgG subclasses were determined using ELISA by coating rADAMTS13 and detecting bound anti-ADAMTS13 autoantibodies with HRP-labeled anti-human IgG_1, IgG_2, IgG_3, or IgG_4 antibodies. (A) Percentage of patients with iTTP having the different IgG subclasses of anti-ADAMTS13 autoantibodies is depicted. (B) Number of patients with iTTP with different combinations of IgG subclasses is depicted. Plus signs denote the presence of a subclass, minus signs denote the absence of a subclass.

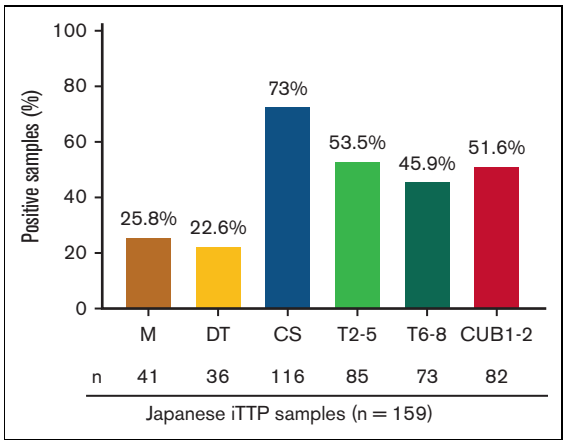

Figure 4. Fine-mapping of anti-ADAMTS13 autoantibodies in the Japanese iTTP cohort. Fine-mapping of anti-ADAMTS13 autoantibodies was done using ELISA by studying the binding of the anti-ADAMTS13 autoantibodies to M, DT, CS, T2-T5, T6-T8, and CUB1-2 domains. The percentage of patient samples containing anti-M, anti-DT, anti-CS, anti-T2-T5, anti-T6-T8, or anti–CUB1-2 autoantibodies among the 159 patients are depicted. C, cysteine-rich; CUB, complement components C1r and C1s, sea urchin protein Uegf, and bone morphogenetic protein-1; D, disintegrin-like; M, metalloprotease; S, spacer; T, thrombospondin type 1 repeat.

25.8% anti-M autoantibodies, and 22.6% anti-DT autoantibodies. Then, we stratified the patients according to their immunoprofile to identify the most prevalent combination of domain-specific autoantibodies. As shown in Figure 5, we found 28 profiles in the Japanese iTTP cohort. Of these, 22 profiles were the same profiles identified in the mainly Caucasian cohorts (profiles in white background, Figure 5) and 6 profiles were unique to the Japanese cohort (profiles in orange background, Figure 5). In the Japanese cohort, we found only 1 dominant profile (defined as being present in >8% of the patients[13]): profile 1, where only anti-CS autoantibodies were present (28.9% of the patients), in contrast to the profiles in the mainly Caucasian cohorts, where profiles 1, 2, and 3 were most prevalent. All other profiles in the Japanese iTTP cohort were present in only 0.6% to 6.9% of the patients (Figure 5).

Link between the most dominant immunoprofile, laboratory findings, and clinical outcome in the Japanese iTTP cohort

In analogy with our study with the mainly Caucasian iTTP cohorts, also for the Japanese cohort, we studied whether there was a possible link between the presence or absence of the dominant profile 1 (only anti-CS autoantibodies, Figure 5), laboratory findings, and TTP-related mortality rate. First, the laboratory findings in the acute phase were compared between the dominant profile (profile 1) and other profiles (supplemental Table 4). Patients with profile 1 (n = 46) showed higher hemoglobin levels and elevated total bilirubin levels compared with patients with other profiles (median values 8.4 vs 7.7 g/dL, $P = .031$ and 4.0 vs 2.8 mg/dL, $P = .044$, respectively). The median titer of anti-ADAMTS13 IgG autoantibodies in profile 1 was significantly lower than in other profiles (57.2 vs 95.2% TTP03-A equivalents, $P = .001$). Subsequently, we also compared the 1-year cumulative mortality rate (clinical outcome) related to TTP episodes between these 2 groups, but no significant differences were found (Figure 6A).

Link between the presence of antibodies against different domains, laboratory findings, and clinical outcome in the Japanese iTTP cohort

In correspondence with our study with the mainly Caucasian iTTP cohorts, we also studied whether there was a possible link between the presence or absence of antibodies against each domain, the laboratory findings at the diagnosis, and TTP-related mortality rate (supplemental Table 5A-F). Median platelet counts in patients with anti-M autoantibodies were significantly lower than in patients without anti-M autoantibodies (9.0 vs 12.0 × 109/L, $P = .002$). The median levels of LDH in patients with anti-M and anti–CUB1-2 autoantibodies were higher than in patients without those autoantibodies (1175.0 vs 919.5 U/L, $P = .022$ and 1122.5 vs 914.5 U/L, $P = .021$, respectively) (Figure 7). As for the median levels of serum creatinine, patients who possessed anti-M and anti-T6-T8 autoantibodies showed more impaired renal function than patients without those autoantibodies (1.07 vs 0.87 mg/dL, $P = .038$ and 0.97 vs 0.84 mg/dL, $P = .022$, respectively) (Figure 7). Total anti-ADAMTS13 autoantibodies were higher in patients with anti-M, anti-DT, anti-T2-T5, anti-T6-T8, and anti–CUB1-2 autoantibodies than in patients without those autoantibodies (160.7 vs72.4% TTP03-A equivalents, 152.9 vs 72.1% TTP03-A equivalents, 130.2 vs 53.1% TTP03-A equivalents, 134.9 vs 59.7% TTP03-A equivalents, 136.2 vs 50.3% TTP03-A equivalents; $P < .001$ in all comparisons) (supplemental Table 5A-F). In addition, the 1-year cumulative mortality rate related to TTP episodes was not affected by the presence or absence of autoantibodies against each domain (supplemental Table 5A-F).

Link between the numbers of domain fragments targeted by the autoantibodies, laboratory findings, and clinical outcome in the Japanese iTTP cohort

We next analyzed whether the number of domain fragments targeted by the autoantibodies could be linked with laboratory findings and TTP-related mortality rate. Six domain fragments (M, DT, CS, T2-T5, T6-T8, and CUB1-2) were used for immunoprofiling and hence the 159 patients were divided into 6 groups; antibodies against only 1 domain (n = 57), against 2 domains (n = 29), against 3 domains (n = 19), against 4 domains (n = 18), against 5 domains (n = 27), and against all 6 domains (n = 9) (supplemental Table 6). Regarding the laboratory findings, there were no significant differences between the 6 groups in platelet count, hemoglobin, LDH, total bilirubin, serum creatinine, and D-dimer. Higher levels of anti-ADAMTS13 IgG autoantibodies were observed when patients had autoantibodies against multiple domains. As for the 1-year cumulative mortality rate related to TTP episodes, we did not find any statistical difference between the 6 groups. Finally, we studied whether there was a link between patients with autoantibodies against all 6 domains compared with autoantibodies against 1 to 5 domains. We here found that patients with autoantibodies against all 6 domain fragments had higher ADAMTS13 inhibitor titers (median 8.1 vs 3.3 BU/mL; $P = .004$) and higher total anti-ADAMTS13 autoantibody titers (median 465.0 vs 82.6% TTP03-A equivalents, $P < .001$) compared with the patients with autoantibodies against 5 domain fragments or less. Interestingly, we found that the 1-year mortality rate in patients with autoantibodies against 6 domain fragments was significantly higher than in

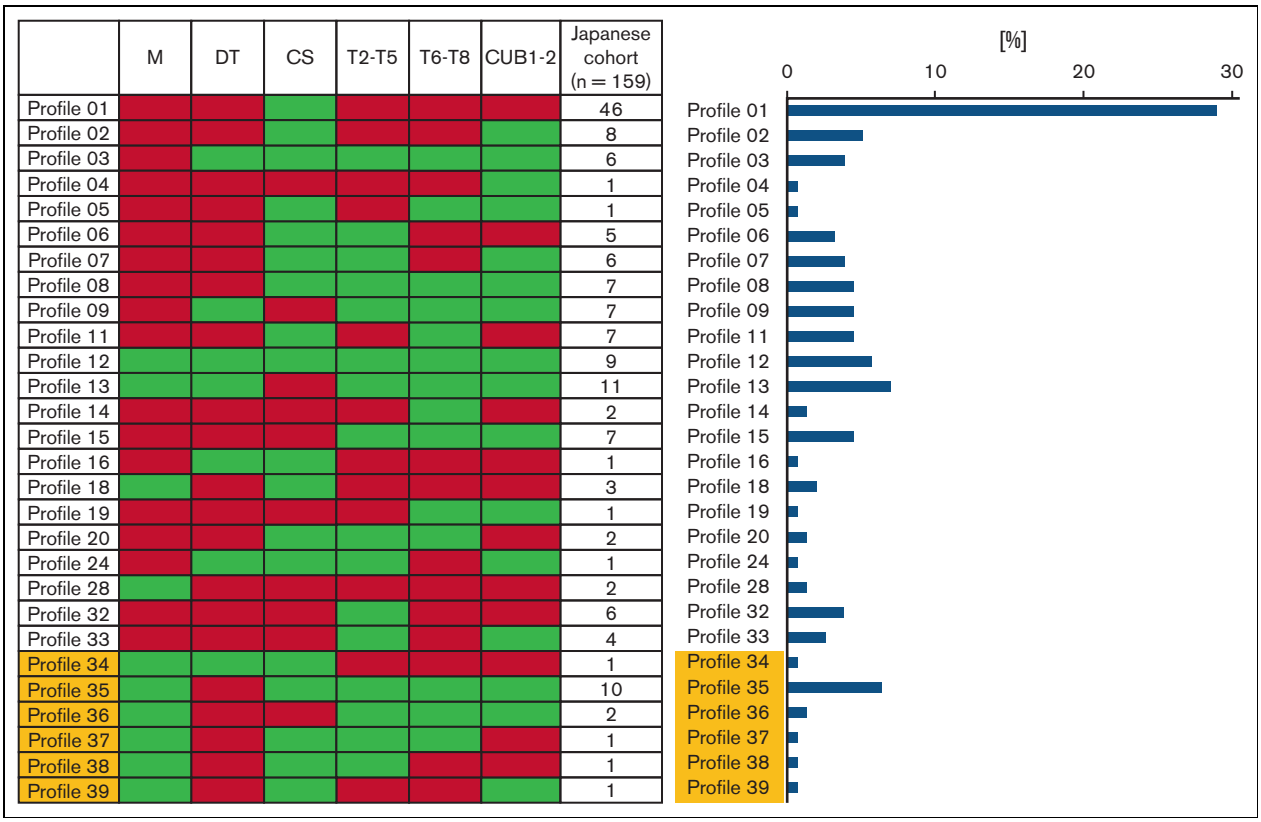

Figure 5. Anti-ADAMTS13 autoantibody immunoprofiles in the Japanese iTTP cohort. Immunoprofiles were formed based on the presence or absence of anti-M, anti-DT, anti-CS, anti-T2-T5, anti-T6-T8, and anti–CUB1-2 autoantibodies. The green and red cells indicate the presence and absence of anti-M, anti-DT, anti-CS, anti-T2-T5, anti-T6-T8, and anti–CUB1-2 autoantibodies, respectively (left graph). The percentage of patients with a specific immunoprofile is depicted in the right graph. The same profiles were found as the ones described in our previous report based on a mainly Caucasian cohort,[13] except for profiles 10, 17, 21, 22, 23, 25, 26, 27, 29, 30, and 31. Profiles 34 to 39 (highlighted in orange) were only detected in the Japanese cohort.

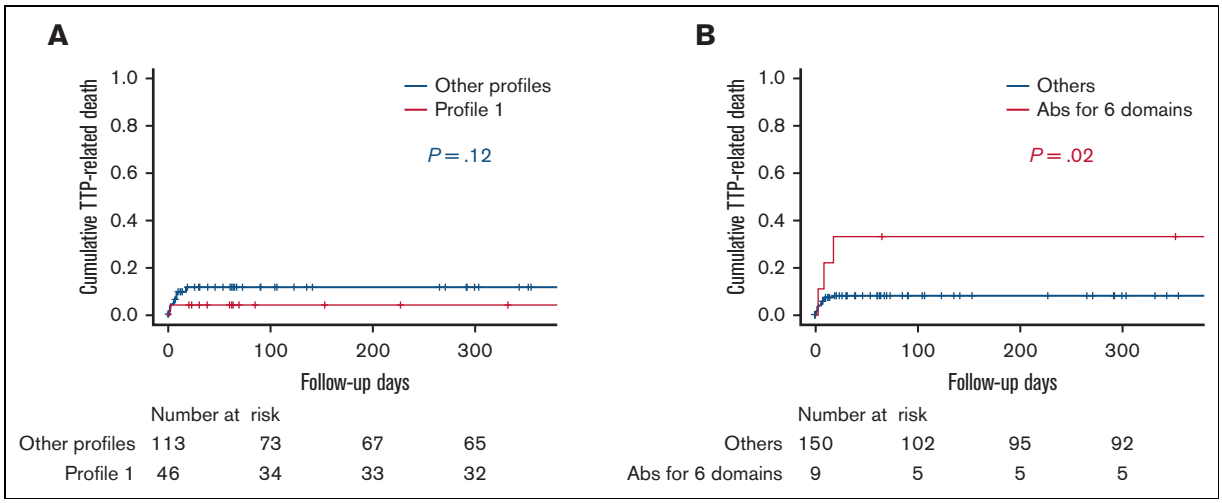

Figure 6. Cumulative TTP-related mortality and anti-ADAMTS13 autoantibody immunoprofiles in the Japanese iTTP cohort. The left panel represents the 1-year cumulative TTP-related mortality in patients with immunoprofile 1 compared with patients with other immunoprofiles. The right panel shows the 1-year cumulative TTP-related mortality in patients with anti-ADAMTS13 autoantibodies against the 6 ADAMTS13 domains (M, DT, CS, T2-T5, T6-T8, and CUB1-2), compared with patients with anti-ADAMTS13 autoantibodies against the 5 or less ADAMTS13 domains.

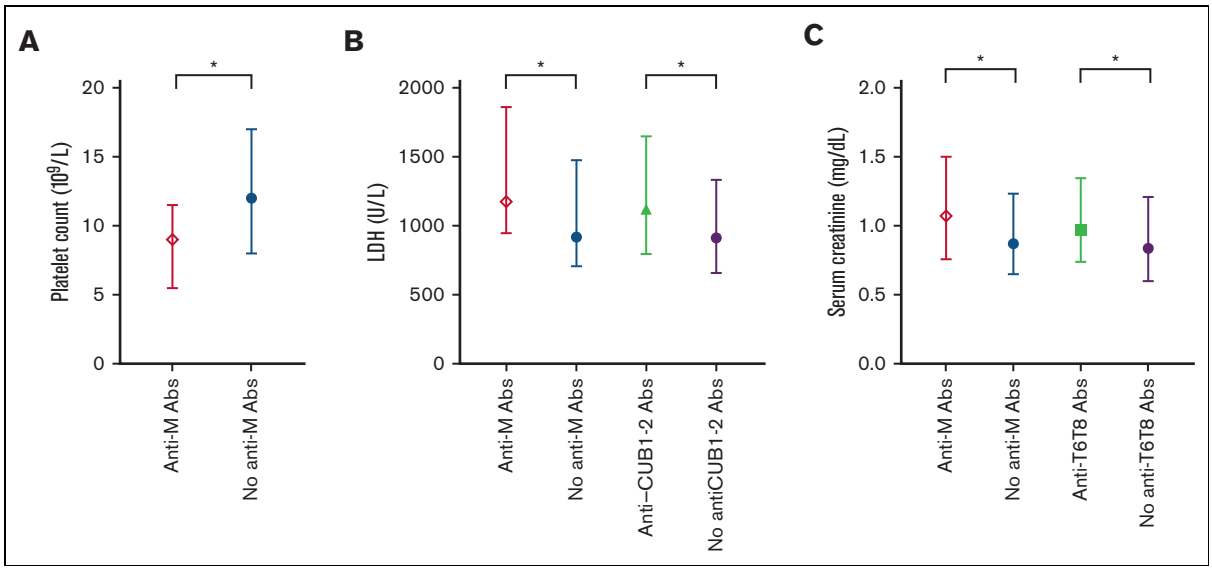

Figure 7. Link between the presence of anti-ADAMTS13 autoantibodies against different domains and clinical outcomes in Japanese patients. (A-C) Patients with anti-M autoantibodies showed more severe thrombocytopenia and higher levels of LDH and serum creatinine than patients without anti-M autoantibodies. (D) Patients with anti-T6T8 autoantibodies had higher levels of serum creatinine than patients without anti-T6T8 autoantibodies. (E) Patients with anti–CUB1-2 autoantibodies had higher levels of LDH than those without anti–CUB1-2 autoantibodies.

patients with autoantibodies against 5 domain fragments or less (33.3% vs 8.1%, $P = .02$) (Figure 6B; supplemental Table 7).

Discussion

Our Japanese patient cohort has been intensively studied over recent years, allowing us to report on the diagnostic and treatment guidelines in Japan,[20] which also contributed to setting up international guidelines for treating and managing this rare disease.[8,25,26] However, although information on ADAMTS13 activity and ADAMTS13 inhibitors is well documented in our cohort, other important ADAMTS13 parameters like ADAMTS13 conformation, IgG subtypes of anti-ADAMTS13 autoantibodies, and anti-ADAMTS13 immunoprofiles have not been studied yet. Indeed, studies in mainly Caucasian cohorts regarding open ADAMTS13 conformation, IgG subtypes, and immunoprofiles of anti-ADAMTS13 autoantibodies have not been studied in a Japanese cohort.[11-13,18] This study included 195 patients with acute-phase iTTP and validated open ADAMTS13 as a novel biomarker for acute iTTP in the Japanese cohort. Hence, these findings encourage the general use of open ADAMTS13 to confirm iTTP diagnosis. We identified IgG_3 and IgG_4 as the major subtypes of anti-ADAMTS13 autoantibodies. Finally, we showed that Japanese patients with iTTP have 1 major immunoprofile, the one with only anti-CS autoantibodies, supporting the further development of ADAMTS13 variants that do not bind anti-spacer autoantibodies for use in different ethnic cohorts.[16,17]

As reported previously, open ADAMTS13 is a novel biomarker for acute iTTP and subclinical disease when patients are in remission and their ADAMTS13 activity is below 50%.[12] In agreement with the findings in mainly Caucasian populations, 94.7% of the Japanese patients with acute iTTP had an open ADAMTS13 in their plasma. Determining ADAMTS13 conformation in acute iTTP is of clinical interest for those patients whose ADAMTS13 activity is between 10% and 20% and in whom anti-ADAMTS13 autoantibodies are not detected. Indeed, although a diagnosis of TTP is clear when ADAMTS13 activity is below 10%, clinicians need additional biomarkers to identify TTP when ADAMTS13 activity is between 10% and 20%. A subset of patients with iTTP do not present with detectable anti-ADAMTS13 autoantibodies in the acute phase. However, open ADAMTS13 can also be used as a surrogate marker for iTTP as it was demonstrated that anti-ADAMTS13 autoantibodies open ADAMTS13. Correct diagnosis of iTTP is crucial to start the correct treatment for these patients: TPE, administration of immunosuppressive agents like rituximab, and/or the anti-von Willebrand factor (VWF) nanobody, caplacizumab.[27] These treatments are demanding for the patients and very expensive, highlighting the importance of a correct diagnosis of iTTP.

Japanese patients with iTTP are diagnosed based on the presence of ADAMTS13 inhibitory antibodies, whereas patients with iTTP in Western countries are nowadays mainly diagnosed based on the presence of anti-ADAMTS13 autoantibodies (mainly IgG) that bind to rADAMTS13. Therefore, we studied the presence of anti-ADAMTS13 IgG autoantibodies bound to ADAMTS13 in the Japanese cohort and determined their isotypes. As expected, there was a significant positive correlation between the anti-ADAMTS13 IgG autoantibody titers and the ADAMTS13 inhibitor titers. However, anti-ADAMTS13 IgG autoantibodies were not detected in 12 Japanese patients with iTTP, although inhibitory antibodies were present. These data suggest these patients might have anti-ADAMTS13 IgA/IgM autoantibodies instead of IgG autoantibodies,[28] however we only detected IgA in 1 and IgM in 3 samples.

Although IgG_1 and IgG_4 anti-ADAMTS13 autoantibodies are the predominant subclasses detected in the mainly Caucasian cohorts,[18,29] mainly IgG_4 and IgG_3 anti-ADAMTS13 autoantibodies were detected in the Japanese iTTP cohort. We also

checked for a possible link between the presence of each IgG subclass antibody and laboratory findings as well as clinical presentation in the Japanese iTTP cohort. However, for none of the IgG subclasses, a link was found (data not shown).

We next identified immunoprofiles in the Japanese iTTP cohort. More than 70% of patients had anti-CS autoantibodies, which is in agreement with what we previously reported for the mainly Caucasian cohorts using the same ELISA[13] (73% vs 74.8%). Notably, anti-M autoantibodies were more frequently found in the Japanese cohort than in the mainly Caucasian cohorts[13] (25.8% vs 9.2%). This is an attractive finding for 2 reasons. First, multiple inhibitory anti-ADAMTS13 autoantibodies were cloned from patients with iTTP, which target the spacer domain and inhibit ADAMTS13 activity by binding to the VWF exosite in the spacer domain.[30,31] Only 1 anti-M autoantibody was cloned from a patient with iTTP so far. This inhibitory anti-M autoantibody was, however, not studied in-depth and it is unclear whether it targets the active site or VWF exosites in the M domain.[32] The possibility of cloning and characterizing more inhibitory anti-M autoantibodies from patients with iTTP would pave the way to further understand which panel of anti-ADAMTS13 autoantibodies inhibit ADAMTS13 activity in patients with iTTP. Second, we previously demonstrated that a murine anti-M monoclonal antibody could inhibit shear-induced VWF cleavage by ADAMTS13 in left ventricular assist devices in vitro and in vivo.[33] The availability of a fully human inhibitory anti-M antibody would facilitate its use in clinical trials. Of note, whether the higher prevalence of anti-M autoantibodies in the Japanese cohort compared with the mainly Caucasian cohorts could be because of the fact that Japanese patients with iTTP were diagnosed based on the presence of inhibitory autoantibodies or because of their difference in the genetic background, remains to be determined.[34-37]

When stratifying the mainly Caucasian iTTP cohort according to their immunoprofiles, 3 dominant immunoprofiles could be identified: profile 1 with only anti-CS autoantibodies (26.7%); profile 2 with both anti-CS and anti–CUB1-2 autoantibodies (12.2%); and profile 3 with anti-DT, anti-CS, anti-T2-T5, anti-T6-T8, and anti–CUB1-2 autoantibodies (8.4%).[13] Interestingly, we here found that the Japanese iTTP cohort only showed 1 dominant immunoprofile, profile 1, where only autoantibodies against the CS domain are present. This finding is of particular interest for the ongoing development of the ADAMTS13 variants that have a reduced binding of the anti-spacer autoantibodies and shows that such therapy will likely apply to patients from different ethnicities.[16,17] Immunoprofiling will be useful in identifying patients that will benefit the most from this type of therapy. These novel findings strongly support the hypothesis that the CS domains are more immunogeneic to autoreactive B cells than any other ADAMTS13 domains, leading to the differentiation of the B cells into plasma cells producing anti-CS autoantibodies.

Studying links between immunoprofiles and clinical outcome might stratify patients according to disease severity and predict clinical outcomes, as has been demonstrated for other autoimmune diseases.[38-40] In our previous report, mainly based on Caucasian patients with iTTP, we found no link between the 3 dominant immunoprofiles and disease severity, but did identify a link between the absence of anti–CUB1-2 autoantibodies and cerebral involvement. In addition, in the Japanese iTTP cohort, we did not find a link between the dominant immunoprofile 1 and clinical outcome, represented by the 1-year TTP-related mortality rate. In contrast, Japanese patients with iTTP with autoantibodies against all 6 ADAMTS13 fragments did have a higher risk for TTP-related death than other patients ($P = .02$). These data and previous reports support the finding that autoantibody epitopes and immunoprofiles are not very strong biomarkers for disease outcome.[14,41]

In conclusion, our data validated the use of open ADAMTS13 as a biomarker in the diagnosis of iTTP in the Japanese iTTP cohort and confirmed the value of immunprofiling of anti-ADAMTS13 autoantibodies to further develop targeted therapies in iTTP. Additional studies in other ethnic cohorts will further expand our knowledge on ADAMTS13 conformation and immunoprofiles.

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Authorship

Contribution: K.S. designed experiments, collected patients' clinical data, performed ELISA assays, performed the statistical analysis, and wrote the manuscript; M.M. provided advice about the study design and wrote the manuscript; L.D.W. and C.D. analyzed the result of ELISA assays; E.H. and M.K. collected patients' clinical information; C.T. and S.F.D.M. interpreted data and provided useful discussions; K.V. designed experiments, interpreted data, wrote the manuscript, and provided funding; and all authors critically reviewed the manuscript.

Conflict-of-interest disclosure: M.M. is a member of the advisory board of Takeda Yakuhin and Sanofi. He is also an inventor of the ADAMTS13 activity ELISA. K.V. is a member of the advisory board of Takeda. The remaining authors declare no competing financial interests.

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