Severe deficiency of VWF-cleaving protease (ADAMTS13) activity defines a distinct population of thrombotic microangiopathy patients

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BACKGROUND: Severe deficiency of ADAMTS13 activity is a biologic risk factor for thrombotic microangiopathy (TMA). It was hypothesized that severe ADAMTS13 deficiency is associated with a distinct TMA subpopulation.

STUDY DESIGN AND METHODS: ADAMTS13 activity before treatment was determined retrospectively in 107 adult TMA patients treated with plasma exchange. Patients were not clinically categorized, but divided between severely deficient (n = 50) and nonseverely deficient (n = 57) ADAMTS13 activity. Laboratory and clinical factors before treatment were compared between the groups.

RESULTS: Median PLT counts were 44,000 per μL in nonseverely deficient ADAMTS13 patients and 13,000 per μL in severely deficient ADAMTS13 patients ($p < 0.001$). Median serum creatinine levels were 2.7 mg per dL in nonseverely deficient patients and 1.2 mg per dL in severely deficient patients ($p < 0.001$). In surviving patients, median plasma exchange procedures were 9 in nonseverely deficient patients and 14.5 in severely deficient patients ($p < 0.01$). Rates of relapse following remission were 4 of 47 in nonseverely deficient patients and 16 of 46 in severely deficient patients ($p < 0.01$). Among analyzed factors only mortality rates were not significantly different.

CONCLUSION: In a heterogeneous population of TMA patients treated with plasma exchange, ADAMTS13 activity defined two subpopulations with distinct clinical and laboratory features. These results suggest that TMA with severe ADAMTS13 deficiency is a distinct pathologic process.

Several observations provide evidence for a central pathogenic role of severely deficient VWF-cleaving proteolytic activity (hereafter ADAMTS13 activity) in thrombotic microangiopathy (TMA). 1) Severe ADAMTS13 deficiency is prevalent in TMA.[1-7] 2) Both inhibitor-mediated and mutational defects are observed, indicating that multiple mechanisms of ADAMTS13 dysfunction are associated with TMA.[1,3,8-10] 3) ADAMTS13 loss-of-function mutations were sufficiently strongly associated with a TMA phenotype to allow positional cloning of the gene.[8] 4) Plausible pathogenic models of microvascular thrombosis can be derived from known functions of ADAMTS13. Despite this array of evidence for the pathogenicity of severe ADAMTS13 deficiency, no studies have reported whether severe ADAMTS13 deficiency is associated with a TMA patient population that is distinct from TMA patients without severely deficient ADAMTS13 activity.

Previous reports have analyzed ADAMTS13 activity in TMA subpopulations categorized by the clinical terms hemolytic uremic syndrome (HUS) and thrombotic

ABBREVIATIONS: HUS = hemolytic uremic syndrome; TMA = thrombotic microangiopathy; TPE = therapeutic plasma exchange; TTP = thrombotic thrombocytopenic purpura.

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thrombocytopenic purpura (TTP).[1,3,4,6,7,11,12] Nevertheless, uniform case definitions for HUS and TTP do not exist, and studies therefore conflict on the degree of correlation between TMA subtypes and ADAMTS13 activity.[7,12-16] In this study we retrospectively analyzed a heterogeneous, plasma exchange-treated TMA patient population, without clinical classification as HUS or TTP, to determine whether ADAMTS13 activity levels alone could define separate subpopulations with distinct laboratory and clinical factors.

MATERIALS AND METHODS

Patients and blood specimens

A total of 107 consecutively enrolled patients referred to The Blood Center of Southeastern Wisconsin (Milwaukee, WI) for plasma exchange during the acute phase of TMA were studied following informed consent. Diagnostic criteria included thrombocytopenia, microangiopathic hemolytic anemia, elevated LDH, and minimal or no evidence of disseminated intravascular coagulation. A Blood Center physician evaluated each patient to confirm the TMA diagnosis and recommend plasma exchange. Plasma exchange was the primary method of patient treatment; however, adjunctive therapies were utilized in some cases at the discretion of referring physicians.

A total of 107 analyzed sets of patient data represent unique patients studied during one episode of TMA, in most cases the initial episode, as reported by the patient. Plasma samples used in the ADAMTS13 activity assay were obtained from sodium citrate-anticoagulated whole-blood samples collected before initial plasma exchange or from the first 200-mL waste plasma bag (anticoagulated with ACD) obtained during the initial plasma exchange procedure. Validation studies with normal plasma samples indicated no biased difference in ADAMTS13 activity between ACD and sodium citrate-anticoagulated plasma (data not shown). Plasma samples were stored at –80°C until use.

ADAMTS13 activity assay

ADAMTS13 activity was determined as previously reported.[17] Briefly, 98 µL of undiluted plasma mixed with 50 µL of purified VWF (final concentration 3 U/mL), 10 mmol per L Pefabloc SC (Boehringer, Mannheim, Germany), and 8 µL of 10 mmol per L $BaCl_2$, was dialyzed (1.5 mol/L urea, 0.005 mol/L Tris, pH 8.0) on a hydrophilic filter membrane (VSWP, 47-mm diameter; Millipore, Bedford, MA) for 15 hours at 37°C. The reaction was quenched with 10 µL of 0.2 mol per L EDTA, pH 7.4. Products of VWF proteolysis were electrophoresed into 0.65 percent agarose in the presence of SDS, and the mobility of VWF multimers was detected with a mixture of nine ^{125}I-labeled VWF MoAbs recognizing multiple VWF subunit domains. Use of pooled of VWF MoAbs rather than affinity-purified rabbit anti-VWF for VWF multimer detection has been validated in our laboratory.

The activity of ADAMTS13 against purified, multimeric, substrate VWF is demonstrated by the progressive disappearance of larger VWF multimers and accumulation of smaller multimers. Patient ADAMTS13 activity was determined as previously reported[17] by comparing the electrophoretic range of substrate VWF remaining after digestion by patient plasma, to the electrophoretic range of undigested VWF in the same gel. The interpretation scheme divided patients into two groups: 1) nonseverely deficient activity—the remaining substrate VWF electrophoretic pattern revealed disappearance of more than 15 percent of the largest VWF multimers; and 2) severely deficient activity—the remaining VWF substrate electrophoretic pattern revealed disappearance of less than 15 percent of the largest VWF multimers. The informativeness of the interpretation scheme was demonstrated in a previous study.[17] Samples with severely deficient ADAMTS13 activity in initial results were confirmed by repeat assay. Exposure of substrate VWF to digestion conditions without an added source of ADAMTS13 revealed no endogenous VWF proteolytic activity. Incubation of recombinant VWF protein constructs under identical conditions yielded protein fragments of molecular weights consistent with cleavage at the Tyr842-Met843 peptide bond (data not shown).

Clinical data

Clinical data were obtained from patient medical records and apheresis procedure records maintained by The Blood Center of Southeastern Wisconsin. Laboratory values reported were those closest to the first therapeutic plasma exchange (TPE) procedure. The number of TPE procedures reported per patient represents total procedures performed to achieve stable remission (>2 weeks without clinically determined need for additional TPE). The endpoint of treatment for patients was determined by consensus between a Blood Center physician and referring physician and included clinical stability with normal PLT count and normal or near normal LDH. Relapse is defined as a subsequent course of plasma exchange performed by the Blood Center in a patient who had previously been treated for TMA and had achieved remission.

Statistical analysis

Laboratory and clinical parameters were compared between groups with the nonparametric Wilcoxon rank-sum test for difference in medians, the chi-squared test, or Fisher's exact test.

RESULTS

Of 107 TMA patients, 50 (47%) were severely deficient in ADAMTS13 activity; 57 (53%) had nonseverely deficient activity (Table 1). The rates of mortality that occurred between presentation and 1 week after the last TPE were not different between the groups. Of the 93 patients who achieved remission, the proportion who had at least one episode of relapse is significantly different between the two groups: normal ADAMTS13, 9 percent (4/47); and deficient ADAMTS13, 35 percent (16/46) ($p < 0.01$).

As shown in Fig. 1A, the group with nonseverely deficient protease activity had significantly higher PLT counts at presentation than the severely deficient group ($p < 0.001$). The median Hct was slightly but significantly higher in patients with nonseverely deficient ADAMTS13 activity ($p < 0.05$, Fig. 1B). The group with nonseverely deficient ADAMTS13 had significantly higher creatinine values (2.7 mg/dL) than the severely deficient group (1.2 mg/dL, $p < 0.001$, Fig. 1C).

The numbers of TPE procedures required to achieve remission were compared in patients who survived at least 1 week after their last plasma exchange procedure. The distribution between the two groups was significantly different ($p < 0.01$, Fig. 2).

DISCUSSION

This study demonstrates that separating a heterogeneous population of TMA patients on the basis of severely deficient or nonseverely deficient ADAMTS13 activity levels before treatment identifies two patient subpopulations with overlapping but distinct laboratory and clinical profiles. Other recent studies have compared TMA patient populations with combinations of ADAMTS13 activity and clinical criteria and have observed differences in a few measures, consistent with our findings.[2,6] The distinguishing feature of our analysis is the comparison of two large TMA subpopulations separated strictly on the basis of ADAMTS13 activity. Perhaps because of the relatively large number of patients in the two populations, we observed differences that were significant in five of six analyzed measures.

Although the demonstration of distinct but overlapping profiles of TMA populations based on ADAMTS13 levels may not have an immediate impact on patient care, the observation supports the important principle that

TABLE 1. Incidence of mortality and relapse

ADAMTS13 activity	Number	Expired*	Relapsed*
Nonseverely deficient	57	10/57 (18)	4/47 (7)†
Severely deficient	50	4/50 (8)	16/46 (35)†

* Data are reported as number (%).
† Fisher's exact test, $p < 0.01$.

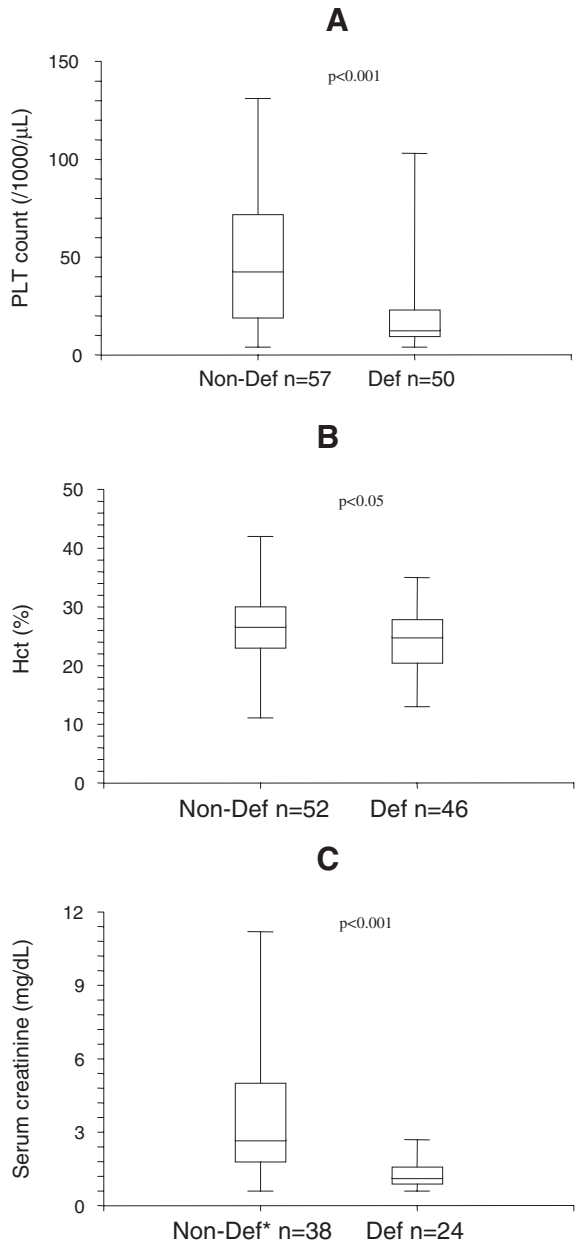

Fig. 1. Distributions of laboratory values by ADAMTS13 activity group displayed as box plots. (A) PLT count distributions compared between severely deficient (Def) and nonseverely deficient (Non-Def) ADAMTS13 activity groups. (B) Hcts compared between groups. (C) Serum creatinine values compared between groups. Boxes represent the 25th to 75th percentiles with medians indicated by central horizontal lines. Vertical lines extend to minimum and maximum observations. Comparisons were generated with the Wilcoxon rank-sum test for difference in medians. *One patient had a value of 30 and is not shown on the graph.

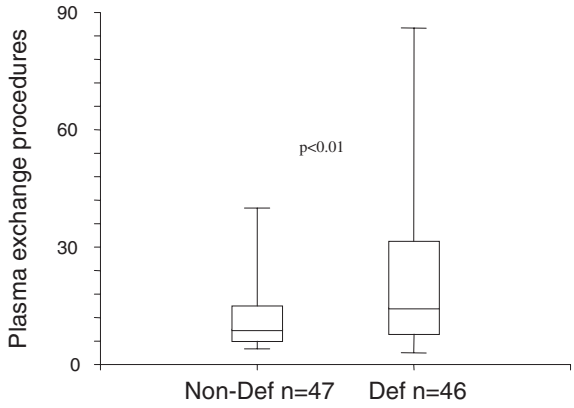

Fig. 2. Distribution of plasma exchanges by ADAMTS13 activity group displayed as a box plot. The number of plasma exchange procedures in patients who survived 1 week or more after the final procedure compared between severely deficient (Def) and nonseverely deficient (Non-Def) ADAMTS13 activity groups. Boxes represent the 25th to 75th percentiles with medians indicated by central horizontal lines. Vertical lines extend to minimum and maximum observations. Comparisons were generated with the Wilcoxon rank-sum test for difference in medians.

severe ADAMTS13 deficiency exerts a dominant pathologic effect in TMA. This and other evidence strongly supports the proposition that severe ADAMTS13 deficiency is a critical pathologic factor in TMA that warrants a central role in disease classification.

Since the discovery of ADAMTS13 activity investigators have held differing opinions about whether deficient activity constitutes a specific pathologic finding in TTP.[7,12,18] Some studies reported nearly perfect correlations between the diagnosis of TTP and severely deficient ADAMTS13 activity[1,3,12] leading to suggestions that TTP is defined by severely deficient ADAMTS13 activity.[19-22] Other studies challenged that conclusion, finding up to 35 percent overlap between ADAMTS13 activity levels and the clinical diagnoses HUS and TTP.[4,7,23] Arguments framed in these terms seem destined to persist because of a lack of uniform case definitions for HUS and TTP.

With the discovery of the important and prevalent risk factor of ADAMTS13 deficiency in TMA, the question may be raised whether TMA disease classification based on such pathologic factors might be more useful than continued debates about the correlation between ADAMTS13 activity and the clinical diagnoses HUS and TTP. The limitations of HUS and TTP terminology in TMA have long been recognized,[24] and the discovery of ADAMTS13 deficiency has rekindled the debate.[14,25,26] It is reasonable to consider for the future a revised nomenclature incorporating both relevant clinical and pathologic factors.

TMA patient management based on ADAMTS13 activity levels is currently investigational. Nevertheless, future advances in clinical management, treatment, and scientific understanding of TMA will depend on case definitions of TMA based on clinical and pathogenic factors, including ADAMTS13 activity. Differentiating TMA by pathogenic features such as ADAMTS13 activity levels may lead to discovery of new pathogenic factors and clinical associations. Some correlations have already emerged, including the 12-fold increased prevalence of the FV Leiden gene mutation in TMA patients with normal ADAMTS13 activity,[17] the association between ticlopidine and ADAMTS13 deficient TMA,[27] and the association of normal ADAMTS13 activity with both Shiga toxin- and bone marrow transplantation-associated TMA.[11,28] It is highly likely that differentiating among TMA populations with immune-mediated ADAMTS13 deficiency, hereditary ADAMTS13 deficiency, and other pathogenic factors will be critical to future treatment advances and clinical trials.

Inclusion of ADAMTS13 activity in TMA disease classification will require a greater standardization of assay methods and a greater understanding of pathologic ranges. Data in this and several other studies[2,12,17,20] suggest that in TMA patients ADAMTS13 activity in the range of less than 5 to 15 percent is informative. Future studies with standardized test methods will further define informative ADAMTS13 activity ranges.

This study shows that ADAMTS13 activity levels can be used to define major divisions within a broad TMA population typically treated with plasma exchange. We propose that clinical and pathogenic factors including ADAMTS13 activity may offer an improved basis for classification of TMA that will facilitate scientific advancement, treatment, and patient management.

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