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Commentary

Thrombotic Thrombocytopenic Purpura: 2012 American Society for Apheresis (ASFA) Consensus Conference on Classification, Diagnosis, Management, and Future Research

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The American Society for Apheresis (ASFA) conducted a 1 day consensus conference on Thrombotic Thrombocytopenic Purpura (TTP) during its annual meeting in Atlanta, GA, on April 10, 2012. The authors of this article, a subcommittee of ASFA's Clinical Applications Committee, developed several questions with regard to definitions, classification, pathophysiology, diagnosis, management, and future research in TTP. These questions were provided to the seven invited speakers who are the experts in the field of TTP. Two moderators conducted the proceedings of the conference which was attended by more than 100 participants. After each presentation, there was an open discussion that included moderator-selected written questions submitted by the audience. A medical writer-generated transcript of the proceedings as well as each presentation was made available to the authors. Each summary was reviewed and approved by the respective speaker before submission of this article. The subcommittee also developed seven key questions for blinded, electronic polling conducted by the moderators to generate a consensus amongst the speakers. This article includes these presentation summaries as well as results of the electronic poll. J. Clin. Apheresis 00:000–000, 2013.

Key words: ADAMTS13; microangiopathic hemolytic anemia; thrombotic thrombocytopenic purpura; therapeutic plasma exchange; Upshaw Shulman Syndrome

Thrombotic Thrombocytopenic Purpura (TTP) is a rare disorder characterized by the presence of microangiopathic hemolytic anemia (MAHA) and thrombocytopenia. Many patients present with neurological symptoms, some have renal involvement, and others may have a fever. In the past a pentad consisting of these features was considered to be diagnostic of TTP. Currently, however, unexplained MAHA and thrombocytopenia are considered to be sufficient features to initiate therapeutic plasma exchange (TPE), which is the standard of care therapy. Nevertheless, there are many other clinical conditions that may present with MAHA and thrombocytopenia as a result of thrombotic microangiopathy (TMA); these conditions generally do not benefit from TPE. The etiology of congenital TTP (Upshaw Shulman Syndrome (USS) is attributed to a genetic defect in the *a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13* (*ADAMTS13*) gene, which results in a severe deficiency of ADAMTS13 enzyme causing persistence of unusually large von Willebrand factor (UL-VWF)

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TABLE I. Panel Participants and Moderators at the 2012 ASFA Consensus Conference (see Appendix B for More Details)

Name	Position	Institution/location
Speakers		
James N. George, MD	George Lynn Cross Professor in the Departments of Medicine, Biostatistics & Epidemiology	University of Oklahoma Health Sciences Center, Oklahoma, OK
Johanna Kremer Hovinga, MD	Consultant and Head of the Hemostasis Research Laboratory	University Clinic of Hematology, Bern University Hospital and the University of Bern, Bern, Switzerland
Thomas Raife, MD	Clinical Professor of Pathology and Medical Director	University of Iowa DeGowin Blood Center, Iowa City, IA
Gail Rock, MD, PhD	Professor of Pathology and Laboratory Medicine	University of Ottawa, Ottawa, Ontario, Canada
J. Evan Sadler, MD, PhD	Chief of the Division of Hematology, Professor of Medicine, and Professor of Biochemistry and Molecular Biophysics	Washington University School of Medicine, St. Louis, MO
Ravi Sarode, MD	Chief of Pathology, Clinical Laboratory Services, Professor of Pathology and Director of the Division of Transfusion Medicine and Hemostasis	UT Southwestern Medical Center, Dallas, TX
Han-Mou Tsai, MD	Director, Blood Coagulation Center. Previously: Professor of Medicine and Associate Head of the Unified Division of Hematology	E-Da Hospital in Kaohsiung, Taiwan; Albert Einstein College of Medicine and Montefiore Medical Center, Bronx, New York
Moderators		
Harvey G. Klein, M.D	Chief, Department of Transfusion Medicine at Clinical Center	National Institutes of Health, Bethesda, MD
Darrell Triulzi, MD	Professor of Pathology and Director of the Division of Transfusion Medicine	University of Pittsburgh, Pittsburgh, PA

multimers that induce VWF-rich platelet microthromboses in small blood vessels with high shear rate [1]. Acquired TTP has also been attributed to a severe ADAMTS13 deficiency due to an IgG autoantibody [2,3]. The published literature, however, does not uniformly support a severe ADAMTS13 deficiency as an etiology of TTP [4]. Despite reversal of mortality from >90% to <20% following the introduction of TPE [5], many patients have recurrence of the disease or are refractory to TPE and require additional therapeutic interventions [4]. No randomized clinical trials have been conducted to address any of these issues faced by treating physicians. Therefore, on April 10th 2012 during its 33rd annual meeting in Atlanta GA, the American Society for Apheresis (ASFA) conducted a 1 day consensus conference on TTP to address some of these practical concerns.

Experts in the field of TTP (Table I) were invited to deliberate questions generated by the authors of this manuscript, a subcommittee of ASFA's Clinical Applications Committee. The subcommittee developed questions after face-to-face meetings and conference calls. Two renowned moderators, Drs. Darrell Triulzi and Harvey Klein, were selected to conduct the proceedings of the conference. More than 100 attendees also participated in the discussion by submitting questions in writing to the moderators after each presentation. Following the conference, the authors had access to all presentations and proceedings of the meeting as recorded by an independent medical writer. Before submission for publication, each speaker reviewed a summary of their presentation. Additionally, the moderators performed an electronic, blinded polling and scoring of seven questions formulated and selected by the committee; this was designed to generate a consensus amongst the speakers.

Summaries of Presentations

The first presentation: Classification and definition of thrombotic thrombocytopenic purpura.

Dr. Sadler's presentation answered each question submitted to him as follows:

1. **How should TMA be classified so that TPE with plasma is administered judiciously?**
   Thrombotic Microangiopathies, commonly characterized by Coombs negative MAHA, thrombocytopenia, and variable pattern of tissue injury from microvascular thrombosis, encompass many clinical conditions and pathophysiologies.
   Thrombotic thrombocytopenic purpura (TTP), which is one of the TMAs, was first described as a constellation of five signs: MAHA, thrombocytopenia, neurological dysfunction, renal insufficiency, and fever. The advantage of the classical pentad is its simplicity and almost immediate availability of the

TABLE II. Disease Conditions to be Considered Before Selecting Therapy

Thrombotic microangiopathy	
Identified mechanism	Other relatively specific syndromes
1. ADAMTS13 deficiency	1. Post transplantation
2. Complement regulatory defects	2. Infection (e.g. HIV)
3. Shiga toxin	3. HELLP syndrome, preeclampsia
4. Disseminated malignancy	4. Catastrophic antiphospholipid antibody syndrome
5. Malignant hypertension	5. Medications—autoimmune (ticlopidine, quinine)
6. Vasculitis	6. Medications—toxic (calcineurine inhibitors, gemcitabine, mitomycin C, clopidogrel, bevacizumab)
7. Neuramidase-associated (*S. pneumoniae*)	
8. Cobalamine metabolic defects	

HELLP = hemolysis, elevated liver enzymes, low platelets.

information to make this diagnosis. A particular, important disadvantage is low specificity. Over the last 20 years, the major effort in the field was aimed at understanding the pathophysiology of TTP to move away from clinical diagnosis toward a mechanism-based diagnosis. We now know that TTP is a disorder of VWF proteolysis caused by either an autoimmune process directed against ADAMTS13 [2,3] or congenital deficiency of this enzyme [1,6].

Hence, a plausible clinical description of TTP consists solely of TMA, without a predisposing condition and without oliguric renal insufficiency at presentation. Among patients matching this description, 40% to 100% have an ADAMTS13 level less than 10% at diagnosis and at least 80% have a good response to TPE (i.e., an *ex juvantibus* condition). Dr. Sadler noted that the TTP Syndrome correlates with severe ADAMTS13 deficiency, which in turn correlates with relapse risk [7,8]. As ADAMTS13 testing has become more widely used, it has been found that some patients with severe ADAMTS13 deficiency may present with life threatening microvascular thrombosis without MAHA. Such observations suggest that, for some patients, ADAMTS13 deficiency may be a more important diagnostic criterion than TMA.

2. **What are the differential diagnoses for patients considered to have TTP?**
   Although TMA is often a major feature of TTP, it also occurs in many other disease conditions that should be considered before therapy is selected (Table II). The patient's history (i.e. antecedent symptoms, previous TMA, age of onset, comorbidities, oliguria, and drug exposure) as well as laboratory values indicative of renal injury (e.g., creatinine, hematuria, casts) and possibly bone marrow examination to detect metastasis are important elements to rule out or rule in during the differential diagnosis process.
3. **Are there TTP or TTP-like disorders when TPE should not be used?**
   The differential diagnosis of TTP is often very challenging. However, because of the overlap in clinical presentation and high mortality among patients with TTP, there are some conditions where early initiation of TPE is ineffective and possibly harmful. For example, Hemolytic Uremic Syndrome (HUS) (i.e., TMA with acute oliguric or anuric renal failure) is rarely associated with ADAMTS13 deficiency. In particular, Shiga-toxin associated-HUS (Stx-HUS) has not been shown to respond to TPE; thus, it should not be attempted in these patients. Atypical HUS (aHUS), which is associated with a complement regulatory defect in over 60% of patients [9], has been shown to have limited response to TPE [e.g., membrane co-factor protein (MCP) deficiency does not respond to treatment]; alternative treatment modalities, i.e., eculizumab, have been approved for treatment of aHUS. Therefore, a course of TPE may be warranted in the aHUS patient.
4. **What are the minimum clinical and laboratory criteria to consider TMA or TTP for initiation of TPE?**
   The proposed definition of TTP (see above) relies on information available before initiation of TPE (i.e., TMA without a predisposing condition and without oliguric renal insufficiency). Dr. Sadler advocated for initiation of TPE without delay in patients with TTP syndrome without other known alternative explanation. Importantly, other elements of TTP diagnosis should be pursued (i.e., ADAMTS13 deficiency) as well as continuous vigilance for other possible causes of TTP syndrome.
5. **How should drug induced TTP be classified?**
   Several medications associated with TTP syndrome can be generally divided into two groups according to the mechanism of TTP: autoimmune and non-autoimmune (likely toxic). Ticlopidine and quinine constitute the former group, while calcineurin

TABLE III. Proposed Definitions for TTP

Term	Definition
Treatment response	Platelet count above 150×10^9/L for 2 consecutive days accompanied by normal or normalizing LDH and stable or improving neurological deficits.
Durable treatment response	Treatment response (as defined above) which is lasting at least 30 days after discontinuation of plasma exchange.
Exacerbation	Recurrent disease within 30 days after reaching treatment response.
Relapse	Recurrent disease 30 days or longer after reaching treatment response.
Refractory disease	No treatment response by day 30 and/or no durable treatment response by day 60.

inhibitors, gemcitabine, mitomycin C, clopidogrel and bevacizumab belong to the latter group. Responses to TPE as well as ADAMTS13 levels vary between drug-induced TTPs; therefore the mechanistic classification might be more relevant, as suggested by Dr. Sadler.

6. **Can standard definitions of response, relapse, exacerbation, remission, etc. be established?** Standardization of basic terms related to disease definition, management and response evaluation is critical to any progress. Several important benefits include (1) ability to compare across studies, (2) ability to facilitate multi-institutional studies, (3) recognition of misdiagnosis, and (4) ability to identify subgroups with distinct pathophysiology, responsiveness to therapy and complications [4,5,10]. Table III summarizes proposed definitions for TTP.
7. **Different patterns of response to TPE have been recognized, i.e., steady progressive improvement and steady improvement followed by unexpected dramatic decrease in platelet count during treatment. Are these patterns clinically significant?** Several different courses of TTP treated with TPE have been identified. According to Dr. Sadler these different scenarios are likely to be associated with either (1) differences in ADAMTS13 rate of recovery among individuals or (2) the mechanism of the disease in these individuals which may not be directly related to ADAMTS13 deficiency. In order to provide meaningful conclusions regarding this variability, well designed studies would be necessary.

The second presentation: Pathophysiology of TTP. Dr. Tsai described the pathophysiology of TTP and other microangiopathies, including aHUS; he answered questions at the end of his presentation.

Our understanding of the pathophysiology of TTP has gradually evolved over 30 years since the description of abnormalities of VWF in congenital TTP. However, several other clinical conditions mimic TTP and replicate laboratory findings of TTP. USS, a congenital TTP, is diagnosed based on a genetic defect in the ADAMTS13 gene. Also, HUS often is considered to belong to the spectrum of the disease.

Dr. Tsai first described the unique pathology found in tissue specimens from patients with TTP: microthrombi consisting of VWF and platelets in arterioles and capillaries with no or little angiopathy (inflammatory changes). The microthrombi are seen only in these vessels because these are areas of high shear stress. With no shear stress, VWF is in globular form; with shear stress it is in an elongated, extended form that is capable of binding platelets [11]. There are differential effects of shear stress on VWF in normal and TTP plasma. *In vitro*, when increasing shear force is applied using plasma from patients with TTP containing unusually large VWF (ULVWF), the VWF activity (measured as ristocetin cofactor assay) increases in comparison to a slight decrease when normal plasma is used [2].

The genetics of TTP was briefly discussed. In the original description, Levy et al. studied 12 patients of 7 kindred and found 12 distinct nonconservative *mis-sense* or *frame-shift* mutations resulting in the deficiency of ADAMTS13 as the molecular mechanism responsible for TTP [1]. Dr. Tsai noted that despite having severe ADAMTS13 deficiency with identical genetic mutations, there is phenotypic heterogeneity in clinical presentation. For example, in four families with at least two affected siblings there were two concordant clinical cases and two discordant cases (as reflected by different severity of illness and organ dysfunction). He concluded that phenotypic severity in TTP is determined by both genetic and environmental modifiers. In his original report describing 39 episodes of acquired TTP in 37 patients, all un-transfused/exchanged patients had severe ADAMTS13 deficiency at baseline while there was no severe deficiency in control patients with hematologic disorders including heparin induced thrombocytopenia (HIT). IgG inhibitors were found in a high proportion of ADAMTS13 deficient patients. Based on mixing studies, only 80 to 90% of autoimmune TTP patients have a detectable inhibitor at presentation; however, over 90% will manifest an inhibitor at some time during their course. ELISA autoantibody assays have higher sensitivity than mixing studies but may yield false positive results in 5 to 10% of individuals without autoimmune TTP. Detectable IgG inhibitor exists as more of a continuum, and inhibitor levels may vary in a given patient. Inhibitor strength may impact initial response to TPE

[12], however, the titer does not necessarily define prognosis (i.e. time to remission and/or relapse), since it may change over time.

In an updated series using an immunoblot assay reported in 2009 [13], all 179 patients with TTP had severe ADAMTS13 deficiency (<10%). With the exception of ticlopidine-induced and HIV-associated TTP (autoimmune mechanism), none or few of the patients with other etiologies in his series (Stx-HUS, aHUS, pregnancy, autoimmune connective tissue disease, hematopoietic stem cell transplant, clopidogrel and other drugs, and cancer) were associated with severe ADAMTS13 deficiency. Because assay sensitivity and specificity vary (both falsely high and low results), this should be recognized in the context of clinical decision-making. In particular, the fluorescence resonance energy transfer (FRETS-VWF73) assay may give higher activity results (>10%) than gel-based VWF assays. Dr. Tsai also discussed a case of acute thrombocytopenia and intravascular hemolysis unrelated to TTP where FRETS-VWF73 was falsely low at 10% with detectable inhibitor; two other methods gave normal or essentially normal values (105% and 65%, respectively). To illustrate where severe ADAMTS13 deficiency may not be diagnostic of TTP, Dr. Tsai discussed technical factors that can affect ADAMTS13 measurements. ADAMTS13 is quite stable in normal plasma but may be unstable in pathological samples, including those from patients with sepsis, liver disease, disseminated intravascular coagulation (DIC), pregnancy, HELLP (Hemolysis, Elevated Liver enzymes, Low Platelets) syndrome, pancreatitis, and other active inflammatory conditions. In some assays the presence of free hemoglobin, bilirubin, and high urea concentrations may inhibit activity of the enzyme or affect the signal detection leading to falsely low values. In his discussion of TTP without severe ADAMTS13 deficiency, Dr. Tsai also cited the possibilities of exogenous ADAMTS13 activity after transfusion of blood components (plasma, red blood cells, or even platelet concentrates) and presence of non-inhibitory antibodies of ADAMTS13. Importantly, he believes that many discrepancies reflect differences in the case definition of TTP, i.e., the stringency of clinical criteria used in making the diagnosis.

Clinical risk factors for TTP include HIV infection [relative risk (RR) 38.5, 95% confidence interval (CI) 19.7–75.0] and female gender (RR 2.7, 95% CI 1.3–5.7). Other reported risk factors, including Black vs. other race and age 30 to 49 versus other ages were not significant in a multivariate analysis. Serial analysis of TTP patients appears to show a threshold in which ADAMTS13 levels >10% do not result in VWF-platelet aggregation. Conversely, the critical ADAMTS13 level causing VWF-platelet aggregation may vary depending on the "shear stress profile" in the circulation of an individual patient. Plausible modifiers include platelet number and reactivity, endothelial secretion of VWF, thrombospondin, and other unknown modifiers.

In patients without intravascular devices, the presence of microangiopathic hemolysis signifies microvascular stenosis, which may result from any one of five different types of pathology: (1) VWF-platelet thrombi in TTP, (2) fibrin-platelet thrombi in various disorders such as DIC, HELLP syndrome, antiphospholipid antibody syndrome, HIT, etc., (3) intravascular cancer cells in patients with metastatic neoplasm, (4) autoimmune or infectious vasculitis, and (5) TMA of Stx-HUS, neuraminidase-HUS, or aHUS. Although these disorders share the common features of MAHA and thrombocytopenia, Dr. Tsai concluded that the immunoblot assay reliably segregates the TTP from the non-TTP groups, irrespective of the assay sensitivity used (<5% or 10%). The "conundrum" of phenotypic classification is giving way to an improved pathophysiologic classification of MAHA. In addition to ADAMTS13 deficiency due to genetic or autoimmune etiologies, other molecular mechanisms have been elucidated [14–16].

Stx-HUS, previously also known as "typical" HUS, is the result of direct endothelial cytotoxicity mediated by shigatoxin-Gb3 receptor binding. Excessive complement activation may also play a role. In pneumococcal sepsis-associated HUS, elaboration of a bacterial neuraminidase leads to exposure of the otherwise cryptic T-antigen on endothelial cells with cytotoxic injury as a result of binding of naturally occurring IgM antibodies. TPE may be harmful in this case due to the presence of anti-T IgM in donor plasma. In aHUS endothelial injury caused by unregulated complement activation results in microvascular stenosis and thrombosis, thus leading to organ dysfunction, thrombocytopenia, and abnormal shear stress which fragments the red blood cells [17]. Organ injury primarily affects the kidneys (severe renal insufficiency is rare in TTP). Interstitial edema as a consequence of abnormal microvascular permeability may also contribute to organ dysfunction, particularly the brain. Uncontrolled complement activation has been associated with mutations or autoantibodies affecting the complement system in more than 50% of patients. Mutations and deletions affecting alternative pathway of complement inhibitor factors H, I, MCP, thrombomodulin (THBD), and complement factor H-related protein 1(CFHR1) have been identified. Further, gain of function mutations in complement factor B (CFB) and C3 and autoantibodies that inhibit complement factor H have also been found. In addition, combinations of polymorphisms or haplotypes involving more than one gene are also found to increase the risk of aHUS. These defects lead to complement-mediated endothelial injury and fibrin/platelet occlusion as well as increased vascular permeability by elaboration of anaphylotoxins C3a and C5a.

Eculizumab, a humanized anti-C5 complement monoclonal antibody, has been approved in the United States and Europe for the treatment of aHUS.

Dr. Tsai's answers to the questions for this session are summarized as follows:

1. **What is the role of ADAMTS13 in TTP?**
   ADAMTS13 deficiency is the molecular mechanism of microvascular thrombosis in TTP. When ADAMTS13 is severely deficient, VWF becomes progressively activated by shear stress in the circulation, thus leading to VWF-platelet aggregation and microvascular thrombosis.
2. **What is the definition of severe (<5% or <10%) versus moderate (<30%) deficiency of ADAMTS13? Can this classification be standardized?**
   The cut-off level of 5% or 10% for active thrombosis in TTP depends on the assays used; the difference is not critical. The critical question to ask of any ADAMTS13 assay is whether it can correctly discriminate the severe decrease of ADAMTS13 in TTP from the normal or mild decrease of ADAMTS13 in other disorders of MAHA.
3. **Clarify the significance of severe deficiency with and without autoantibody. Are these conditions pathophysiologically distinct?**
   The routine plasma mixing study to detect ADAMTS13 inhibitors only yields positive results in 80% to 90% of patients with autoantibodies. Because the inhibitor level of an individual patient fluctuates, it does not correlate with the time to sustained remission or the risk of subsequent relapse. A critical question that clinicians have to address is how to distinguish autoimmune ADAMTS13 deficiency with negative inhibitor test results from genetic ADAMTS13 deficiency. A partial or complete recovery of the ADAMTS13 level during remission or inadequate response to plasma therapy supports autoimmune ADAMTS13 deficiency, whereas partial or severe deficiency in the parents, siblings or offspring favors genetic ADAMTS13 deficiency.
4. **When a patient presents with severe deficiency but without an inhibitor yet later develops a strong inhibitor during TPE, what does this suggest about TTP pathophysiologic mechanisms?**
   As mentioned above, the ADAMTS13 inhibitor level is quite unstable, especially during the first few weeks of acute episodes; thus, using the conventional plasma mixing study, the inhibitor level may be too low for detection.
5. **Are there alternative etiologies of TTP?**
   Presently, TTP is defined as a prothrombotic disease resulting from genetic mutations or autoimmune inhibitors of ADAMTS13. There is currently no exception to the association between widespread microvascular VWF-platelet thrombosis as observed in TTP and severe ADAMTS13 deficiency.
6. **What is the role of complement factor deficiencies (congenital and acquired) in HUS?**
   Defective regulation of the alternative complement system is detected in 50 to 70% of patients presenting with aHUS. Most, if not all patients with aHUS benefit from anti-complement therapy with eculizumab, suggesting that the TMA results from uncontrolled complement activation even in most of those patients without detectable defects in complement regulation.
7. **Are there differences in clinical outcome as a result of different molecular defects in congenital TTP? What is the relationship between ADAMTS13 and ABO blood types, obesity, liver disease, etc, which have been identified as possible risk factors for TTP?**
   How different mutations differ in affecting the severity of TTP is unknown, since with few exceptions ADAMTS13 mutations are non-recurrent, having been detected only in single pedigrees. On the other hand, siblings with the same mutations may exhibit vastly different phenotypic severity. This is believed to reflect the consequence of genetic modifiers (epistasis) or environmental factors.
   The association of ABO with TTP is based on a study in which the definition of TTP was imprecise, and the evidence linking ABO blood type or obesity with TTP is not convincing. Such studies are often biased due to improper selection of the control group for comparison. Except for a few patients who contract hepatitis C or HIV infection from plasma transfusion, the majority of TTP patients do not have liver disease.

The third presentation: Laboratory testing for TTP. Dr. Johanna Kremer Hovinga reviewed older and newer methods to assess ADAMTS13 activity.

1. **What is the sensitivity/specificity and predictive value of available ADAMTS13 assays (including inhibitor and autoantibody assays)?**
   Three multicenter studies have been published comparing the performance of the different assays to detect ADAMTS13 activity [18–20]. The Berne study (30 samples) and the Milan study (60 plasma samples) used 5 assays and 11 assays, respectively [18,20]. The results demonstrated that most methods were able to reliably detect a severe ADAMTS13 deficiency of <5%, which corresponds generally to their lower limit of detection. The studies also showed good correlation between the expected and the observed values of ADAMTS13 activity in the

TABLE IV. Activity Assays for ADAMTS13

Using VWF (plasma-derived or recombinant) Turnaround time: 2-4 days	Using VWF peptide Turnaround time: 1-4 hours (except Western blot)
SDS-PAGE	Western blot of degraded VWF A1-A3
Multimer analysis (immunoblotting)	VWF78 & VWF115
IRMA & ELISA	ELISA of degraded VWF73 (A2)
Residual VWF function	FRETS-VWF73
1.Collagen Binding Activity	SELDI-TOF VWF73
2.Ristocetin Cofactor Activity	Several others, including commercially available ones, based on VWF73
Flow-based cleavage of VWF strings	
Whole blood (Plate & Cone Analyzer)	

VWF = von Willebrand factor, SDS-PAGE = sodium dodecyl sulfate polyacrylamide gel electrophoresis, IRMA= immunoradiometric assay, FRETS= fluorescence resonance energy transfer, SELDI-TOF = surface enhanced laser desorption/ionization time-of-flight mass spectrometry.

low range ($\leq$20%). The reported detection limit as a measure of sensitivity is the highest for the Seldi TOF (surface enhanced laser desorption/ionization time-of-flight mass spectrometry) assay with a detection limit of 0.5%, followed by the SDS-PAGE (Sodium dodecyl sulfate polyacrylamide gel electrophoresis) of VWF cleavage products, at 1%. The quantitative immunoblotting assay (IB) and the FRETS-VWF73 based-assays have a detection limit between 1 and 5%, the GST-VWF ELISA of 3%, IRMA (immunoradiometric assay) of 5%, and SDS-PAGE of VWF peptides, collagen binding activity assay (CBA), and the residual ristocetin cofactor activity assay (RCo) of around 6%. In these reports, plasmas with higher levels of ADAMTS13 activity yielded worse correlations between laboratories as well as between observed and expected ADAMTS13 activity.

2. **What are the pre-analytical, analytical and post-analytical variables affecting testing? What are the criteria for an ideal specimen (timing of collection, types of anticoagulants used)? What is the impact of transfusion of blood products (RBC, platelets, or plasma) on the assay?**
   Because several pre-analytical factors may affect the assays, the choice of the optimum specimen to measure ADAMTS13 has an important effect on the result [7,21,22]. Most laboratories use citrated plasma or even serum, as these have been shown to give very comparable results when using the IB, CBA and the FRETS-VWF73 methods. Because EDTA inhibits the enzyme, EDTA-plasma is not suitable. Specimens collected after the patient has received plasma or plasma containing component transfusions may provide erroneously higher ADAMST13 levels due to exogenous ADAMTS13. In order to preserve enzymatic activity, plasma or serum samples should be shipped frozen to reference laboratories for ADAMTS13 testing.
3. **Is there an assay available that can provide real time results for clinical decision making? Is this possible?**
   At this time, despite the variety of approaches to measure enzymatic activity, none provide real time results for clinical decision-making (Table IV). For that reason, readily available laboratory tests such as platelet count, lactate dehydrogenase (LDH), and creatinine are to be used in the clinical diagnosis of TTP to initiate emergent TPE.
4. **What are the pitfalls of ADAMTS13 assays currently available for accurate measurements of ADAMTS13?**
   Assay-specific interferences are also known from the literature. The FRETS-VWF73 method, the most commonly used in the United States is affected by hyperbilirubinemia and free plasma hemoglobin [22,23]. Essentially, bilirubin acts as a fluorescence quencher at the emission wavelength of 450 nm. In plasmas with bilirubin levels of 100 μmol/L (5.8 mg/dL) or higher, the interference may cause a falsely severe ADAMTS13 deficiency suggestive of idiopathic TTP in patients with other TMAs who have ADAMTS13 activity in the lower range of normal. Since this degree of hyperbilirubinemia is not uncommon in patients with brisk hemolysis, this is a potential testing confounder. Free hemoglobin at 0.2 g/dL or more also acts as a quencher in fluorescence-based assays and may cause falsely low ADAMTS13 activity. In Dr. Kremer Hovinga's laboratory, all samples with an ADAMTS13 activity $\leq$20% are reflexively tested for the presence of a functional ADAMTS13 inhibitor. Based on the observation that plasma samples are sometimes collected only after the administration of plasma-containing blood products, this threshold was set in order to detect the reappearance of an inhibitor early on during remission and to assess the nature of residual ADAMTS13 activity up to 20%.
   Various studies have evaluated ADAMTS13 activity

in patients with sepsis/DIC to assess the specificity of ADAMTS13 for TTP. Utilizing the FRETS-VWF73, none of 40 patients from Switzerland (which also used the IB method) and none of 30 patients from France had <5% ADAMTS13 activity [24,25]. However, in Germany, Bockmeyer and colleagues found ADAMTS13 activity <10% using the CBA assay in 19 of 267 (7.1%) patient samples with sepsis/DIC in an intensive care unit [26]. Among Japanese patients, 15.6% of septic patients had ADAMTS13 <5% when tested with IB [27]. Notably, patients in this study were not tested for the presence of an ADAMTS13 SNP P475S (common in the Japanese population with an allelic frequency of 9.6%), which is urea-sensitive and associated with lower ADAMTS13 activity in urea-based assays, such as the IB [16]. Asymptomatic siblings of patients with hereditary TTP with severe ADAMTS13 deficiency also influence the specificity of the assay; therefore, tests should be interpreted in light of the clinical manifestations. Rare cases of ADAMTS13 <5% determined by IB have also been reported during the acute episode of Stx-HUS but not in remission [28]. During the German outbreak of Stx-HUS caused by *Escherichia coli* 014:H4 in 2011, Dr. Kremer Hovinga's laboratory detected ADAMTS13 <5% in two of six patients with neurological symptoms [10]. One of them later relapsed, suggesting idiopathic TTP instead of Stx-HUS.

The hallmark classification of TTP as acquired instead of congenital is the presence of a functional ADAMTS13 inhibitor by Bethesda-like assays. Other commercially available assays detect autoantibodies by ELISA with the recombinant (r) ADAMTS13 as target. While these assays are easy to perform, between 4% and 13% of healthy control individuals appear to harbor such antibodies. Western blots and the glycosylphosphatydil inositol (GPI)-anchored tests are limited to specialized or research laboratories, which also employ rADAMTS13 as targets. Table V lists several studies which determined the prevalence of ADAMTS13 functional inhibitors using a variety of assays [29–33]. In three of them, the specimens were also tested for ADAMTS13-specific IgG. The data show that prevalence of the latter is higher than that of functional inhibitors by 7%, 10%, or 14% [34–36]. At this time, the clinical significance of non-inhibitory IgG antibodies is unknown.

TABLE V. Prevalence of ADAMTS13 Inhibitor in Various Studies

Study	Percentage of patients with functional inhibitor (%)	Percentage of patients with anti-ADAMTS13 IgG (%)
Furlan et al., 1998 [3]	83	NA
Tsai et al., 1998 [2]	79	NA
Veyradier et al., 2001[29]	51	NA
Rick et al., 2002 [30]	90	NA
Kremer Hovinga et al., 2004 [31]	62	NA
Coppo et al., 2004 [32]	55	NA
Peyvandi et al., 2004 [33]	87	NA
Rieger et al., 2005 [36]	83	97
Tsai et al., 2006 [34]	93	100
Ferrari et al., 2009 [35]	90	100

5. **Is there a role for ADAMTS13 monitoring in the management of TTP (e.g., initiating TPE or stopping TPE, or adding an intervention when no overt symptoms or when there is a sudden deterioration in clinical response to treatment)?**

Treatment decisions regarding initiation of TPE, monitoring of response and/or when to discontinue it, still need to be based on clinical and routine laboratory parameters such as platelet count and signs of hemolysis. Although patients with ADAMTS13 activity <10% and an ADAMTS13 antibody (with or without inhibitory capacity) are more likely to present with a TTP relapse [7,37] than patients without enzyme deficiency and/or antibodies, there are no interventions shown to prevent recurrences. Since ADAMTS13 deficiency may persist without thrombocytopenia or MAHA, this finding does not justify TPE in the absence of clinical signs and symptoms [7]. Clinical experience in patients with ADAMTS13 <5% and strong inhibitors suggest the need for an acute event ("second hit") to precipitate TTP. Thus, the ADAMTS13 result alone does not justify TPE. In the United Kingdom, France and Italy, such patients now often preemptively receive rituximab, a monoclonal anti-CD20 antibody (off-label indication) [38].

The fourth presentation: TPE in the Management of TTP was given by Dr. Gail Rock who answered two questions at the end of her presentation.

The near 100% mortality rate of TTP was significantly reduced after plasma infusion therapy was introduced. For patients with renal failure, intolerance of the necessary volume of plasma led to the use of TPE, where roughly equivalent amounts of plasma are removed and infused. In a landmark randomized controlled trial (RCT) in 1991, The Canadian Apheresis Group (CAG) demonstrated that survival of TTP was improved with TPE compared with plasma infusion (78% versus 49%, $n = 102$, $P = 0.002$) [5]. That same year comparable but slightly higher survival (91%) was demonstrated in a clinical report of 108 patients treated with corticosteroids alone or TPE combined with corticosteroids [39]. As a result, TPE became firmly established as primary standard therapy for TTP.

A subsequent publication by the CAG demonstrated that anuric or oliguric patients, not eligible to be randomized in the 1991 trial, were treated with TPE and had survival similar to that of patients in the RCT [40].

With an incidence of less than 20 cases per million, TTP is an ultra orphan disease. Multicenter trials to achieve adequate patient enrollment and statistical power have allowed for the collection of meaningful epidemiologic and laboratory information. For example, the CAG database from 1982 to 1995 demonstrated that the frequency of the 5 classic TTP findings or pentad at presentation were thrombocytopenia 100%, anemia 100%, neurologic signs 64%, fever 22%, and renal involvement 18%. A 10-year follow-up of patients in the 1991 CAG RCT cohort showed more than one-third of surviving patients experienced a relapse of TTP. There was no apparent pattern of the frequency or timing of the relapses or any role of splenectomy in preventing relapse. While TPE is successful in treating relapses, patients remain at risk of mortality with each TTP disease episode just as with initial presentations.

Advances to uncover the pathophysiology of TTP have enabled refinement in therapy over the past several decades and offer promise to improve morbidity and mortality. The discovery of VWF as part of the histopathology of intravascular thrombi in TTP [41] was accompanied by the discovery ULVWF multimers in four relapsing TTP patients during their remission [6,42]. These findings have not been reproducible in the plasma of patients with acquired acute TTP.

Compared with fresh frozen plasma (FFP), cryosupernatant plasma (CSP) contains reduced levels of the largest VWF multimers, providing a theoretical advantage in restoring the VWF derangements seen in TTP. In fact, initial investigations using CSP as replacement fluid in TPE for TTP showed benefit [40]. A RCT comparing CSP with FFP was planned for 236 subjects, which also incorporated correlates of VWF and ADAMTS13 measurements. Unfortunately, this study was abruptly discontinued due to withdrawal of funding when solvent-detergent treated plasma was introduced in Canada. Of the 52 patients enrolled, no definite advantage of CSP was seen [43].

Laboratory parameters collected in the CAG database showed several relatively consistent observations in acquired TTP: normal VWF multimers, ADAMTS13 levels normal in as many as 40 to 50% of patients, lack of an inhibitor to ADAMTS13 in more than 33% of patients treated, and the persistence of the inhibitor (when present) but with normal platelet counts at 6 months follow-up. The presence of anti-CD36 antibodies in the plasma of TTP patients was another consistent observation. CD36 or GPIV is a membrane glycoprotein present on platelets and the endothelial microvasculature. Eighty five percent of TTP patient samples tested demonstrated anti-CD36, depending on the assay used to detect them [44]. CD36 has been demonstrated in microparticles during DIC and may contribute to its pathophysiology. Similarly, plasmas from 11 patients with HUS were reactive with CD36, which in turn appears to share homologous structures with *E. coli* 0157:H7 verotoxin. The cross reactivity of these antibodies with the parent antigen on both platelets and endothelial cells could result in thrombotic complications and vascular damage seen in TTP or HUS [44].

Dr. Rock's answers to two provided questions are as follows:

1. **What does the evidence support for optimal TPE therapy? What is unknown?**
   Optimal therapy for TTP is still based on TPE using at least 1 plasma volume (PV) exchange with plasma (1.5 PV on days 1–3) over a 5- to 7-day course. While the majority (85–90%) of patients respond to this regimen, it is sometimes necessary to introduce adjuvant therapies including splenectomy and/or drugs. Recent experience with rituximab has shown decreased mortality of less than 8% in patients refractory to TPE alone. Finding the right combination of adjuvant therapies remains a future challenge, as a zero mortality rate should be the goal in this group of patients.
2. **What clinical or laboratory findings should be used to determine when to discontinue TPE?**
   The ultimate clinical marker is, of course, patient survival. This is usually, but not always, noted after a first course of therapy. At present, the best laboratory marker is the platelet count with a progressive increase in number and a sustained count over several days of successful treatment.

The fifth presentation: Adjunct/rescue Therapies in the Management of TTP. Dr. James George prefaced his presentation by stating that the data discussed would be relevant only to those patients with acquired, autoimmune TTP associated with severe ADAMTS13 deficiency (activity <10%). He addressed questions posed to him as he presented his talk.

1. **Is there evidence to support concomitant immunosuppression along with TPE to reduce treatment failures?**
   In addressing the utility of corticosteroids there is limited data; however, the Bell et al study from Johns Hopkins compared 54 patients with TTP/HUS who were treated with TPE plus corticosteroids (prednisone 200 mg/d) versus 54 patients treated with corticosteroids alone [39]. Interpretation of the study was complicated by the fact that 24 patients in the corticosteroid-only group were switched to TPE plus corticosteroids during the study. Although

survival was equal in both groups (70/79—90% vs. 28/30—93% for combination therapy vs. corticosteroids alone, respectively), intention to treat would bias the equivalent outcome in favor of the corticosteroid arm. Corticosteroids may help patients achieve a remission.

Each of the three studies presented by Dr. George resulted in somewhat different conclusions regarding the clinical utility of rituximab in TTP. In the first study 40 patients (34 of whom were experiencing their first episode of TTP) were treated with rituximab plus methylprednisolone (1,000 mg/d for 3 days) "per local protocol" vs. 40 historical control patients (selected from the previous year). There was no difference in deaths (study = 3, control = 2) or days of TPE to achieve remission (study = 16.5 days, control = 18). There was a significant difference, however, in frequency of relapse (study = 3 of 37 [11%], control 21 of 38 [55%], $P = 0.001$) [38]. This unusually high frequency of relapse among the control patients suggests the possibility of bias in the retrospective selection of these patients. The second study compared 22 patients with ADAMTS13 <10%. Nineteen were experiencing their first episode of TTP and were treated with rituximab on days 1, 3, 7, and 14, if considered refractory (defined as a platelet increment less than two times initial count after 4 days of TPE/corticosteroids) or undergoing an exacerbation compared with 57 historical controls [45]. Patients who received rituximab experienced shorter times to remission, fewer relapses in the first year, but no difference with a longer follow-up (median follow-up of 33 and 35 months, respectively, $P = 0.68$). A third study from his own institution reviewed 68 consecutive patients with ADAMTS13 deficiency (<10% activity at their initial episode). Nine of the 10 patients treated with rituximab because they were refractory to TPE or had an exacerbation survived; one patient relapsed at 31 months. There was no statistical difference in the relapse free survival ($P = 0.166$) among all survivors (9 treated with rituximab and 46 without), indicating that corticosteroids and rituximab may be effective for decreasing the duration of TPE required to achieve a remission; rituximab may also be effective to delay or prevent relapses. The effectiveness of corticosteroids and rituximab has been supported by the observation that the duration of TPE required to achieve a durable remission has decreased over the past 15 years, and this has resulted in a comparable decrease in the frequency of TPE-related complications [46].

There was also a single case series describing the use of cyclosporine as initial adjunctive treatment for the treatment of TTP [47]. This study suggested that, compared with corticosteroids, the use of cyclosporine can decrease the time to remission and occurrence of exacerbations. Patients were often continued on cyclosporine for 6 months; however, the effectiveness for prevention of relapse was uncertain. Anecdotal experience with the use of splenectomy in refractory or frequently relapsing TTP patients has been mixed. However, Kremer et al. has reported decreased relapses after splenectomy [48], in addition to unpublished data from the authors' experience until 2011. It is possible that the removal of the spleen might be useful in reducing the B cell mass capable of forming autoantibodies. Other adjuvant therapies such as cyclophosphamide, vincristine, bortezomib or twice daily TPE are poorly studied but might be of use "when everything else fails." Although antiplatelet agents such as aspirin and dipyridamole have been used as adjunctive therapy, the current use appears to be limited to patients with specific neurologic indications, e.g., stroke or TIA [5].

2. **What is the risk-benefit profile of platelet transfusion?**

   The reluctance to transfuse platelets in patients with active TTP has been largely based on dramatic anecdotal reports of adverse outcomes that occurred in the era before effective treatment with TPE. A retrospective study at Dr. George's institution reviewed the outcome in 54 TTP patients with ADAMTS13 activity <10% [49]. Thirty-three of the 54 (61%) patients received platelet transfusions. There were eight deaths (24%); five of these were due to thrombosis in patients who received platelets and three (24%), due to thrombosis in patients who did not receive a platelet transfusion. The incidence of death was not different between the two groups ($P = 0.971$). Similarly, there were no differences in the frequency of severe neurologic events ($P = 0.190$): 17 (52%) patients received platelet transfusions (5/17 patients experienced neurologic events before platelet transfusions) and 7 (33%) patients did not receive platelet transfusions.

   Dr. George concluded that platelet transfusions for patients with TTP are indicated when appropriate reasons are present, such as thrombocytopenia with overt hemorrhage and prevention of bleeding with a surgical procedure that involves a risk for clinically important bleeding; central line insertion may not be considered a surgical procedure with increased bleeding risk in the majority of these patients.

The sixth presentation: Management of Congenital TTP (Upshaw Shulman Syndrome, USS). Dr. Ravi Sarode answered each question sequentially during his presentation.

1. **What are the clinical differences in USS?**

   Genetic mutations (*mis-sense*, nonsense and splice site mutations) in the ADAMTS13 gene lead to

TABLE VI. Potential Alternative/Adjunctive Therapies for Thrombotic Thrombocytopenic Purpura

Agent	Mechanism of action
Recombinant ADAMTS13	Increase ADAMTS13 activity to cleave ULVWF multimers
Aurintricarboxylic acid	Block binding of VWF to platelet glycoprotein Ib
ARC1779	Block binding of VWF to platelet glycoprotein Ib
Nanobodies	Block binding of VWF to platelet glycoprotein Ib
N-acetylcysteine	Increase degradation of ULVWF multimers

ULVWF, ultra large von Willebrand factor; VWF, von Willebrand factor.

severe deficiency (<10% activity) of this enzyme [1]. Generally, both ADAMTS13 alleles are affected causing either reduced secretion or catalytic function [50]. There is no definite correlation between genotype and clinical phenotype of USS at the present time. It was thought that congenital TTP presents at a very young age either during infancy or early childhood; recent studies have demonstrated, however, that USS can occur even later in life. Thus, USS can be classified into two groups: early onset (<18 years of age) and late onset (>18 years of age) [51]. Early onset can occur either in the neonatal period (45%) when patients present with hyperbilirubinemia, likely requiring exchange transfusion, or in infancy or early childhood (29%). These patients may remain either asymptomatic or present with episodes of unexplained thrombocytopenia and/or anemia; the disorder may not be recognized as USS until a more classical picture of MAHA and thrombocytopenia with multiorgan involvement is encountered following a trigger (e.g., infection). Similarly, late onset (or adult onset) generally is precipitated in women by an event like pregnancy, whereas males have been diagnosed with USS after the age of 45 [52]. Thus, although the diagnosis of USS is made when ADAMTS13 activity is <10%, the clinical syndrome might be dependent upon the baseline activity of the enzyme. This is akin to hemophilia presentation where clinical severity correlates well with factor VIII (FVIII) levels. Thus, USS patients with an activity of <2% may present with TTP in infancy (early onset group) as opposed to those with 3 to 9% activity who may develop TTP only upon exposure to infection or stress (e.g., pregnancy) and belong to the late onset group. This hypothesis is supported by the knockout mouse model with undetectable ADAMTS13, where a TTP-like syndrome develops only upon exposure to Shiga toxin [53].

2. **Is there an alternate therapy to plasma infusion that avoids volume overload and other adverse effects of plasma therapy?**
   The ADAMTS13 enzyme is present in FFP, plasma frozen within 24 h, CSP and even cryoprecipitate [54]. The amount of ADAMTS13 present is quite similar in all these components except for a higher concentration in cryoprecipitate [54]. In the United Kingdom, several USS pediatric patients are treated with intermediate purity FVIII concentrate at 15 to 30 U/kg [55]. There are several advantages of intermediate purity FVIII concentrates, including (1) small volume, (2) virally inactivated product, and (3) ability to be administered for prophylactic therapy in the outpatient setting or for home therapy [55]. Similarly, cryoprecipitate can be used with no risk for volume overload when compared with plasma infusions.
3. **Do patients with USS develop alloantibodies?**
   Several hundred patients with USS have been reported in the literature; to date, there are no reports of any patient developing an alloantibody causing poor response to plasma therapy following frequent exposures to allogeneic ADAMTS13. A systematic follow up of 43 USS patients in Japan showed no inhibitory antibody by Bethesda type assay [51]. However, 7 of 43 patients had IgG antibody against rADAMTS13 detected by an ELISA. The clinical significance of such a finding is unknown, as none of these patients were refractory to plasma therapy. These findings probably suggest that many patients may have a small amount of ADAMTS13 in their plasma, thus precluding making an alloantibody to the transfused enzyme.
4. **How to manage other congenital deficiencies (e.g., complement factor deficiency—aHUS)?**
   aHUS is characterized by presence of MAHA, thrombocytopenia, normal ADAMTS13 and acute renal failure. As covered by Dr. Tsai, aHUS can present at any age akin to USS. aHUS results from defects in (1) complement pathway regulators (CFH and CFI deficiencies, CFHR proteins and MCP, (2) complement factor activators (CFB and C3), (3) thrombomodulin, which inactivates C3a and C5a, or (4) autoantibody against CFH [56]. The initial diagnosis of aHUS is not very straightforward unless there is a family history of aHUS. Therefore, most patients are initially diagnosed as

TTP and started on TPE. The response to TPE is generally good in regards to improvement of MAHA and thrombocytopenia; however, renal failure may not respond well. A clinical diagnosis of aHUS is entertained only when a normal (or not severely deficient) ADAMTS13 activity is obtained. Current investigation to identify a complement pathway factor defect is limited to a few research laboratories and only approximately 50% of patients are identified as having such a defect. Similar to USS, most cases of aHUS are diagnosed at a young age; many patients are diagnosed later in life, perhaps due to a better understanding of the complement pathway. According to the European Working Group on HUS plasma should be given at a dose of 20 to 30 mL/kg; TPE (1.5 PV (plasma volume)) should be performed daily for 5 days and gradually tapered [56]. Eculizumab, a chimeric monoclonal antibody against C5, prevents formation of the membrane attack complex. It is FDA licensed for the treatment of aHUS. Generally, patients are treated with 300 to 600 mg/week; a response is seen within 48 h. Rarely, combined renal and liver transplantation is performed for MCP defect since this protein is synthesized by the liver.

The last presentation: Future Directions and Research. Dr. Thomas Raife addressed questions in the course of his presentation.

1. **What research might be needed to further understand the pathophysiology and outcomes in TTP?**
   Animal models offer a valuable resource for further understanding the pathophysiology of TTP and HUS. Animal models for TTP include ADAMTS13 deficient mice [53] and ADAMTS13 knockout mice [57]. A canine model of Stx-HUS closely resembles human HUS [58]. Animal models will be critical to future advances in the understanding and treatment of TTP. There is a lack of understanding of the extreme variability in the presentation and clinical course of TTP. Some of the variability may be due to the absence of established diagnostic criteria for TTP, without which clinicians are unable to *confirm* a diagnosis of TTP and can only operate on the basis that they *cannot rule out* TTP. Evaluation of new treatment protocols and therapies will be challenging under these circumstances.
   The heterogeneity of TTP may result from variable factors whose roles are poorly understood. Examples may include disorders of endothelium, red blood cells, complement, and leukocytes in the pathogenesis of microvascular thrombosis. The role of immune dysregulation remains poorly understood. Further research is needed to elucidate contributing pathogenic factors in TTP.
2. **Will there be a role for the use of rADAMTS13 in the treatment of TTP?**
   Functional rADAMTS13 [59] has demonstrated the ability to overcome inhibitory ADAMTS13 autoantibodies [60] and to correct TTP features in a mouse model [57]. Similar to the use of porcine FVIII in hemophilia treatment, forms of ADAMTS13 that are unaffected by antibodies have been explored and may offer advantages over the normal human form.
3. **While replacing ADAMTS13 activity represents one possible alternate therapy, could it be possible to prevent the formation of the platelet/VWF microthrombi seen in TTP?**
   Experimental agents have been explored that inhibit binding of VWF to platelet glycoprotein Ib and may prevent microvascular thrombosis in TTP. Aurintricarboxylic acid, which blocks VWF binding to platelet glycoprotein Ib [61,62] and platelet aggregation [61,63], was considered decades ago. The aptamer ARC1779 is an oligonucleic acid that binds to the A1-domain of VWF and inhibits platelet aggregation. [64,65]. ARC1779 has been used successfully in conjunction with TPE in a few patients [66,67]. Nanobodies, which are functional fragments of single-chain antibodies, are currently being explored as well as a phase II clinical trial as adjunct to TPE [68].
   The discussion panel also suggested the therapeutic potential of *N*-acetylcysteine, which was observed to reduce the size and activity of VWF in human plasma and to reverse TTP-like symptoms in an ADAMTS13 deficient mouse model [69]. A clinical trial using *N*-acetylcysteine as an adjunct to TPE in the treatment of TTP is being considered.
4. **Will TPE still be the standard of care in 5 years?**
   Because the length of time necessary to bring new drugs to market is such that any promising alternative therapy for TTP will not soon be available, TPE will remain the standard of care for the next 5 years.

In summary, there remain many unanswered questions concerning the pathophysiology and treatment of TTP. Disease definitions are needed. Animal models will help elucidate the pathophysiology. There is a need to understand the unpredictable variability in TTP patients. A number of promising pharmaceutical therapies are being explored that may represent alternate or adjunctive therapies to TPE (Table VI).

TABLE VII. Results of Electronic Polling of the Speakers

	Question	Result
1.	Untreated TTP carries a high mortality rate. If a patient presents with (1) unexplained microangiopathic hemolytic anemia (Coombs' negative anemia), (2) thrombocytopenia (platelet count less than 100×10^9/L), and (3) without oliguric renal insufficiency, should emergent TPE be initiated with plasma as a replacement fluid?	Yes 7/7 = Unanimous agreement
2.	Do you agree with the following definitions related to treatment of TTP: Term — Definition Treatment response — A platelet count above 150×10^9/L for two consecutive days accompanied by normal or normalizing LDH and stable or improving neurological deficits. Durable treatment response — Treatment response (as defined above) lasting at least 30 days after discontinuation of TPE. Exacerbation — Recurrent disease within 30 days after reaching treatment response. Relapse — Recurrent disease 31 days or longer after reaching treatment response. Refractory disease — There is no treatment response by day 30 and/or no durable treatment response by day 60.	Yes 6/6 = Unanimous agreement (1 recused)
3.	Congenital TTP (Upshaw Shulman Syndrome) diagnosis is based on a genetic defect in the ADAMTS13 gene which is phenotypically associated with a severe enzyme deficiency (<10%) in the plasma. Should a diagnosis of acquired TTP also be based on a severe deficiency of ADAMTS13?	Yes 4/7 = Simple majority agreement
4.	Current evidence strongly supports autoimmunity as a cause of acquired TTP. Management of autoimmune disorders often includes immunosuppression therapy. Does currently available evidence support routine use of corticosteroids in the treatment of newly diagnosed TTP?	Yes 4/7 = Simple majority agreement
5.	Current literature suggests that despite aggressive TPE, 30 to 50% of TTP patients have exacerbations, relapses or refractory disease. Case reports and case series in such scenarios have shown rituximab, an anti-CD-20 chimeric monoclonal antibody, to be effective. Should rituximab be used routinely in patients with the features listed above?	Yes 6/7 = Strong agreement
6.	TTP microthrombi consist of platelets and von Willebrand factor. Platelet transfusion in a patient with a suspected diagnosis of TTP (e.g., for a central line placement) may theoretically cause worsening of platelet-von Willebrand microthromboses. Should platelet transfusion be reserved for life threatening bleeds (e.g., an intracranial bleed)?	Yes 5/7 = Majority agreement
7.	Congenital TTP patients are generally treated with plasma infusion therapy. Studies show that fresh frozen plasma, thawed plasma and frozen plasma at 24 hours (FP24) contain nearly the same amount of ADAMTS13 per unit volume; compared with plasma, cryoprecipitate has a higher amount of ADAMTS13 per unit volume. The literature shows that congenital TTP has also been treated with cryoprecipitate and intermediate purity factor FVIII concentrates that contain a large amount of von Willebrand factor. Can cryoprecipitate be substituted for plasma (in a fluid restricted clinical situation) to treat a congenital TTP episode?	Yes 5/6 = Strong agreement (1 no opinion)

The blinded, electronic poll developed by the subcommittee of Clinical Applications Committee of ASFA after the conference had a format that required a "yes" or "no" response to each question with an option to submit any comments. A 7/7 "yes" response indicated unanimous agreement, 5 to 6/7 "yes" was considered majority agreement, 4/7 "yes" was graded as simple majority agreement, 3/7 "yes" was scored as simple majority disagreement, 1 to 2/7 "yes" was scored as majority disagreement and 0/7 "yes" was scored as unanimous disagreement. Comments by the speakers are listed in no particular order in the Appendix A.

ELECTRONIC POLLING

Results of the seven key questions formulated by the subcommittee and used by the moderators in a blinded-electronic polling of the speakers to generate a consensus are given in Table VII. The speakers also provided comments on these questions, which are given in Appendix A.

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APPENDIX A: SPEAKERS COMMENTS TO ELECTRONIC POLLING QUESTIONS

Question 1: (When to Initiate TPE)

1. The key word here is "unexplained" MAHA. Lab testing might prove useful to provide an explanation. For example, knowing about ADAMTS13 deficiency or soluble complement factor deficiency could be quite useful.
2. Although it is prudent to initiate TPE therapy when TTP is considered a possibility, because TTP is not the only disorder associated with the syndrome of thrombocytopenia and MAHA, it is essential to collect blood samples for ADAMTS13 assays and other diagnostic studies before any blood products are administered. Once the diagnosis of TTP is excluded either by ADAMTS13 assays or other clinical or laboratory results, the treatment should be modified according to the nature of the alternative diagnosis.
3. The complications associated with long term TPEs are not insignificant, therefore, it is essential that TPE be used judiciously, avoiding unnecessary exposures to plasma and diligently looking for other causes of TMA if ADAMST13 is found not to be severely deficient.

Question 2: (Definitions)

1. An early relapse (between day 31 and 60) may occur; therefore, treatment can easily pass the 60-day margin. I wouldn't necessarily want to consider this patient as refractory, and would rather call it as relapsed.
2. Probably the most important risk factor for an acquired TTP survivor when it comes to risk of relapse is the fact that he/she has had a relapse already.
3. I would agree to these definitions, as opposed to agreeing that these are the definitions of these terms. They are not based in "truth," but rather, are reasonable and useful conventions by which to standardize practice. Also, for the definition of response, a distinction should be made between *clinical* and *immunological* (ADAMTS13) responses.
4. It should be noted that there is no biological basis for using 2 days in the definition of response and 30 days in distinguishing between exacerbation, relapse and refractory disease.
5. When clinical response is arbitrarily defined as at least 2 consecutive days with normal platelet counts during TPE, there is no guarantee that the patient will remain in remission when TPE is discontinued. Consequently, TPE is generally tapered over 1 to 2 weeks when the platelet count is closely monitored.
6. Immunological response may be defined as ADAMTS13 stabilizing to a level $>10\%$ in serial assays after TPE is discontinued. Patients with immunological responses will have hematological response and generally are not at immediate risk of recurrent thrombocytopenia or thrombosis.
7. Refractory disease: since persistent thrombocytopenia may result from a concurrent disease such as ITP, HIV, or other infections, HIT, etc., the

diagnosis of refractory disease should be validated by demonstration of ADAMTS13 remaining <10%.
8. The above approach to the assessment of treatment response only applies to patients with TTP as defined by severe ADAMTS13 deficiency. Patients without ADAMTS13 deficiency require different approaches to therapeutic assessment according to their underlying diseases.

Question 3: (ADAMTS13 as a Diagnostic Criterion)

1. A diagnosis leads to a treatment plan. Today, we can't refute a diagnosis of TTP based on the ADAMTS13 activity value. When ADAMTS13 is >10% it is prudent to consider and evaluate other diagnoses (which may create other treatment plans that are less "urgent").
2. Acquired TTP is a clinical diagnosis necessitating initiation of TPE treatment. TPE should not be withheld on the basis of an ADAMTS13 value.
3. It is possible to link a clinical disease of acquired TTP to a pathophysiology of ADAMTS13 even in some cases of only mildly reduced or even normal ADAMTS13 activity by currently available assays. These cases are rare so far, yet frequent enough not to exclude TTP when ADAMTS13 is >10%.
4. In this question, "diagnosis" cannot be considered to be equivalent to the indication for treatment as defined in Question 1; a "diagnosis", as defined here, is not necessary to initiate TPE treatment, and it is not appropriate to require this definition of diagnosis to initiate TPE treatment. There are exceptions to this rule, but I believe the rule is appropriate for almost all patients. This rule is appropriate to establish a distinct manner of follow-up, because risk for relapse is essentially restricted to these patients, with a severe, acquired deficiency of ADAMTS13.
5. It seems premature to me to limit "acquired TTP" to severe ADAMTS13 deficiency. Put another way, TTP associated with severe ADAMTS13 deficiency probably deserves a better, more specific etiologic name.
6. For the purpose of establishing diagnostic criteria for TTP, I would agree to this as a part of the definition. I would agree that 10% is a reasonable cut-off. I would suggest that there would be many cases that would not meet such a definition of TTP, and that these might be renamed something like atypical TTP.
7. The conventional pentad, triad or diad criteria are not specific for the diagnosis of TTP and will include patients with MAHA due to other causes such as aHUS, vasculitis, cancer cell embolism, paroxysmal nocturnal hemoglobinuria, etc.
8. Conversely, a TTP patient may present with thrombocytopenia alone, TIA/stroke with thrombocytopenia but without MAHA, or even TIA/stroke without either thrombocytopenia or MAHA. Thus, one cannot rely on the criteria of thrombocytopenia and MAHA as the basis of TTP diagnosis, although the syndrome is the most common cause, thus raising the possibility of the disease.
9. The diagnosis of TTP should only be applied to patients with evidence of ADAMTS13 mutations causing its severe deficiency (<10% of normal) or ADAMTS13 inhibitors.
10. The conventional concept that it is not essential to distinguish between TTP and aHUS because both are treated similarly with TPE is invalid because aHUS is more effectively treated with eculizumab and requires different follow-up assessment.
11. Clinical laboratories may occasionally yield inaccurate ADAMTS13 activity results. Therefore, interpretation of the ADAMTS13 assay results should correlate with clinical features.
12. Although TTP is a clinical syndrome and it is prudent to initiate TPE in a patient who does not have plausible explanation of MAHA and thrombocytopenia or stroke, the final diagnosis of TTP should be based on severe ADAMTS13 deficiency for better management of the patient, both current and future. Otherwise many patients are misdiagnosed as TTP and continue to be treated with TPE rather than with appropriate therapy like eculizumab for aHUS.

Question 4: (Role of Corticosteroids)

1. The evidence is not high-grade, but I'm convinced.
2. Not as a standard of care. I would not object to their use, but I would object to their use being considered a standard of care.
3. There is no evidence that the conventional doses of corticosteroids decrease the levels of ADAMTS13 inhibitors, or autoantibody levels in any types of autoimmune disorders. Routine use of corticosteroids for TTP only causes side effects without providing discernible benefits for most patients. Its use may be understandable, albeit not evidence-based, in patients with refractory disease.
4. Conventionally almost all autoimmune diseases are treated with corticosteroids as a first line of therapy and therefore, TTP being autoimmune disorder should be treated with corticosteroids.

Question 5: (Role of Rituximab)

1. Rituximab is off-label and there are not enough data on long-term outcome in this situation.

2. Roche has recently released a warning (at least in Switzerland) of severe, though rare side effects of rituximab in autoimmune disorders—Steven-Johnson syndrome/toxic epidermal necrolysis.
3. Rituximab has not been shown to be superior to splenectomy in this situation. Long-term data for splenectomy are available for ITP. Therefore, I think that an individual decision is necessary and should include the patient's preferences as well as local possibilities (e.g., experienced surgeons performing laparoscopic splenectomy, etc.).
4. One could argue about exacerbations, but I think routine use of rituximab for relapses or refractory disease is justified.
5. With a huge caveat that this should not be elevated to the level of standard of care. I would make it a recommendation. But there should be alternatives, such as other immunomodulating agents.
6. Rituximab should be considered only for patients with ADAMTS13 inhibitors, either directly demonstrated by laboratory assays or inferred from severe ADAMTS13 with less than expected increase following plasma therapy.
7. A common malpractice is to give rituximab to a patient carrying the diagnosis of 'TTP' without evidence of ADAMTS13 deficiency mediated by autoimmune inhibitors. Such patients may have aHUS or other causes of MAHA that require different therapeutic approaches. To avoid confusion, such patients should not be given the diagnosis of TTP.
8. The role of rituximab in TTP treatment is considered separately for the following stages of TTP:
   a. Acute stage: there is no evidence that immediate use of rituximab decreases the early mortality of TTP. There is preliminary evidence that preemptive rituximab may decrease the duration of TPE and delay the time to relapse. Further studies are needed before preemptive rituximab can be universally recommended for patients presenting with acute TTP.
   b. Subacute: Rituximab is appropriate for patients who are unable to achieve sustained response with TPE or have frequent relapses. The definitions of sustained response and frequent relapses are flexible, taking into consideration factors such as cost, convenience, concern of adverse effects, etc.
   c. Remission: Rituximab may be used to prevent impending late relapse when serial ADAMTS13 levels show a trend of decrease during remission.

Question 6: (Platelet Transfusion)

1. My answer is no, as it shouldn't be forbidden. I take a pragmatic approach. I try not to use platelet transfusions; however, if the anesthesiologist requires it for central line placement, then so be it—better to have a speedy central line placement to start TPE as soon as possible than delay TPE because of insufficient venous access.
2. I believe that the appropriate indications for platelet transfusion in patients with TTP are the same as for all other clinical situations: thrombocytopenia with overt bleeding, or to prevent bleeding from an invasive procedure or surgery in a patient with severe thrombocytopenia. For TTP patients, insertion of a central venous catheter should not be considered to be "an invasive procedure" requiring platelet transfusion support; however, some surgeons or interventional radiologists will insist on a platelet transfusion, and their insistence usually has to be followed.
3. While the evidence against use of platelets is not strong, the evidence for a threshold below which prophylactic platelets should be used is also not strong. For me, the issue is not that platelets can feed the fire, but that prophylactic platelets are not likely to be useful when they have an extremely short survival in the circulation.
4. Although I don't think there are compelling data about the danger of platelet transfusions, caution is warranted. Importantly, there are probably equally uncompelling data about the utility of platelet transfusions in this setting.
5. Severe bleeding is exceedingly rare in TTP. Platelet transfusion should rarely be needed (if ever) for TTP. (It should be noted that this statement only applies to TTP as defined by severe ADAMTS13 deficiency.)
6. For patients without severe ADAMTS13 deficiency (e.g., "TTP without severe ADAMTS13 deficiency" according to some), the indication of platelet transfusion should be assessed according to the nature of the underlying disease.
7. There is no evidence that platelet transfusion increases the platelet count sufficiently to allow for a central line placement in a TTP patient. Also, it can potentially delay the line insertion due to time required for obtaining and then transfusion of platelets.

Question 7: (Cryoprecipitate for USS)

1. Any plasma product containing ADAMTS13 is fine. Cryoprecipitate is not available in many European countries.
2. Yes you CAN use cryo, but the advantage seems marginal. Scott (2007) reported that cryo had about twice the ADAMTS13 concentration as FFP or cryo-supernatant plasma. One would have to give a

large amount of fibrinogen, factor VIII and VWF to achieve a similar level of ADAMTS13 in a patient with congenital TTP, compared with treatment with FFP. For example, a 70 kg patient treated with 20 mL FFP/kg would get 1400 mL = 5 to 6 units of FFP (250 mL/unit), and the equivalent ADAMTS13 dose for cryo would be 10 mL cryo/kg, or 50 to 75 units, or at least twice the full replacement dose of factor VIII, and perhaps 15 times a full replacement dose of fibrinogen. The decrease in volume administered would be only one-half. Seems like cryo would be useful only when FFP were simply not available for some reason, or if the huge factor VIII/fibrinogen/VWF load were not considered to be a thrombotic risk. Unless my math is incorrect, I don't think it's worth it, except in an emergency caused by lack of plasma.

3. ADAMTS13 exists at the same levels in the whole plasma and its cryosupernatant fraction. Therefore, there is no basis that ADAMTS13 exists at higher levels in the cryoprecipitate. The observation that ADAMTS13 exists at higher levels in cryoprecipitate has not been validated in other labs.
4. Frequent treatment with cryoprecipitate may markedly raise the plasma levels of factor VIII, VWF and fibrinogen, causing concern of thrombotic complications.
5. Since congenital TTP requires as little as 2 mL/kg body weight of plasma to reverse its platelet thrombosis, volume overload should rarely, if ever, be a problem. Each FFP treatment at 7.5 mL/kg generally will maintain the platelet count in the normal range for two weeks. Plasma therapy will need to be administered more frequently if smaller amounts are given.
6. For patients unable to tolerate plasma infusion due to allergic reactions or other reasons, Octaplas or factor VIII concentrates containing ADAMTS13 (e.g., BPL 8Y) may be an alternative. However, frequent treatment or a large amount of factor VIII concentrates may markedly raise the plasma levels of factor VIII and VWF, causing concern for thrombotic complications.
7. Personally I had used cryoprecipitate for a patient with USS. She always seemed to respond well to cryo rather than plasma especially in regards to volume and allergic reactions.

APPENDIX B: PROFILES OF SPEAKERS AND MODERATORS

1. James N. George, MD, is George Lynn Cross Professor in the Departments of Medicine, Biostatistics & Epidemiology, University of Oklahoma Health Sciences Center, Oklahoma, OK, where he was Chief of the Hematology-Oncology Section from 1990 to 1999. He is the past president of the American Society of Hematology (ASH) and the recipient of the ASH Wallace Coulter Award for lifetime achievement in hematology. Throughout his career Dr. George's research has focused on platelets. He characterized the structure and function of platelet membrane glycoproteins in normal subjects and in patients with congenital platelet dysfunction. Dr. George's interests concentrate on patients with thrombocytopenia, describing the demographics, clinical course, and outcomes of patients with ITP, drug-induced thrombocytopenia, and TTP.
2. Johanna Kremer Hovinga, MD, is the Consultant and Head of the Hemostasis Research Laboratory at the University Clinic of Hematology, Bern University Hospital and the University of Bern, Switzerland, where the first link between a severe deficiency of the Von Willebrand factor-cleaving protease (now denoted ADAMTS13) and TTP was established in 1997. Dr. Kremer Hovinga's laboratory contributed to the development of several ADAMTS13 assays, and initiated the first multicenter study on ADAMTS13 assay comparison in 2003. Together with Anthony Hubbard (NIBSC, Potters Bar, United Kingdom), Dr. Hovinga was assigned by the Scientific and Standardisation Committee of the International Society on Thrombosis and Haemostasis (ISTH) to develop the first WHO International Standard of ADAMTS13 function and antigen in plasma. Of particular interest to the Hemostasis Research Laboratory is the investigation into possible causes of discrepancies in ADAMTS13 results between different assays; this will hopefully lead to a better understanding of the physiological roles of ADAMTS13.
3. Harvey G. Klein, MD, is Chief, Department of Transfusion Medicine at the Clinical Center of the National Institutes of Health where he has also been Special Assistant to the Director for Science. He is co-author of the latest edition of Mollison's Blood Transfusion in Clinical Medicine. He has been Chair of the US Pharmacopoeia Blood and Blood Products Committee and a member of the FDA Blood Products Advisory Committee. He is a former Council President of the National Marrow Donor Program and Past President of the AABB. He is the recipient of numerous honors and awards including the NIH Clinical Center Director's Award, the Emily Cooley Award of the AABB, the Latham Award of the European Society of Hemapheresis, the Cohn-de-Laval Award of the World Apheresis Association (WAA), and the John B. Alsever Award for contributions to the blood banking sciences. Dr. Klein's area of research includes transfusion-transmitted disease, the management of immunosuppressive effects

of blood transfusion, and the impact of biotechnology on transfusion medicine.

4. Thomas Raife, MD, is a Clinical Professor of Pathology and Medical Director of the University of Iowa DeGowin Blood Center, Iowa City, IA. His research interest in TMAs was developed during his fellowship training and has become a 20-year devotion to the study of TTP and related disorders. The pathophysiology and treatment of microvascular thrombosis was the focus of two awarded grants from the NIH where he participated as co-investigator and principal investigator, respectively.
5. Gail Rock, MD, PhD, is Professor of Pathology and Laboratory Medicine in the Faculty of Medicine at the University of Ottawa, Ottawa, Ontario, Canada. She is the current Editor-in-Chief of the international journal *Transfusion and Apheresis Science,* Executive Vice-President of the Canadian Hematology Society, and Chair of the Canadian Apheresis Group. She is past president of ASFA, WAA, and Canadian Hematology Society. She was the recipient of the WAA Cohn-de-Laval Award, the President's Award from ASFA, the Achievement Award from the Canadian Blood Services, and the Francis S. Morrison Memorial Lectureship from ASFA. The focus of Dr. Rock's research is TTP.
6. J. Evan Sadler, MD, PhD, is currently Chief of the Division of Hematology, Professor of Medicine, and Professor of Biochemistry and Molecular Biophysics at Washington University School of Medicine in St. Louis, MO. He is a past president of ASH and past long term Investigator at Howard Hughes Medical Institute at St. Louis, where his research focused on the pathophysiology of hemorrhagic and thrombotic diseases including von Willebrand disease and TTP. He was the recipient of the ASH William Dameshek Prize, and the Distinguished Career Award and Investigator Recognition Award from the ISTH.
7. Ravi Sarode, MD, is Professor of Pathology and Director of the Division of Transfusion Medicine and Hemostasis at UT Southwestern Medical Center in Dallas, TX. He is the immediate past president of ASFA. His clinical research has concentrated on transfusion medicine, hemostasis and therapeutic apheresis leading to appropriate utilization of blood components, laboratory testing and apheresis techniques. For over two decades, his special interest has been the management of TTP and TMAs.
8. Darrell Triulzi, MD, is Professor of Pathology and Director of the Division of Transfusion Medicine at the University of Pittsburgh, Pittsburgh, PA. He also serves as the Medical Director of the Institute for Transfusion Medicine and the Director of the Centralized Transfusion Service serving 15 hospitals in Pittsburgh. Dr. Triulzi is the immediate Past-President of AABB. He is an associate editor for the journal *Transfusion* and a member of the American Board of Pathology Test Committee on Blood Banking/Transfusion Medicine. He is the PI for the NHLBI Transfusion Medicine/Hemostasis Clinical Trials Network and NHLBI REDSIII network. Dr. Triulzi is a recognized expert in transfusion support of organ transplant recipients, leukoreduction, platelet transfusions, and the development of a model for centralized transfusion services.
9. Han-Mou Tsai, MD, presently directs the establishment of Blood Coagulation Center at E-Da Hospital in Kaohsiung, Taiwan. His past appointments include Professor of Medicine and Associate Head of the Unified Division of Hematology at Albert Einstein College of Medicine and Montefiore Medical Center, Bronx, NY, and Professor of Medicine and Pathology, M. Elaine M.D. Professor of Hematology at Penn State University Hershey Medical Center, Hershey, PA. Dr. Tsai's research in the investigation of a von Willebrand factor-cleaving protease in the plasma was seminal in the identification and cloning of the protease as ADAMTS13 and the association of TTP with autoimmune inhibitors or mutations of the protease. The rationale of using rituximab or cyclosporine in patients with refractory or relapsing TTP and development of rADAMTS13 for the treatment of TTP were based on these discoveries. He demonstrated that shear stress not only increases the susceptibility of von Willebrand factor to cleavage by ADAMTS13 but also enhances its platelet aggregating capacity, thus providing a lucid example of physical forces directly affecting biochemical reactions in vivo.